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The atypical antidepressant mirtazapine attenuates expression of morphine-induced place preference and motor sensitization

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Abstract

Opioid abuse and dependence remains prevalent despite having multiple FDA-approved medications to help maintain abstinence. Mirtazapine is an atypical antidepressant receiving attention for substance abuse pharmacotherapy, and its action includes alterations in monoaminergic transmission. As monoamines are indirectly altered by opioids, the current investigation assessed the ability of mirtazapine to ameliorate morphine-induced behaviors. Conditioned place preference (CPP) is a behavioral assay wherein a rewarding drug is paired with a distinct environmental context resulting in reward-related salience of cues through learning-related neuronal plasticity. A second behavioral assay involved motor sensitization (MSn), wherein repeated administration results in an enhanced motoric response to an acute challenge, also reflecting neuronal plasticity. Attenuation of CPP and/or MSn provides two behavioral measures to suggest therapeutic potential for addiction therapy, and the present study evaluated the effectiveness of mirtazapine to reduce both behaviors. To do so, morphine-induced CPP was established using an eight day conditioning paradigm, and expression of CPP was tested on day 10 following a 24hr or 30min mirtazapine pretreatment. To determine if mirtazapine altered the expression of MSn, on Day 11, rats received a pretreatment of mirtazapine, followed 30min later by a challenge injection of morphine. Pretreatment with mirtazapine 24hr prior to the CPP test had no effect on CPP expression. In contrast, a 30min pretreatment of mirtazapine attenuated the expression of both CPP and MSn. Collectively, these results indicate that mirtazapine may help to maintain abstinence in opioid dependent patients.

Keywords

antidepressant; conditioned place preference; motor sensitization; morphine

1. Introduction

Opioid abuse is debilitating to the using individual, their families and society. Current pharmacotherapies aimed at promoting abstinence in opioid-addicted patients include opioid replacements such as methadone, which poses abuse liability, or opioid antagonists which can be anhedonic and may add to the difficulty in maintaining abstinence (Schaefer,

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1988;Stolerman, 1985). Further exploration of therapeutic options is needed, and we propose that non-opioid receptor targets should be considered. As the monoaminergic system is clearly involved in the brain effects of opioid exposure (Bland, Schmid, Watkins, and Maier, 2004;Chefer, Denoroy, Zapata, and Shippenberg, 2009;Fadda, Scherma, Fresu, Collu, and Fratta, 2005;Porrás, Di, V, Fracasso, Lucas, De, Caccia, Esposito, and Spampinato, 2002;Tao and Auerbach, 1995;Tao, Ma, and Auerbach, 1998;Willins and Meltzer, 1998), these systems may provide useful targets.

Mirtazapine, an FDA-approved atypical antidepressant, antagonizes noradrenergic (NE)_{α2}, histamine₁, and serotonin (5-HT)_{3/2A/2C} receptors (de Boer, Maura, Raiteri, de Vos, Wieringa, and Pinder, 1988;Kooymán, Zwart, Vanderheijden, Van Hooft, and Vijverberg, 1994) with indirect agonist activity at 5-HT_{1A} receptors (Kakui, Yokoyama, Yamauchi, Kitamura, Imanishi, Inoue, and Koyama, 2009) and inverse agonist (i.e., negative intrinsic efficacy) properties at constitutively active 5-HT_{2C} receptors (Chanrion, Mannoury la, Gavarini, Seimandi, Vincent, Pujol, Bockaert, Marin, and Millan, 2008;Labasque, Meffre, Carrat, Becamel, Bockaert, and Marin, 2010). Momentum is gaining for the use of mirtazapine in several clinically relevant aspects of opioids, including opioid-mediated antinociception (Milne, Sutak, Cahill, and Jhamandas, 2008;Schreiber, Rigai, Katz, and Pick, 2002;Sikka, Kaushik, Kumar, Kapoor, Bindra, and Saxena, 2011) and acute tolerance to this effect (Milne, Sutak, Cahill, and Jhamandas, 2008), nausea and vomiting (Chang, Ho, and Sheen, 2010), as well as reward, physical dependence and withdrawal associated with repeated opioid treatments (Kang, Wang, Li, Hu, Zhang, and Li, 2008). Toward the goal of characterizing the potential for addiction therapy, we explored the utility of mirtazapine to mitigate previously established cellular (McDaid, Tedford, Mackie, Dallimore, Mickiewicz, Shen, Angle, and Napier, 2007) and behavioral (Graves and Napier, 2011;Herrold, Shen, Graham, Harper, Specio, Tedford, and Napier, 2009;McDaid, Tedford, Mackie, Dallimore, Mickiewicz, Shen, Angle, and Napier, 2007;Voigt, Mickiewicz, and Napier, 2011) consequences of abused drugs, with a focus on methamphetamine. The current project extends this work and the opioid literature, to advance the development of mirtazapine as addiction pharmacotherapy by (i) providing a direct comparison of two behavioral indices of opioid-induced brain effects, conditioned place preference and motor sensitization, and (ii) exploring two time frames for mirtazapine treatment that target different aspects of drug-induced brain plasticity, i.e., maintenance and expression.

