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Mirtazapine Alters Cue-Associated Methamphetamine-Seeking in Rats

Steven M. Graves and **T. Celeste Napier**
Department of Pharmacology, and Center for Compulsive Behavior and Addiction Rush University Medical Center, Chicago, IL

Abstract

Background—Methamphetamine (METH) is a potent psychostimulant, repeated use of which can result in a substance abuse disorder. Withdrawn individuals are highly prone to relapse which may be driven, at least in part, by a hyper-responsivity to METH-associated cues that can prompt METH-seeking. There is a critical need to identify clinically efficacious pharmacotherapies for METH abuse. Mirtazapine (Remeron®) is an atypical antidepressant, that antagonizes activated norepinephrine$_{\alpha 2}$, histamine$_1$ serotonin (5-HT)$_{2A/C}$, and 5-HT$_3$ receptors. This pharmacological profile prompted our interest in its potential for preventing relapse to METH-taking. The current study tested the hypothesis that mirtazapine would attenuate METH-seeking in rats trained to self-administer METH.

Methods—Rats were trained to self-administer METH in a lever-pressing operant task. The effect of mirtazapine on METH-seeking was determined by evaluating lever pressing in the presence of cues previously associated with METH, but in the absence of METH reinforcement. Two paradigms were utilized: cue reactivity, wherein rats do not undergo extinction training, and a cue-induced reinstatement paradigm after extinction.

Results—Mirtazapine (5.0mg/kg) pretreatment reduced METH-seeking by ~50% in the first 15min of cue reactivity and cue-induced reinstatement testing. This mirtazapine dose did not significantly impact motor performance.

Conclusions—This study revealed the overlapping nature of cue reactivity and cue-induced reinstatement procedures and provided preclinical evidence that mirtazapine can attenuate METH-seeking behavior.

Keywords

methamphetamine; reinstatement; cue reactivity; atypical antidepressant; self-administration

Introduction

Methamphetamine (METH) is a potent psychostimulant responsible for a $23.4 billion socioeconomic burden in 2005 (http://www.rand.org/news/press/2009/02/04/meth.html). Mirtazapine (Remeron®) is an atypical antidepressant approved for the treatment of

Corresponding Author: Steven M. Graves, Dept. of Pharmacology, Rush University Medical Center, 1735 W Harrison Street, Cohn Research Building #463, Chicago, IL 60612, Phone: 312-563-4887, FAX: 312-563-2403, Steven_graves@rush.edu.

moderate to severe depression and is an antagonist at the norepinephrine$_{\alpha 2}$, histamine$_1$, serotonin (5-HT)$_3$, 5-HT$_{2A/C}$ receptors (1–3) and an indirect acting 5-HT$_{1A}$ receptor agonist (4–6). Recent evidence indicates inverse agonist properties (ie, negative intrinsic efficacy) at constitutively active (ie, displays agonist independent activation) 5-HT$_{2C}$ receptors (7). 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors regulate dopamine function throughout the mesoaccumbens pathway (8–11,11–13) (for reviews, see (14,15)) and may provide opportunities for psychostimulant abuse pharmacotherapy (for reviews, see (16,17)). Human imaging studies reveal brain regions that are activated by drug-associated cues which correlates with ratings of craving, dopamine release, and severity of the substance abuse disorder (18–23). We contend that mirtazapine is well-suited to blunt such cue-associated responses and in so doing, effectively attenuate relapse.

Mirtazapine used off-label to treat patients with substance abuse disorders provides encouraging results. Mirtazapine, reduces hyperarousal and anxiety that occurs during amphetamine detoxification (24), improves sleep pattern disturbances during withdrawal from METH (25), reduces craving in alcohol-dependent patients with co-morbid depressive disorders (26–28), improves tolerability of alcohol detoxification (28,29), maintains abstinence in opioid-dependent patients (30), and alleviates insomnia and depression during benzodiazepine withdrawal (31). While most clinical evidence consists of case reports or studies with small sample sizes, collectively this literature indicates the potential for mirtazapine to provide therapeutic benefit for relapse reduction.

Our preclinical studies further indicate the therapeutic potential of mirtazapine in brain and behavioral effects of METH. We revealed that repeated administration of mirtazapine attenuates the expression of METH-induced behavioral sensitization and associated electrophysiological changes in the limbic (ventral) pallidum (32). We also determined that mirtazapine attenuates METH-induced conditioned place preference (33). The current study focused on the potential of mirtazapine to reduce “seeking-like” behaviors in rodents. To do so, rats were trained to self-administer METH and tested for cue-associated METH-seeking using a classical cue-induced reinstatement (C-IR) paradigm and more novel cue reactivity (CR) assessments. The tested hypothesis was that mirtazapine would reduce METH-seeking as shown by two different models of the abstinent human METH abuser. As mirtazapine was shown to be effective during the first three days after withdrawal from amphetamine in dependent humans (24), for the current pioneering studies in laboratory rats, we opted to also test the effects of mirtazapine during a short term (ie, one day) withdrawal.

Methods and Materials

Animals

Male Sprague-Dawley rats were purchased from Harlan (Indianapolis, IN) weighing 250–275g and housed 2/cage under a 12hr light/dark cycle in an environmentally controlled facility; food and water were provided *ad libitum*. Rats were handled in accordance with procedures established in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, Washington DC) and protocols were approved by the Rush University Institutional Animal Care and Use Committee.

