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Repeated mirtazapine nullifies the maintenance of previously established methamphetamine-induced conditioned place preference in rats

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Abstract

The atypical antidepressant mirtazapine enhances monoaminergic transmission; thus, mirtazapine therapy may counter the hypo-activation of monoamine systems associated with withdrawal from methamphetamine abuse. Human addiction therapy will likely require chronic administration that is given after brain and behavioral maladaptations are established. To emulate this scenario in rats, we ascertained if acute or repeated mirtazapine treatments could antagonize previously established consequences of repeated methamphetamine. Methamphetamine-induced conditioned place preference (CPP) was used, wherein methamphetamine (1mg/kg, i.p.) was administered in a unique environmental context once-daily for three days interposed by saline injections in an alternate context. Subsequently, mirtazapine (5mg/kg, i.p.) was administered in the home cage either as 10 once-daily injections or a single injection. The expression of CPP was determined in drug-free rats three days after the last mirtazapine injection. Expression of methamphetamine-induced CPP was inhibited by 10 home cage administrations of mirtazapine but not by a single injection of mirtazapine. These findings reveal that mirtazapine can inhibit the maintenance of methamphetamine-induced CPP and that treatment duration and/or treatment timing contributes to this effect of mirtazapine.

Keywords

methamphetamine; psychostimulant; mirtazapine; addiction; conditioned place preference

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1. Introduction

Methamphetamine is a potent, highly abused psychostimulant, and relapse to drug-taking behavior in the abstinent methamphetamine abuser is alarmingly high. In rodents, repeated methamphetamine administration induces long-term changes in brain protein expression [1,2] and neuronal function [3,4]. Such persistent maladaptations likely contribute to the high rate of relapse that occurs in methamphetamine-withdrawn addicts.

Exposure to cues previously associated with an abused drug can elicit drug-craving and seeking in individuals with substance abuse disorders [5,6]. The associative learning that occurs between the rewarding effects of abused substances and the associated contextual cues can be modeled in rodents using conditioned place preference (CPP). CPP measures the tendency to spend more time in an environmental context previously paired with the rewarding effects of an unconditioned stimulus, such as methamphetamine. A pharmacological mean to uncouple the contextual cue from the unconditioned stimulus should aid in reducing drug-seeking and relapse by the abstinent addict. Toward that objective, the current study evaluated the potential of the atypical antidepressant mirtazapine, to reverse *previously established* methamphetamine-induced CPP.

Mirtazapine enhances noradrenergic, serotonergic [7,8], and dopaminergic transmission [8,9]. As these systems are hypo-active during psychostimulant withdrawal [10–13], enhancing dopamine and serotonin levels in the brain may help alleviate the negative affect, drug-seeking, and relapse propensity that are associated with withdrawal [14]. We have revealed that one injection of mirtazapine administered 24h prior to CPP testing attenuates the expression of CPP induced by a two-day methamphetamine conditioning protocol [15]; however, this short protocol does not represent the human condition in which repeated drug-taking results in robust and persistent mnemonic processes that contributes to persistent drug-seeking in the abstinent addict. To better emulate the human scenario in the current study, multiple pairings were employed to strengthen the context association and promote endurance of the drug memory [16], and a longer methamphetamine-withdrawal period was imposed. Successful pharmacotherapy will likely necessitate repeated administration to significantly reduce the neuropathology associated with substance abuse disorders. Repeated mirtazapine administration results in profound adaptations in neuronal function (e.g., tonic activation of 5-HT_{1A} receptors) and gene expression (e.g., density of 5-HT₂ and β_1 adrenergic receptors) [17–20] that may influence the maintenance of methamphetamine-induced CPP; therefore, it was important to ascertain if repeated mirtazapine administration retained the ability to nullify previously established methamphetamine-induced behaviors. Additionally, as our prior work with single methamphetamine pairing showed successful antagonism of preference with a single mirtazapine injection, we sought to determine if a single injection of mirtazapine could also disrupt preference induced by multiple methamphetamine pairings.

2. Materials & Methods

2.1. Animals

One-hundred eleven male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 250–300g at the start of the experiments were acclimated to the vivarium for at least one week prior to the onset of experimentation. Rats were housed in pairs in a climate-controlled environment on a standard 12h light/dark cycle (lights on 7am, lights off 7pm) and allowed *ad libitum* access to food and water. Cage mates were given identical treatments. Housing facilities were accredited through the Association for Assessment and Accreditation of Laboratory Animal Care, and all experiments were carried out in accordance with the conditions set forth by the National Institutes of Health *Guide for the Care and Use of*

Laboratory Animals (National Research Council, 1996) and with the approval of the Institutional Animal Care and Use Committees at Loyola University Medical Center and Rush University Medical Center.