Conditioned place preference (CPP) is a behavioral model that assesses drug reward. Through learning-related neuronal plasticity, cues such as an environmental context become associated with the reinforcing properties of a substance like morphine; this is referred to as acquisition or development. As a result, the subjects prefer to spend more time in environments previously associated with the rewarding drug, and expression of place preference can be determined even in the absence of the reinforcing drug (Bardo, Rowlett, and Harris, 1995;Tzschentke, 1998). The salience of drug-associated cues and environment can contribute to drug-craving and drug-seeking in humans (Childress, Mozley, McElgin, Fitzgerald, Reivich, and O'Brien, 1999;Volkow, Fowler, and Wang, 2003;Volkow, Wang, Ma, Fowler, Zhu, Maynard, Telang, Vaska, Ding, Wong, and Swanson, 2003;Volkow, Wang, Telang, Fowler, Logan, Childress, Jayne, Ma, and Wong, 2006;Volkow, Wang, Telang, Fowler, Logan, Childress, Jayne, Ma, and Wong, 2008), and diminishing the effects of cues and environments may aid in maintaining abstinence from drug use in the drug-abusing individual. Motor sensitization (MSn) refers to an enhancement in the motor effects that occurs with repeated, intermittent injections of abused drugs, including morphine (Babbini and Davis, 1972;Dallimore, Mickiewicz, and Napier, 2006;McDaid, Dallimore, Mackie, and Napier, 2006;Mickiewicz, Dallimore, and Napier, 2009;Mickiewicz and Napier, 2011;Tzschentke and Schmidt, 1999). MSn is another useful behavioral index of drug-induced neuronal plasticity, and like CPP, MSn consists of both a development and

expression phase. Early arguments regarding the neuroanatomical substrates that undergo drug-induced adaptations were thought to be similar for both CPP and MSn (Robinson and Berridge, 1993); however, investigations have shown these two behaviors can be distinct (Hemby, Jones, Justice, Jr., and Neill, 1992; Olmstead and Franklin, 1994; Rademacher, Kovacs, Shen, Napier, and Meredith, 2006; Shen, Meredith, and Napier, 2006; Swerdlow and Koob, 1984; Voigt, Mickiewicz, and Napier, 2011). To expand this literature, we selected a morphine dosing protocol that is sufficient for expression of CPP (i.e., four injections of 10mg/kg morphine given every other day) (Tzschentke and Schmidt, 1999), but it is not known if this protocol results in expression of MSn. With this approach, we determined the effects of mirtazapine on morphine-induced behaviors, and tested the hypothesis that mirtazapine would attenuate the expression of MSn with both measures being conducted in the same rats.

Relevant to the processes that would be acted upon by post-conditioning mirtazapine is whether the morphine-associated context engages transmitter systems that are antagonized by mirtazapine (necessitating that mirtazapine be “on-board”), or if mirtazapine blocks processes that are involved in the maintenance of the drug-induced maladaptions (i.e., whether mirtazapine has effect when administered during a period of abstinence). Prior work suggests that mirtazapine blocks processes involved in the maintenance of drug-induced maladaptions (Herrold, Shen, Graham, Harper, Specio, Tedford, and Napier, 2009; Voigt, Mickiewicz, and Napier, 2011). However, recent evidence using morphine CPP suggests that mirtazapine may also antagonize neurotransmitter systems involved in the expression of CPP (Kang, Wang, Li, Hu, Zhang, and Li, 2008). To provide new insights into this issue, we tested the effects of both a 24hr and a 30min pretreatment period prior to testing for place preference to determine if it was necessary for mirtazapine to be on-board to alter CPP previously induced by morphine.

2. Results

2.1 Post-conditioning treatments with mirtazapine and place preference

Saline-conditioned rats receiving sham injections as either a 30min (n=10 rats) or 24hr (n=28 rats) pretreatment did not differ for chamber preference during the CPP expression test (Fig. 1A, $t_{(9)}=1.8$, $p=0.11$ and Fig. 1B, $t_{(25)}=0.93$, $p=0.36$, respectively). Administration of mirtazapine 30min (n=12) or 24hr (n=7) prior to testing did not significantly affect behavior of saline-conditioned animals (Fig. 1C, $t_{(11)}=1.40$, $p=0.20$ and 1D, $t_{(6)}=1.84$, $p=0.12$, respectively).

Morphine-conditioned rats displayed a significant preference for the morphine-paired chamber; 30min (n=22 rats) and 24hr (n=22 rats) sham pretreatment had no effect on behavior (Fig. 2A, $t_{(19)}=4.55$, $p=0.0002$ and Fig. 2B, $t_{(19)}=17.33$, $p<0.0001$, respectively). Morphine-induced place preference was attenuated by a 30min pretreatment of mirtazapine (Fig. 2B, n=12, $t_{(11)}=1.38$, $p=0.20$). In contrast, expression of CPP was retained in rats that received a 24hr pretreatment of mirtazapine (n=7) (Fig. 2C, $t_{(6)}=4.97$, $p<0.01$). To correct for use of multiple *t*-tests and protect from Type II errors, α was divided by 4 and significance set at $p<0.125$ for all CPP analyses as described in the methods.