Test Drugs

Mirtazapine (isolated from tablet form by Plantex; Hackensack, NJ) was dissolved in HCl, diluted in sterile water, pH adjusted to ~6.8 with NaOH and administered intraperitoneally (ip) as 0.5, 1.0 or 5.0mg/kg for CR, C-IR, and rotorod assessments. Pentobarbital (10mg/ml; Cardinal Health, Chicago, IL) was administered as 10mg/kg ip for a positive control for

rotorod assessments. METH (Sigma St. Louis, MO) was self-administered intravenously as 0.1mg/kg/0.1ml sterile saline per infusion.

Intravenous Catheter Implantation

Rats were anesthetized and implanted with custom built catheters constructed of silastic tubing (0.3mm i.d. × 0.64mm o.d.; Dow Corning Co., Midland, MI) in the right jugular vein. The distal end of the cannula passed subcutaneously over the mid-scapular region and exited through a metal guide cannula (22 gauge; Plastics One Inc., Roanoke, VA). Five days post surgery recovery was provided before beginning self-administration. Catheters were flushed daily with 0.1–0.2ml sterile saline (heparin was not included) to maintain catheter patency. Catheter patency was implicated by ease of flushing and consistent METH self-administration.

Self-Administration

Operant chambers (Med-Associates, St. Albans, VT) equipped with two levers (the left lever was designated as “active” and the right as “inactive”), a stimulus light above each lever, and a house-light on the opposite wall were enclosed in sound attenuating, ventilated cabinets. Infusions were delivered by a syringe attached to an infusion pump. Self-administration sessions lasted for 3hr/day. Depressing the active lever resulted in infusion of 0.1mg/kg/0.1ml METH over 6sec accompanied by illumination of a cue light above the lever. Subsequently, the house-light was illuminated and remained lit for 20sec indicating a “*time-out*” period. During time-out, active lever presses were recorded but had no programmed consequence. Inactive lever presses never had programmed consequences. Number of active lever presses, inactive lever presses, and number of infusions were recorded. Rats were trained for 14 days to self-administer METH; animals were not food restricted and minimal shaping was used. On days 1–7, rats self-administered on a fixed-ratio 1 (FR1; 1 lever press/infusion) schedule of reinforcement and on day 8, all rats were moved to an FR5 schedule (see Figure 1) to increase behavioral responses and increase resistance to extinction (34). Rats were administered mirtazapine vehicle ip 15min prior to self-administration sessions on days 8–14 to acclimate rats to injection procedures. Stable self-administration was operationally defined as less than 15% reinforcement variability between days 13 and 14; rats failing to meet this criterion were excluded from the study.

Cue-Reactivity/Extinction Responding (CR)

Responding to contextual (operant chamber) and explicit cues (cue light, time-out light, and activation of infusion pump) associated with METH self-administration was assessed by a 1hr CR test session. CR testing consisted of explicit cues contingently presented on an FR1 schedule in the operant chamber context. To ensure that no METH was received, lines were flushed with saline. Saline-filled syringes remained connected to infusion lines to prevent air from entering and were disconnected from the infusion pump. Using a within-subjects design, rats were evaluated for CR in five, once-daily sessions. The first CR test (CR1; day 15) was performed 15min after vehicle pretreatment. CR1 served to acclimate rats to the protocol and determine baseline METH-seeking (CR1 was not included as part of the dose-response assessment). CR2-CR5 tests were performed 15min after pretreatment with vehicle, 0.5, 1.0, or 5.0mg/kg mirtazapine ip (in randomized order) on days 18, 21, 24 and 27 (Figure 1A). METH-seeking was quantified by number of active lever presses. The number of inactive lever presses, contingently presented cues, active lever presses during time-out periods, and latency to first response were also recorded. To prevent extinction, CR sessions were interposed with two days of self-administration following the same FR5 3hr/day procedure used on days 8–14 (referred to as “*intermittent self-administration*”). For the rat to be retained in the study, the number of infusions during one of the two consecutive days had to be at least 50% the number of reinforcements achieved on the last day of the

FR5 baseline (ie, day 14). Stability and reliability of the CR behavior was validated by comparing CR across the randomly administered vehicle-treated controls, and the behavior during intermittent self-administration was compared throughout the paradigm.

Cue-induced Reinstatement (C-IR)

Rats were trained to self-administer METH as described above for 14 consecutive days (FR1 days 1–7; FR5 days 8–14) after which rats underwent extinction training using a modified protocol from Rogers *et al.*, and Reichel & See (35,36). Extinction training sessions (3hr/day) continued for a minimum of 10 days (refer to Figure 1B) during which lever pressing was recorded but had no programmed consequences (ie, explicit cues were not extinguished). Extinction was defined as two consecutive days in which the number of active lever presses during the first hr of the session was less than 20% of that achieved during the first hr of day 14 self-administration, for two consecutive days. After active lever pressing was extinguished, separate groups of rats were subjected to a 15min pretreatment of vehicle, 0.5, 1.0, or 5.0mg/kg mirtazapine, and tested for C-IR. C-IR sessions commenced with a single 20sec presentation of the cue light followed by contingent presentations of the explicit cues (cue light, time-out light, and activation of infusion pump) on an FR1 schedule, in the absence of METH reinforcement. C-IR sessions lasted for 1hr. Number of active lever presses, inactive lever presses, contingently presented cues, and active lever presses during time-out periods were recorded.