2.2. Drugs

(+)-Methamphetamine HCl (Sigma, St. Louis, MO) was dissolved in 0.9% sterile saline and the dose, 1mg/ml/kg, was calculated as the salt. Mirtazapine (1,2,3,4,10,14b-hexa-hydro-2-methylpyrazino [2,1-a] pyrido [2,3-c] benzazepine) (isolated from tablet by Plantex (Hackensack, NJ) a division of Teva Pharmaceutical Industries, Ltd. (North Wales, PA)) was dissolved in HCl, then sterile water was added to the final volume, with the final pH titrated to ~6.3 with acid/base. Mirtazapine was administered as 5mg/ml/kg. All injections were given intraperitoneally (i.p.).

2.3. Apparatus for Assessing Behavior

The test room was dimly lit (54–108 lux) with white noise (white noise generator, San Diego Instruments, San Diego, CA) continuously present. The CPP apparatus (63cm × 30cm × 30cm) consisted of three chambers divided by Plexiglas sliding doors (AccuScan Instruments, Inc., Columbus, OH); two large conditioning chambers (25cm × 30cm × 30cm) were separated by a small center chamber (13cm × 30cm × 30cm). Each chamber had distinct visual and tactile cues (chamber 1, vertical lines on walls and an overturned paint dish glued to the center of a randomly patterned floor; chamber 2, horizontal lines on walls and a square patterned floor; center chamber, no stripes on walls and a smooth, slightly raised platform floor). Time spent in each chamber and motor activity was monitored *via* two sets of photobeams (24 in the horizontal plane and 12 vertical in the vertical plane).

2.4. Conditioned Place Preference

The rats were transported from the animal housing room to the adjacent test room at least 30min prior to the start of the experiment. Rats were subjected to a 15min pre-test at least 72h prior to initiating conditioning to determine unconditioned preference. Pre-test results verified that the box configuration did not engender a group bias for either chamber; however individual rats tended to spend more time in one chamber than the other so a counterbalanced design was employed. Due to computer malfunction, pre-test data were not collected for 6 out of the 111 rats; thus, for these rats the day 1 chamber was randomly assigned. The average time spent for each group in the day 1 and day 2 paired chambers were approximately equal before conditioning. As illustrated in the experimental timelines (Fig. 1A & 2A), conditioning occurred over five days; methamphetamine-conditioned rats were given a methamphetamine injection every other day for three days and a saline injection on the alternate two days; saline-conditioned rats were administered saline for all five days. This conditioning protocol has been successfully used in the past to reliably produce amphetamine [21,22] and methamphetamine CPP that is sensitive to pharmacological interventions [23,24]. During conditioning, rats were placed into the appropriate chamber of the CPP box immediately after being injected (methamphetamine or saline) for 45min. *Experiment 1* was designed to ascertain if 10 days of home cage mirtazapine treatments inhibited the maintenance of methamphetamine-induced CPP (days 8–17, time line illustrated in Fig. 1A) rats were assigned to one of three treatment groups: (1) saline-conditioned rats subsequently treated with 10 days of vehicle, (2) methamphetamine-conditioned with 10 days of vehicle, or (3) methamphetamine-conditioned with 10 days of mirtazapine. *Experiment 2* was designed to determine if a single mirtazapine injection in the home cage inhibited the maintenance of CPP when administered three days prior to the CPP test (day 17, protocol time line illustrated in Fig. 2A). Rats were assigned to one of the following two treatment groups (1) methamphetamine-conditioned with mirtazapine vehicle treatment or (2) methamphetamine-conditioned with mirtazapine. To determine the effects

of mirtazapine administration on the expression of methamphetamine-induced CPP, all rats were given a drug-free CPP test on day 20 (i.e., three days after the last mirtazapine administration). As more complete metabolism of mirtazapine is observed in rat hepatocytes compared to human hepatocytes [25] and the half life in humans is 22h [26], this three day drug-free period likely allowed mirtazapine to be cleared from the system prior to testing for CPP. For the CPP test, rats were placed into the center chamber and the sliding doors were immediately removed allowing the rat free access to the entire CPP box. The test session lasted 30min and time spent in each chamber and motor activity was monitored. For *Experiment 2*, an initial CPP test was administered on day 9 or 10 to verify the development of CPP. This repeated CPP testing protocol does not result in extinction of the preference (both groups express methamphetamine-induced CPP).