Motor Assessments

Rotorod tests were conducted to determine the effect of mirtazapine on motivated motor function. To do so, experimentally naïve rats were subjected to five days of training wherein rats were placed on the rotorod at an initial speed of five revolutions per min (rpm). Every 30sec, the speed was increased by 5rpm increments to a maximum of 40rpm for up to 2min. Rats that failed to remain on the rotorod at 20rpm on day 5 were excluded from the study. Rotorod performance was assessed following vehicle, 0.5, 1.0, and 5.0mg/kg mirtazapine ip, given in randomized order (within-subjects design). Performance was tested 15, 30, 45, 60, and 75min after the injection. Motor assessments were conducted every other day. During the intervening days, the drug-free rats were tested for maintenance of minimum performance, ie, rats failing to reach a speed of 20rpm were excluded from the study. After completion of the mirtazapine dose-response study, rats were tested with 10mg/kg pentobarbital (ip) as a positive control. The pentobarbital dose was selected based on pilot studies showing that 10mg/kg did not impair the righting reflex (data not shown).

To determine if chronic METH could alter responding to mirtazapine, a separate group of METH self-administering rats (n=14; from an ongoing study) were used to evaluate motor function in a small (25″ L × 12″ W) automated activity chamber (Accuscan Instruments, Columbus, OH). This was conducted six days after last METH exposure. The rats were habituated to the activity chamber for 1hr, administered 5.0mg/kg ip mirtazapine or its vehicle, and 15min later, motor activity was monitored by photo beams for 45min.

Statistical Analysis

Data are presented as mean ± SEM. Data from self-administration, CR, C-IR, and intermittent self-administration sessions were analyzed separately. A one-way repeated measures ANOVA (rmANOVA) was used to analyze the number of active lever presses, inactive lever presses, the number of infusions per session for days 10–14 of self-administration, the latency to first response, and for sessions of intermittent self-administration. A Student's *t*-test was used to compare behavior from the last day of self-administration (ie, active lever presses, inactive lever presses, and infusions) between groups assigned to CR and C-IR protocols. Reinstatement was verified using a two-way rmANOVA

comparing active lever pressing behavior (15min intervals) from the last day of extinction training *vs*. C-IR. Dose-response assessments of CR and C-IR tests were analyzed using a two-way rmANOVA for active lever presses, inactive lever presses, contingently presented cues, and time-out lever pressing. A two-way rmANOVA was used to analyze the latency to fall for rotorod testing comparing doses of mirtazapine, vehicle and pentobarbital. When appropriate, a Newman-Keuls *post-hoc* analysis was used. For all analyses $\alpha=0.05$. Data were analyzed using Graphpad Prism Software v5 (La Jolla, CA) or GB-STAT professional v10 (Dynamics Microsystems; Silver Springs, MD). For each comparison, statistical outliers were calculated as being greater than two standard deviations from the mean and were not included in the analysis (rats failing to meet minimum criteria described above were removed from all analyses); for time course data (CR and C-IR tests), outliers were calculated using the cumulative average over the 60min test session.

Results

Self-Administration

Sixty-one rats were trained to self-administer METH; 4 rats were excluded from analysis due to >15% infusion variability between days 13 and 14, and 3 rats died overnight after a self-administration session. Of the remaining 54 rats, 17 were assigned to the CR and 37 to the C-IR protocol. The average daily METH infusions for the last 2 training sessions for rats in the CR protocol was 20.9±2.5 for day 13 and 21.7±2.6 for day 14, and for rats in the C-IR protocol was 21.7±1.6 for day 13 and 21.3±1.5 for day 14 (Figure 2); there was no difference between rats assigned to CR *vs.* C-IR protocols in number of active lever presses ($p=0.82$), inactive lever presses ($p=0.18$), or infusions ($p=0.90$) from the last day of self-administration (Student's *t*-test; data not shown). For rats assigned to C-IR protocol (between-subjects design) there was no difference in pre-extinction self-administration behaviors (ie, day 14 session), including number of active lever presses ($p=0.93$), inactive lever presses ($p=0.13$), or infusions ($p=0.95$) for the treatment groups (one-way ANOVA; data not shown).

Cue Reactivity/Extinction Responding

For the CR protocol, a within-subjects design was used wherein each rat was tested with all treatment conditions. This design required verification that the measured outcome, CR, remained stable for the extent of the assessment period (days 18–27). Cues used in the current study for CR testing were the explicit cues contingently presented upon active lever pressing in the operant chamber context. Repeated exposure to cues in the absence of METH may induce extinction learning. Thus, to minimize possible extinction training and maintain stable METH-seeking, CR tests were interposed with two days of intermittent METH self-administration. Two rats were removed from the study as their responding for two consecutive days fell below 50% of the number of reinforcements received on day 14 of self-administration. Data collected from vehicle CR sessions for the remaining 15 rats are shown in Figure 3. Due to randomizing the treatment order, the number of animals tested for vehicle CR on days 18, 21, 24, and 27 were 5, 3, 3, and 4, respectively. Active lever pressing during CR2-5 was not significantly different ($p=0.78$; one-way ANOVA), nor were any trends apparent (eg, 'cumulative extinction' did not occur). These observations demonstrate that METH-seeking was persistent and stable. Also presented in Figure 3 are the intermittent self-administration data from rats that completed the full dose-response assessment tested across the five CR tests (n=15). The number of active lever presses ($p=0.15$), inactive lever presses ($p=0.32$), and infusions ($p=0.33$) did not differ statistically (one-way rmANOVA) throughout periods of intermittent self-administration. These assessments demonstrate that despite repeated CR testing, extinction training did not occur

and self-administration and METH-seeking were consistent throughout the study. These conclusions are validated by our other studies with this protocol (37).