2.5. Statistical Analysis

A mixed factor ANOVA was employed using the between subjects factor of treatment and the within subjects factor of chamber. A *post hoc* Newman-Keuls test was used to identify between-chamber differences; CPP was identified by a significantly greater amount of time spent in the methamphetamine-paired chamber compared to the time spent in the saline-paired chamber. Data are presented as mean $\pm$ SEM. Statistical outliers were determined as those rats that spent greater than two standard deviations above or below the mean time spent in any chamber during the CPP test on day 20; six rats were excluded based on this criterion.

3. Results

The 15min pre-test revealed no bias for either chamber (Experiment 1, $n=73$, time spent chamber 1, $402\pm 20s$ vs. chamber 2, $381\pm 20s$, paired t -test: $p=0.059$, $t_{(72)}=0.540$; Experiment 2, $n=22$; time spent in chamber 1, $341\pm 27s$ vs. chamber 2, $416\pm 26s$, paired t -test: $p=0.162$, $t_{(21)}=1.448$). Moreover, a bias (i.e., preference or aversion) did not develop in rats that were saline-conditioned (i.e., administered saline in both chambers during conditioning) (time spent in the chamber saline-paired on days 1, 3, & 5 = $912\pm 89s$, vs. the chamber paired on days 2 & 4 = $760\pm 87s$, paired t -test $p=0.395$, $t_{(21)}=0.868$, $n=22$). In contrast, as detailed in the results for the respective Experiments (below), methamphetamine-induced CPP developed with three, once every-other-day injections of 1mg/kg methamphetamine and this preference persisted for at least 15 days. Moreover, the magnitude of the preference in the current study was similar for the methamphetamine-conditioned rats subsequently given vehicle in both Experiment 1 and Experiment 2 (compare white bar graphs for methamphetamine-paired chamber in Figs. 1B & 2B; $901\pm 49s$ vs. $993\pm 58s$, Student's t -test; $p=0.301$, $t_{(39)}=1.048$); thus, the addition of CPP Test 1 to verify the development of CPP in Experiment 2 has not altered CPP expressed on day 20.

3.1. Experiment 1

For this experiment, the place preference test was conducted on day 20, three days after terminating 10 days of home cage treatments (days 8–17; Fig. 1A). Two-way ANOVA revealed an effect of Chamber ($F_{(1,114)}=18.6$, $p<0.0001$) and a Treatment $\times$ Chamber interaction ($F_{(1,114)}=4.69$, $p=0.032$) but no effect of Treatment ($F_{(1,114)}=0.076$, $p=0.783$). A *post hoc* Newman-Keuls analysis revealed that methamphetamine-conditioned rats that subsequently received 10 injections of vehicle (days 8–17) demonstrated significant CPP ($n=30$; $p<0.01$); however, when 10 once-daily treatments of mirtazapine were administered to methamphetamine-conditioned rats, the *post hoc* Newman-Keuls no longer identified a significant difference between time spent in the methamphetamine- and saline-paired chambers ($n=29$; $p>0.05$). These results reveal that 10 days of repeated post-conditioning

treatments with mirtazapine can antagonize processes that maintain methamphetamine-induced CPP.

During the CPP test motor activity was monitored; there was no difference in motor activity between the methamphetamine-conditioned rats that received 10 injections of vehicle or mirtazapine: Horizontal activity, methamphetamine / vehicle (n=30) 3808 ± 198 vs. methamphetamine / mirtazapine (n=29), 3884 ± 160 , Student's *t*-test, $p=0.768$, $t_{(57)}=0.297$; Vertical activity, methamphetamine / vehicle (n=30) 1017 ± 67 vs. methamphetamine / mirtazapine (n=29) 975 ± 66 , Student's *t*-test, $p=0.657$, $t_{(57)}=0.446$.