Independent of the CR test number or pretreatment, METH-seeking behaviors occurred most intensely within the first 15min of CR testing (Figure 4A); by 30min, levels emulated those expressed on the inactive lever (Figures 4A *vs.* 5A for 30–60min). A 15min pretreatment with 5.0mg/kg ip mirtazapine reduced the number of active lever presses (Figure 4A) during the first 15min. Nevertheless, the ability to discriminate between the active and inactive levers was not impaired by 5.0mg/kg mirtazapine, ie, the number of active lever presses were significantly higher than inactive lever presses during that time period (two-way rmANOVA with *post-hoc* Newman-Keuls; $p<0.001$; data not shown). This conclusion was verified with re-analysis of the data using two way rmANOVA comparing dose and lever for the first 15min (the full time course was not included in order to provide adequate power) indicating a significant Dose x Lever interaction ($F_{(3,14)}=2.79$). Pretreatment with 0.5 and 1.0mg/kg mirtazapine did not alter active lever pressing (Figure 4A). Similar to effects on overall active lever pressing behavior, in the first 15min, 5.0mg/kg mirtazapine reduced the number of contingently presented cues ($p<0.001$), and active lever pressing during time-out periods ($p<0.001$) by approximately 50% (data not shown). There was no significant effect on inactive lever pressing (Figure 5A) or the latency to first response ($p=0.21$) (data not shown).

Cue-Induced Reinstatement

Similar to CR assessments, lever pressing behavior during C-IR was most robust in the first 15min of the session. Rats significantly reinstated active lever pressing in the first 15min of reinstatement testing after a pretreatment of vehicle ($p<0.001$), 0.5 ($p<0.001$), 1.0 ($p<0.001$), and 5.0mg/kg ($p<0.001$) mirtazapine (compared to lever pressing during the last day of extinction; two-way rmANOVA with Newman-Keuls; data not shown). Also, similar to CR assessments, 5.0mg/kg mirtazapine significantly attenuated the number of active lever presses by approximately 50%, while 0.5 and 1.0mg/kg had no effect (Figure 4B). In contrast to CR, C-IR inactive lever pressing was significantly decreased by 5.0mg/kg mirtazapine (Figure 5B). Similar to the effects seen with CR assessments, contingent cues ($p<0.01$) and time-out lever pressing ($p<0.01$) were also attenuated by approximately 50% (two-way rmANOVA, Newman-Keuls *post-hoc*, data not shown). Active lever pressing remained higher than inactive after 5.0mg/kg mirtazapine (two-way rmANOVA, Newman-Keuls; $p<0.001$) again demonstrating the rats' ability to discriminate between levers. Re-analysis of these data using two way rmANOVA comparing dose and lever for the first 15min did not reveal a Dose x Lever interaction ($F_{(3,24)}=0.95$), in contrast to such analyses of CR. This likely reflects greater variability seen with C-IR compared to CR. Mirtazapine at 5.0mg/kg resulted in zero lever presses for two rats, thus, response latency could not be analyzed

Motor Assessments

Two additional experiments were performed to aid in interpreting mirtazapine-induced responding in the CR and C-IR studies, First, a set of METH self-administering rats (n= 14) were tested for effects of 5.0mg/kg mirtazapine (or vehicle) on motor function in a small animal activity chamber. Mirtazapine had no effect on total distance traveled (vehicle, 183±53cm; mirtazapine, 172±45cm, $p=0.89$) or rearing/wall climbing (vehicle, 37±9; mirtazapine, 27±6 beam breaks, $p=0.31$) (paired *t*-test comparisons). Second, rotorod assessments were used to evaluate the effects of mirtazapine on motivated motor behaviors in METH-naïve rats. The positive control, pentobarbital, significantly reduced performance at all time periods assessed (Figure 6). In contrast, mirtazapine had no effect on rotorod performance at any of the doses used for the CR and C-IR tests. These collective results

demonstrate that the effects observed with mirtazapine in the CR and C-IR were likely not to be related to impairments in motor function.

Discussion

Mirtazapine decreased METH-seeking in both CR and C-IR protocols without altering motor performance. These results concur with prior work showing the ability of mirtazapine to reduce expression of METH-induced place preference (33) and behavioral sensitization (32). For each of these studies, mirtazapine was administered after METH had altered the animals' brain and behavior, and the abrogation of METH effects occurred one day (current study and (33)), or 30 days (32) after the last METH exposure. Pilot studies with METH self-administering rats show that after 14 days of abstinence in the home cage, subsequent CR testing with mirtazapine (5mg/kg; n=4) decreased active lever pressing by 40–50% compared to vehicle-treated rats (n=3). These preliminary data are similar to the current results obtained during an early phase of withdrawal. Thus, preclinical evidence continues to emerge that indicates possible therapeutic value of mirtazapine in the abstinent, METH-abusing individual.

The focus of the current study was on behavioral processes that indicate METH-seeking. Toward that end, we compared the effect of mirtazapine between an established model of 'seeking', C-IR, with the less explored CR model. We reveal that subsequent exposure to explicit cues (in the absence of METH reinforcement) resulted in similar outcomes regardless of the presence or absence of extinction training. Behavioral patterns were consistent between CR and C-IR testing showing robust lever pressing within the first 15min of testing followed by a progressive extinction for the remainder of the session similar to that seen by previous investigators (38,39). The C-IR protocol results in robust behavior, with rather large variability as previously reported (35,36,40). The CR protocol was less robust, but with reduced variability. In both CR and C-IR, the number of active lever presses was reduced in the first 15min of testing indicating that the two paradigms can provide similar indications for the capacity of pharmacologics to reduce METH-seeking. In C-IR, the significance of context cues are extinguished, while salience is retained for explicit cues. Our observations demonstrate that the significance of cues that drive C-IR as well as CR behaviors is vulnerable to the effects of mirtazapine. Thus, extinction procedures appear to be unnecessary to explore seeking behavior in rodent models of human abstinence. Recently, others have also successfully examined the ability of potential pharmacologics to alter extinction-like responding similar to the CR tests used here (39,41,42). There are several features of the CR paradigm that make it attractive for screening of potential relapse reduction, including: (i) Repeated CR assessments did not impair the 'reacquisition' of self-administration behaviors during periods of intermittent self-administration, and (ii) lever pressing was not extinguished by repeated CR testing. Thus, CR may provide a valuable addition to current rodent models of human drug-seeking.

The effects of mirtazapine on CR and C-IR may differ for inactive lever pressing behavior. In the current study, the patterns of responding were similar, but only C-IR achieved a statistically significant decrease at 5.0mg/kg mirtazapine. This may reflect the differential significance of contextual cues between CR and C-IR. Reinstatement groups underwent a minimum of 10 days of extinction training in which the significance of operant box context, including both levers, was extinguished; whereas, with CR the contextual salience was maintained with periods of intermittent self-administration. This may explain the larger variance seen in the inactive lever pressing during CR compared to that in C-IR. As 5.0mg/kg mirtazapine resulted in a trend for decreased pressing for the inactive lever, this larger variance seen in the CR protocol may have contributed to a false negative statistical outcome. There are several possible interpretations for changes in inactive lever pressing;

non-specific effects on motor is one. However, there is compelling evidence that argues otherwise. For example, a $GABA_B$ agonist (CGP44542) at doses which decrease inactive lever pressing in an operant task (43), have no effect on performance in an intracranial self-stimulation procedure (44). Such reports, along with the current observation that 5.0mg/kg mirtazapine had no effect on motor function in naïve rats or rats with a METH history calls for alternative interpretations. One attractive conclusion is that that mirtazapine blunts the salience of the levers rather than impairing the ability of the rats to perform the operant task. Future studies aimed at exploring the effect of mirtazapine on lever pressing for non-drug reinforcers would aid in interpreting the behavioral effects of mirtazapine. Such a study should involve a reinforcer other than food as a clinically noteworthy side-effect of mirtazapine is weight gain which may confound food-oriented evaluations.

The decrease in METH-seeking behavior observed with 5.0 mg/kg mirtazapine was robust for the number of active lever presses, contingent cues, and time-out lever pressing for both CR and C-IR testing, with ~50% decrease obtained in each parameter. Emerging preclinical literature indicates 5-HT_{2A} and 5-HT_3 receptor antagonists as potential substance abuse pharmacotherapy; both of these receptors likely contributed to the current findings. Serotonin$_{2A}$ antagonism attenuates cocaine-elicited motor activity (45), the discriminative stimulus properties of cocaine and METH (45,46,46,47), and C-IR of cocaine-seeking (47). Similar effects are observed with 5-HT_3 antagonism which attenuates stress-induced reinstatement of alcohol-seeking (48), cocaine-induced lowering of reward thresholds (49), and intra-ventral tagmental area self-administration of cocaine (50). Mirtazapine engages the 5-HT_{1A} receptor and 5-HT_{1A} receptor activation impacts addictive-like behaviors in both rodents and non-human primates (for review, see 51). For example, a 5-HT_{1A} agonist (8-OH-DPAT), decreases cocaine self-administration by rats (52). Mirtazapine emulates several 5-HT_{1A} agonist actions in the central nervous system (4–6), which may have contributed to the observed effects. Mirtazapine also can be fully substituted by a 5-HT_{2C} antagonist in a drug discrimination paradigm (53). However this property likely did not contribute to the current observations, for 5-HT_{2C} receptor antagonists do not alter cue-induced reinstatement of cocaine-seeking in rats (54,55) and we have found that these drugs also do not influence METH-seeking using CR assessments (37). Recent *in vitro* evaluations indicate that mirtazapine acts as a 5-HT_{2C} inverse agonist, and not an antagonist (7). This is an interesting feature, as 5-HT_{2C} constitutive activity strongly regulates dopamine efflux in the nucleus accumbens (9). Further studies are needed to determine the therapeutic potential of 5-HT_{2C} inverse agonists as substance abuse pharmacotherapy.

Conclusion

Acute administration of mirtazapine attenuated METH-seeking to a similar degree in both CR and C-IR tests at a dose that did not impair motor function. Mirtazapine is well tolerated in humans and we contend it deserves further consideration for relapse reduction in the abstinent stimulant abuser.

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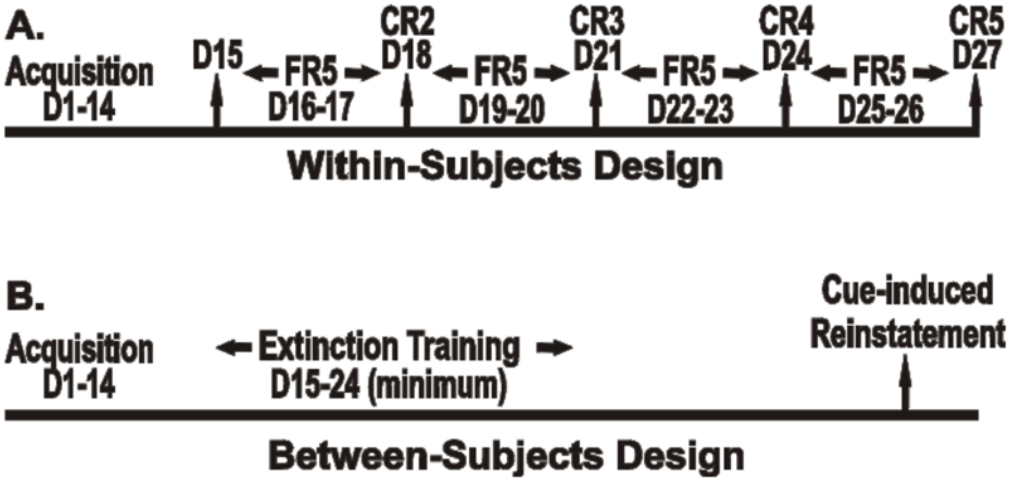