Clinically, chronic mirtazapine administration induces weight gain in some patients [27–29]. However, the repeated mirtazapine treatment used in Experiment 1 was not sufficient to alter body weight over the 10 day treatment period. Methamphetamine-conditioned rats subsequently given 10 days of home cage injections gained an average of 20g during the 10 days with no significant between group differences. The increase in body weight over the 10 treatments was calculated (first mirtazapine injection vs. last mirtazapine injection) for vehicle treated rats (n=15) was 22 ± 2 g vs. mirtazapine treated rats (n=15) 18 ± 1 g (Student's *t*-test, $p=0.067$, $t_{(28)}=1.908$). These results concur with our prior outcomes with 15 injections of 5mg/kg mirtazapine [3].

3.2. Experiment 2

To ascertain whether this antagonism reflected processes that only occurred at the end of the repeated treatment period, we tested the effects of a single injection of mirtazapine given on day 17. For this experiment, we also verified that preference developed prior to administering mirtazapine. To do so, an initial CPP test was administered on protocol day 9 or 10. As time spent in the methamphetamine-paired chamber on day 9 (994 ± 128 s, n=6) was similar to day 10 (976 ± 63 s, n=16) (Student's *t*-test, $p=0.891$, $t_{(20)}=0.139$), these data were pooled. The pooled data were 981 ± 56 s spent in the methamphetamine-paired chamber and 546 ± 54 s in the saline-paired chamber (n=22, paired *t*-test, $p=0.0006$, $t_{(21)}=4.041$). Subsequent CPP testing (on day 20), three days after a single injection of mirtazapine or its vehicle, resulted in place preference being expressed independent of treatment history on day 17 (Fig. 2B). A two-way ANOVA revealed a significant effect of Chamber ($F_{(1,40)}=34.329$, $p<0.0001$) with no effect of Treatment ($F_{(1,40)}=0.791$, $p=0.379$) or a Treatment $\times$ Chamber interaction ($F_{(1,40)}=3.371$, $p=0.074$). A *post-hoc* Newman-Keuls test revealed a significant difference between time spent in the saline- and methamphetamine-paired chambers for both the vehicle (n=11) and mirtazapine (n=11) treated rats. Thus, in methamphetamine-conditioned rats, a single injection of mirtazapine, given on day 17 (which corresponds to the last day of the 10-day repeated treatment in Experiment 1) was not sufficient to alter the expression of preference. The addition of a CPP test on day 9 or 10 did not influence the ability of rats to express a preference on day 20 for either treatment group (methamphetamine / vehicle, time spent in the methamphetamine-paired chamber during the CPP Test on day 9 or 10 was 1004 ± 97 s vs. during the CPP Test on day 20 which was 993 ± 58 s; methamphetamine / mirtazapine, time spent in the methamphetamine-paired chamber during the CPP Test day 9 or 10 was 958 ± 61 s vs. during the CPP Test on day 20 which was 925 ± 86 s). These evaluations also verify that the mirtazapine injection on day 17 did not inhibit CPP on day 20.

Motor activity was monitored during the CPP test on day 20. As in Experiment 1, there were no between-group differences in horizontal activity (methamphetamine / vehicle, 3442 ± 181 vs. methamphetamine / mirtazapine, 3441 ± 160 ; n=11; Student's *t*-test, $p=0.998$, $t_{(20)}=0.003$), or vertical activity (methamphetamine / vehicle, 1051 ± 49 vs. methamphetamine / mirtazapine, 1002 ± 68 ; n=11; Student's *t*-test, $p=0.562$, $t_{(20)}=0.590$). Furthermore, a subset of rats were administered a methamphetamine challenge (1mg/kg) on day 22. The motor

activity of methamphetamine conditioned rats administered mirtazapine or vehicle was nearly identical after the challenge (respectively comparing motor activity of those with a mirtazapine history, $n=8$ vs. vehicle history, $n=8$; horizontal activity, 6110 ± 531 vs. 5656 ± 318 ; Student's t -test, $p=0.475$, $t_{(14)}=0.734$; vertical activity, 2186 ± 281 vs. 1935 ± 132 ; Student's t -test, $p=0.432$, $t_{(14)}=0.809$). Thus, the observation that mirtazapine nullified previously established, methamphetamine-induced CPP were specific to place preference and cannot be generalized to all methamphetamine-induced behaviors.