Figure 1. Cue reactivity and cue-induced reinstatement protocol timelines

Rats were trained to self-administer methamphetamine (METH) on a fixed ratio (FR) 1 schedule of reinforcement on days 1–7 and a FR5 on days 8–14 (acquisition). **A.** Cue Reactivity. On day 15, rats were acclimated to the cue reactivity (CR) protocol; mirtazapine assessments were made on days 18, 21, 24 and 27 (ie, CR2-CR5) during which a 15min pretreatment was administered in a randomized order. Between each CR test, rats were allowed to self-administer 0.1mg/kg/0.1ml infusion METH on an FR5 schedule for 2 consecutive days. **B.** Cue-induced reinstatement. Rats were trained to self-administer for 14 consecutive days (as described above); beginning day 15 rats underwent a minimum of 10 consecutive days of extinction training (3hr/day) during which the context but not explicit cues were extinguished. Twenty-four hours after the last day of extinction training rats were tested for cue-induced reinstatement.

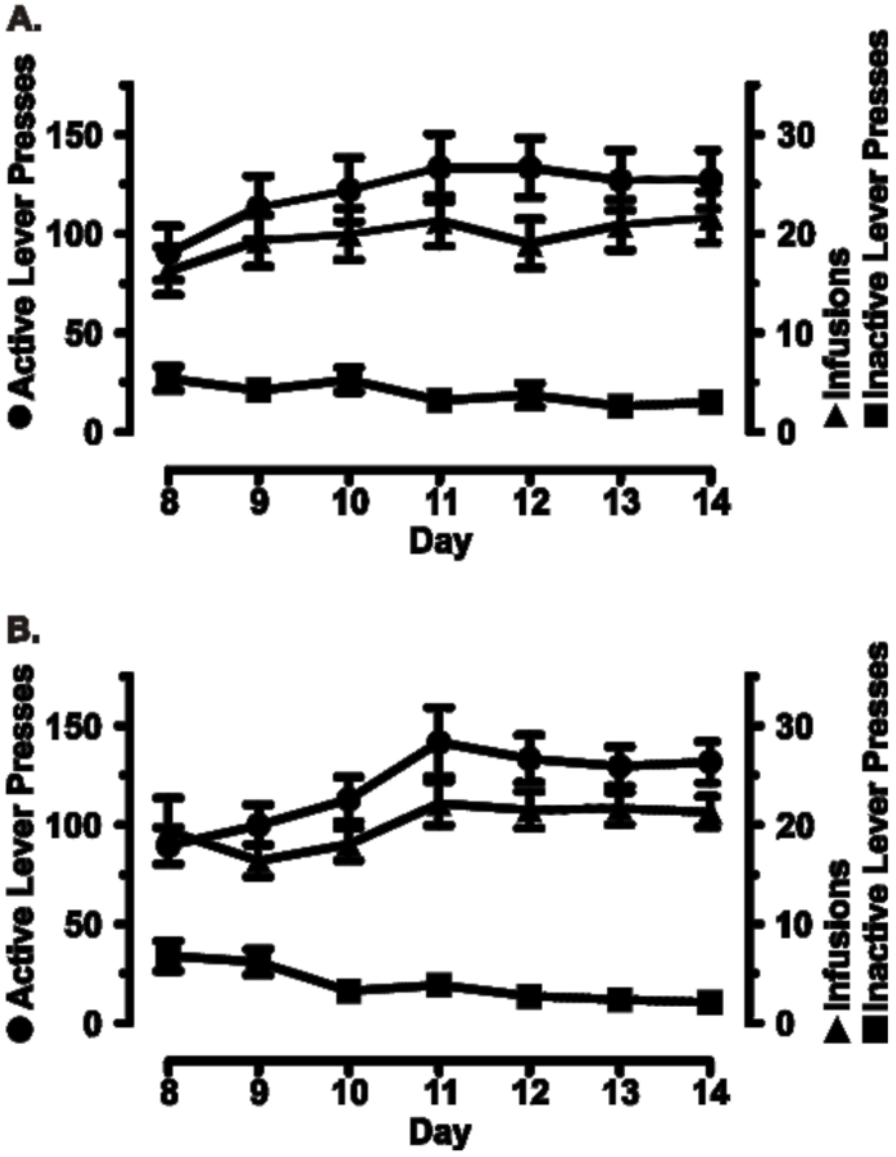

Figure 2. Self-administration prior to cue reactivity and cue-induced reinstatement testing

Illustrated is METH self-administered 0.1mg/kg/0.1ml in a fixed ratio (FR) 5 schedule of reinforcement on days 8–14. Four rats demonstrating >15% infusion variability between days 13 and 14 were excluded from the study. **A.** Cue reactivity. Seventeen rats assigned to CR testing demonstrated stable self-administration behavior and had no significant differences across the last 4 days of self-administration for active lever presses ($F_{(3,16)}$=0.23), inactive lever presses ($F_{(3,14)}$=0.53; 2 statistical outliers excluded), or infusions ($F_{(3,16)}$=0.61) as measured by one-way rmANOVA. **B.** Cue-induced reinstatement. Thirty-seven rats assigned to cue-induced reinstatement testing had no significant differences across the last 4 days of self-administration for active lever presses ($F_{(3,41)}$=0.48; 1 statistical outlier excluded), inactive lever presses ($F_{(3,39)}$=2.02; 3 statistical outliers excluded), or infusions ($F_{(3,41)}$=0.17; 1 statistical outlier excluded) as measured by one-way rmANOVA.

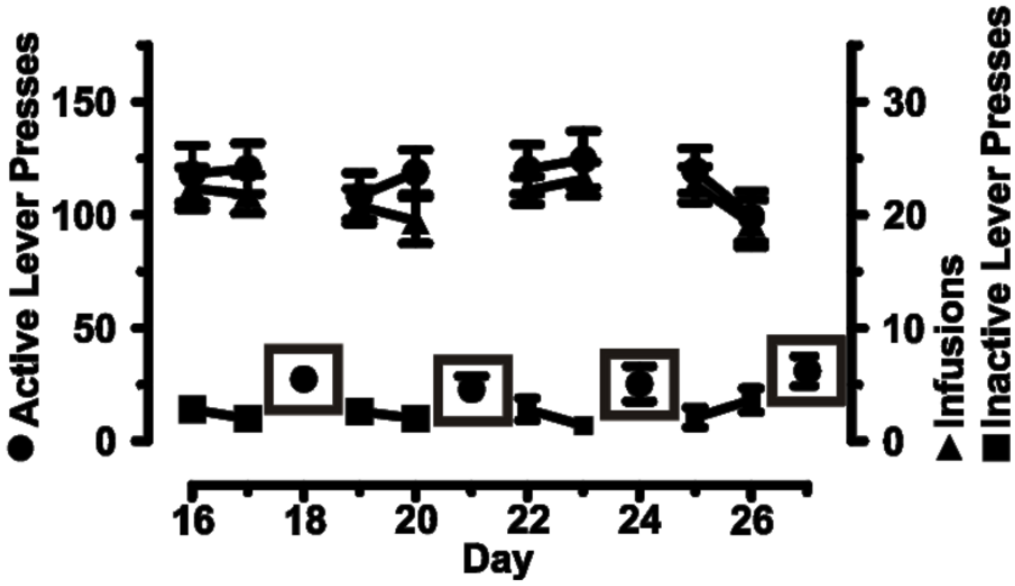

Figure 3. Stability of cue reactivity and interposed self-administration

Shown is cue reactivity (CR; boxed-in closed circles) as measured by active lever presses for the vehicle pretreatments. Number of rats tested in CR2, CR3, CR4, and CR5 were 5, 3, 3, and 4, respectively; thus, the 15 rats who met the inclusion criteria (maintaining no less than 50% the number of reinforcements achieved on day 14 for the 2 consecutive days of intermittent self-administration; 2 of 17 rats did not meet criteria) each received one randomly assigned vehicle test. Vehicle testing was not significantly different between CR2-CR5 ($F_{(3,11)}$=0.37). Each CR session was interposed with 2 days of self-administration (0.1 mg/kg/0.1ml METH; FR5; 3hrs/day). The inactive and active lever presses, as well as number of infusions, are shown for these sessions. Stable self-administration was achieved with this intermittent self-administration protocol; one-way rmANOVA showed no difference in number of active lever presses ($F_{(7,12)}$=1.86; 2 statistical outliers excluded), inactive lever presses ($F_{(7,12)}$=1.19; 4 statistical outliers excluded), or infusions ($F_{(7,12)}$=1.17; 2 statistical outliers excluded) across intermittent self-administration period.