4. Discussion

These experiments revealed that the long-term maintenance of mnemonic associations between the rewarding effects of methamphetamine and the methamphetamine-paired context can be disrupted by 10 once-daily injections of 5mg/kg mirtazapine. This disruption does not occur when mirtazapine is administered only on the last day of the 10 day period (day 17). Neither a single nor 10 once-daily injections of mirtazapine altered motor activity during the CPP test on day 20 (three days after the last mirtazapine injection); thus, the place preference results do not reflect a change in the capacity of rats to successfully execute the task. The findings suggest that processes that participate in memory maintenance and/or expression are vulnerable to repeated mirtazapine administration. Focus was on the behavioral consequences of mirtazapine measured three days after the last mirtazapine injection. Additional studies are needed to ascertain if the mirtazapine treatments can induce longer acting (or shorter acting) effects. The three-day period was imposed to allow for mirtazapine to be eliminated and we did not observe any long-lasting effects on spontaneous motor activity during the CPP test. Thus, the persistent consequences of repeated mirtazapine administration on memory maintenance and/or expression are likely not due to mirtazapine-induced motor consequences observed during the CPP test.

Maintenance of previously acquired CPP is not a static process. Rather, maintenance of behaviors induced by repeated methamphetamine likely reflects continual modifications at molecular, cellular, and circuit levels that differ depending on the time (hours, days, and weeks) after the last exposure [1–4,30–33]. Indeed, successful conversion of experiences into short-term memories, short-term memories into long-term memories, and then to successfully express those memories requires activation of an array of receptors and protein kinases in a time-dependent manner [34–38]. The temporal nature of these events presents opportunities that can be exploited to disrupt the mechanisms critical for the maintenance of methamphetamine-induced CPP. For example, consolidation of newly acquired memories occurs over several days [34,39,40]. We have observed that with a two-day methamphetamine-conditioning protocol (day 1 methamphetamine-pairing & day 2 saline-pairing), a single mirtazapine injection 24h after the last conditioning session inhibits CPP tested on the following day [15]. Given the close temporal nature of the conditioning and mirtazapine treatment, this protocol does not allow separation of mirtazapine effects on consolidation versus maintenance of the methamphetamine-context memory. The current study was designed to evaluate the effects of mirtazapine after the methamphetamine-induced CPP was established. Thus, the 10 day mirtazapine treatment was initiated three days after conditioning which allowed for examination of maintenance independent of consolidation.

This study revealed that 10 injections of mirtazapine were sufficient to inhibit the maintenance of previous established methamphetamine-induced CPP, whereas a single injection (corresponding to the last day of the repeated treatment) was not. One possible interpretation of these results is that adaptations that occur only after repeated mirtazapine treatment are required for mirtazapine inhibition of methamphetamine-induced CPP (i.e., duration-dependent effects). Similar to the current study, we previously demonstrated that

15 injections of mirtazapine inhibit the maintenance of motor sensitization induced by five once-daily treatments of 2.5mg/kg methamphetamine [3]. Moreover, acute *vs.* repeated mirtazapine administration have differential effects on several behavioral assessments in rats [41]. Brain adaptations to repeated administration of mirtazapine (7 to 28 days) include transcription of genes involved in receptor expression/function, signal transduction, and neuronal structure [17,18,20,42–46] that may impact neuronal function. Thus, behavioral consequences of repeated mirtazapine treatment measured in the current study may be due to long-term adaptations that occur as a consequence of repeated mirtazapine treatment. It also is possible that the 10-day mirtazapine administration encompassed a critical window of vulnerability which was necessary to disrupt the maintenance of methamphetamine-induced CPP (i.e., time-dependent effect). Although the last day of the 10-day mirtazapine treatment did not prove to be the critical treatment time, this does not preclude the possibility that a different time frame encompassed by the 10-day treatment is particularly sensitive to the effects of mirtazapine. For example, our lab has previously demonstrated that a single injection of mirtazapine administered one day after conditioning and tested for preference one day later was sufficient to disrupt methamphetamine-induced CPP established with a single methamphetamine-pairing conditioning protocol [15]. Thus, it may be that the period of time soon after conditioning is critical especially in light of the consolidation processes which are known to occur at this time [34,39,40]. Further studies are necessary to determine the contribution of these two possibilities in the current outcomes. These outcomes are corroborated by those where repeated mirtazapine was able to mitigate behavioral sensitization to chronic methamphetamine [3]. Thus, the collective findings underscore that the effectiveness of repeated mirtazapine is independent of the methamphetamine protocol employed (i.e., CPP or behavioral sensitization), a feature that is required for clinical efficacy.