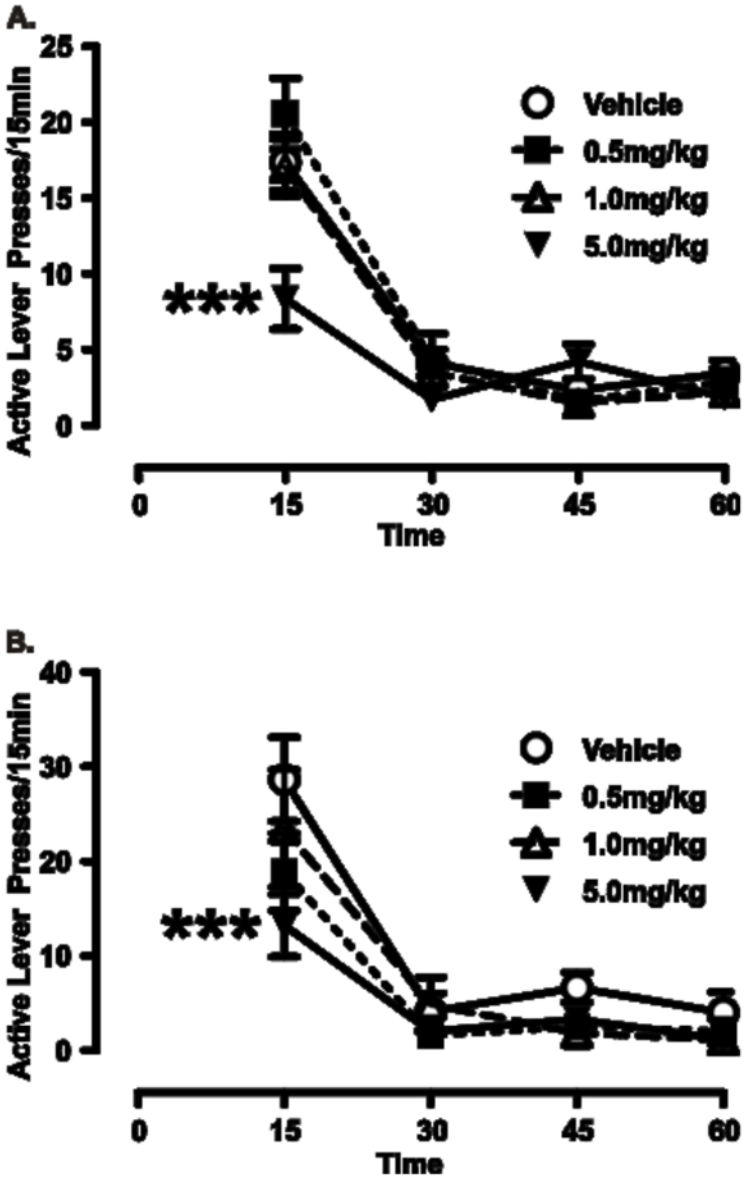

Figure 4. Active lever pressing was altered by mirtazapine

Shown are data from the rats that completed the cue reactivity (n=15) and cue-induced reinstatement (n=37) paradigms. Rats were tested after a 15min pretreatment of vehicle, 0.5, 1.0, or 5.0mg/kg mirtazapine (within-subjects design for cue reactivity and between-subjects design for cue-induced reinstatement paradigms). **A.** Cue reactivity. Each CR session consisted of contingent cue presentations on a fixed ratio (FR) 1 schedule for 1hr. Two-way rmANOVA revealed a significant Treatment effect ($F_{(3,52)}$=3.80), Time effect ($F_{(3,156)}$=116.23), and Treatment x Time interaction ($F_{(9,156)}$=5.09); one statistical outlier excluded from analysis. Pretreatment with 5.0mg/kg mirtazapine attenuated active lever pressing whereas 0.5 and 1.0mg/kg mirtazapine had no effect. (***p<0.001 comparing vehicle *vs*. 5.0 mg/kg mirtazapine pretreatment; Newman-Keuls *post-hoc*) **B.** Cue-induced reinstatement. Each reinstatement session consisted of a passive presentation of the cue light followed by contingent cue presentations on a fixed ratio (FR) 1 schedule for 1hr. Two-way rmANOVA revealed a significant Treatment effect ($F_{(3,9)}$=3.67), Time effect

($F_{(3,124)}$=45.49), and no Treatment x Time interaction ($F_{(9,124)}$=1.24). Pretreatment with 5.0mg/kg mirtazapine decreased active lever pressing in the first 15min (***p<0.001 comparing vehicle *vs*. 5.0 mg/kg mirtazapine pretreatment; Newman-Keuls *post-hoc*.); one statistical outlier removed from vehicle and 0.5mg/kg mirtazapine pretreated groups.

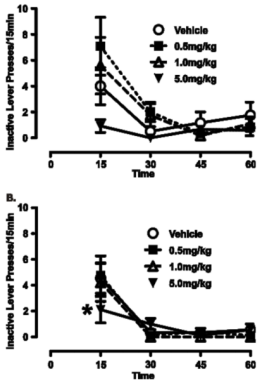

Figure 5. Inactive lever pressing during mirtazapine

Shown are data from the rats that completed the cue reactivity (n=15) and cue-induced reinstatement (n=37) paradigms. **A.** Cue reactivity. A two-way rmANOVA revealed no Treatment effect ($F_{(3,44)}$=2.29), a significant Time effect ($F_{(3,132)}$=13.46) with no Treatment x Time interaction ($F_{(9,132)}$=1.92); three statistical outliers excluded from analysis. **B.** Cue-induced reinstatement. A two-way rmANOVA revealed no Treatment effect ($F_{(3,44)}$=2.29) on inactive lever pressing. A significant Time effect was demonstrated ($F_{(3,132)}$=13.46) with no Treatment x Time interaction ($F_{(9,132)}$=1.92). Newman-Keuls *post-hoc* analysis indicated a significant decrease comparing 5.0mg/kg mirtazapine *vs*. vehicle in the first 15min of testing (*$p<0.05$); one statistical outlier removed from vehicle and 0.5mg/kg mirtazapine pretreated groups.

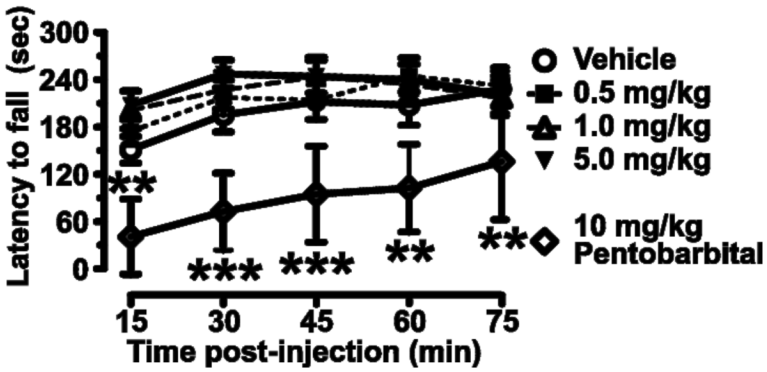

Figure 6. Rotorod evaluations of the motor effects of mirtazapine

Sixteen rats, separate from those used for self-administration, were evaluate for rotorod performance. Two-way rmANOVA revealed a Treatment effect ($F_{(4,75)}$=9.91), Time effect ($F_{(4,300)}$=21.74), and Treatment x Time interaction ($F_{(16,300)}$=2.44). Mirtazapine had no effect on rotorod performance at any dose tested. The positive control, 10 mg/kg ip pentobarbital significantly impairment of rotorod performance across all time points tested (**p<0.01; Newman-Keuls *post hoc).*