The pharmacology that underlies the effects of mirtazapine is complex. It acts as an antagonist with high affinity at histamine H₁, α_2 adrenergic, and serotonin 5-HT_{2A/2C} receptors [7,8,44]. An indirect consequence of mirtazapine administration is activation of the 5-HT_{1A} receptors [7,8,18–20,45], which appears to become more robust with chronic treatments [18,20,20,44–46]. These receptors are known to influence both the behavioral effects of psychostimulants and mnemonic processes as reviewed below. While the effects of selective antagonists for receptor targets of mirtazapine on the *expression* of psychostimulant-induced behaviors have received scientific attention, the studies have yielded conflicting results. Both increases [47–51] and decreases [48,52–59] in psychostimulant-induced motor activity, motor sensitization, and self-administration are observed. These divergent reports make it difficult to attribute the expression results of the current study to a particular receptor target of mirtazapine. While no studies have examined the effects of specific receptors targets of mirtazapine in the maintenance of psychostimulant-induced behaviors, a role of the 5-HT_{1A} receptor (an indirect effect of mirtazapine) in the *maintenance* of mnemonic processes has been explored. Administration of a 5-HT_{1A} receptor agonist *after* training (as mirtazapine was given in the current study) inhibits the maintenance of spatial and fear-conditioned memories [60–62]. Thus, in the current study mirtazapine may have inhibited the maintenance of methamphetamine-induced associative learning *via* a number of different receptor systems and further studies with repeated administration of selective ligands are needed to determine the receptor(s) responsible for the observed behavioral inhibition by mirtazapine in the current study.

Dopamine/serotonin releasers may reduce relapse and alleviate negative aspects of withdrawal [14,63,64]. It follows that mirtazapine administered to human addicts results in positive clinical outcomes with a reduction in withdrawal severity from amphetamine [65] and methamphetamine [66] and promotes abstinence in both opiate and stimulant abusers [67]. While not all studies demonstrate favorable clinical outcomes [68,69], there is ample

data to warrant further investigation of mirtazapine as a treatment for methamphetamine abuse and cue-elicited relapse prevention.

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Research Highlights

- Methamphetamine place preference is disrupted by administration of the atypical antidepressant mirtazapine
- Memory maintenance is disrupted by augmenting 5-HT_{2A/2C} receptor signaling independent of re-exposure to the conditioned (i.e., conditioning environment) or unconditioned stimulus (i.e., methamphetamine)
- Mirtazapine may treat psychostimulant addiction by disrupting the maintenance of associative learning

Fig. 1.

Methamphetamine-induced CPP is inhibited by 10 injections of post-conditioning mirtazapine. A. Illustration of treatment protocol. A pre-test was conducted and rats were subsequently conditioned for five days. Mirtazapine (5mg/kg) or its vehicle was administered in the home cage on days 8–17. Three days later (day 20), rats were tested for CPP. M, Meth, S, saline, Ø, no drug, Trtmt Grps, treatment groups. B. Time spent in each chamber illustrated (S, saline-paired chamber, M, methamphetamine-paired chamber). Methamphetamine-conditioned rats administered 10 vehicle injections (white bars) spent a significantly greater amount of time in the methamphetamine-paired chamber relative to the saline-paired chamber; this preference was no longer observed when 10 injections of mirtazapine were administered in the home cage (black bars). *Post-hoc* Newman-Keuls revealed a significant difference between time spent in the saline- and methamphetamine-paired chamber, $**p < 0.01$.

Fig. 2.

A single injection of post-conditioning mirtazapine did not inhibit the expression of methamphetamine-induced CPP. A. Illustration of treatment protocol. A pre-test was conducted on day 0, conditioning occurred for five days, and a CPP test 1 verified the development of CPP. Mirtazapine (5mg/kg) or its vehicle was administered in the home cage on day 17 and three days later (day 20), rats were given CPP test 2. M, Meth, S, saline, Ø, no drug, Trtmt Grps, treatment groups. B. Time spent in each chamber illustrated (S, saline-paired chamber, M, methamphetamine-paired chamber). Methamphetamine-conditioned rats administered a single vehicle injection on day 17 spent a significantly greater amount of time in the methamphetamine-paired chamber relative to the saline-paired chamber (white bars) as did the methamphetamine-conditioned mirtazapine treated rats (black bars). *Post-hoc* Newman-Keuls revealed a significant difference between time spent in the saline- and methamphetamine-paired chamber, ** $p < 0.01$.