2.2. Effect of mirtazapine on the expression of motor sensitization

Motor behaviors were monitored during acquisition of CPP. Saline-conditioned rats (n=22) habituated to the environment during repeated exposure to the chambers, as evidenced by decreased motor activity on day 7 compared to day 1 (Table 1). In contrast, morphine-conditioned rats (n=30) displayed significant development of MSn as shown by enhanced motor responses on the seventh day of conditioning (i.e., the third every-other day treatment of 10mg/kg morphine) compared to the first day of conditioning (Table 1). However, despite

significant development of sensitization, when tested on day 11 with an acute morphine challenge (5mg/kg), rats (n=14) only showed expression as measured by total distance (see Fig. 3A); expression was not evident by horizontal activity, vertical activity, or stereotypy behaviors (Table 2). This effect of an acute challenge with morphine was antagonized following a 30min pretreatment of mirtazapine (n=16) (Fig. 3B).

3. Discussion

The current study revealed that a 30min pretreatment of 5mg/kg mirtazapine attenuated the expression of morphine-induced CPP, in agreement with previously reported effects of 10 and 20mg/kg morphine (Kang, Wang, Li, Hu, Zhang, and Li, 2008). In contrast, a 24hr pretreatment was not effective. We also show that a 30min pretreatment of mirtazapine attenuated the expression of morphine-induced MSn. As overviewed below, when compared to the existing literature, several new insights are provided by the current outcomes with regard to the ability of mirtazapine to abrogate behaviors mediated by abused drugs.

We previously observed that a 24hr pretreatment of 5mg/kg mirtazapine attenuates maintenance of methamphetamine-induced CPP (Herrold, Shen, Graham, Harper, Specio, Tedford, and Napier, 2009). Comparing these findings to the current report indicate that the neuronal substrates involved in the maintenance of CPP memory for methamphetamine differ from those associated with morphine. There is an emerging literature indicating functional overlaps in monoaminergic and opioid systems, as well as those acted on by mirtazapine, e.g., both methamphetamine-induced MSn (Chiu, Ma, and Ho, 2005) and mirtazapine-induced antinociception (Sikka, Kaushik, Kumar, Kapoor, Bindra, and Saxena, 2011) are both blocked by *mu* opioid receptor antagonists. It is likely that the neuroadaptations associated with methamphetamine vs. morphine differ, both in the temporal characteristics and in makeup. Further investigations that directly compare the two abused drugs with regard to the antagonist capacity of mirtazapine will be an important step in determining the general applicability of mirtazapine for addiction therapy.

An alternative hypothesis explaining the observed effects of mirtazapine relates to memory consolidation and retrieval. Mirtazapine had no effect on morphine CPP when administered as a 24hr pretreatment in the home cage. The observed effects are therefore unlikely a result of interference with memory consolidation. In contrast, a 30min mirtazapine pretreatment attenuated expression of CPP, which may reflect impairment in memory retrieval. However, there are currently no reports indicating memory impairment by mirtazapine. Instead of impairing memory, mirtazapine shows evidence for improving memory and emotional processing (Arnone, Horder, Cowen, and Harmer, 2008; Nowakowska, Chodera, and Kus, 1999). Mirtazapine thus likely diminishes the salience of morphine-associated cues and is not acting in any capacity as an amnesic agent disrupting memory consolidation or retrieval.

Like morphine-induced CPP, a 30min pretreatment of mirtazapine attenuated the expression of morphine-induced sensitization of motor ambulation (i.e., total distance traveled). This discovery shows that the effects of mirtazapine on opioid actions are not paradigm-dependent and that the neuroplasticity that is involved in CPP and MSn induced by the morphine treatment protocol employed here both involved mirtazapine-sensitive systems. The morphine protocol used in the current study was atypical for MSn assessments; most protocols use once-daily treatments, which are widely known to result in the development and/or expression of MSn (Babbini and Davis, 1972; Johnson and Napier, 2000; Kalivas and Duffy, 1987; McDaid, Dallimore, Mackie, and Napier, 2006; Mickiewicz, Dallimore, and Napier, 2009; Spanagel and Shippenberg, 1993). In contrast, we are aware of only one other study has shown that giving morphine every other day was sufficient to induce MSn (Tzschentke and Schmidt, 1999), and the current study is the first to assess expression in this

protocol. Similar to a previously report (Tzschentke and Schmidt, 1999), sensitization developed as measured by most of our motor parameters; however, in contrast to what is reported after withdrawal from daily morphine treatments (Dallimore, Mickiewicz, and Napier, 2006), only total distance showed a significant expression of MSn. A lack of expression in other measured parameters, in conjunction with a modest level of expression by total distance, was likely a consequence of the intermittent morphine treatment regimen employed here. It is also important to recognize that acute morphine treatments can induce catalepsy (e.g., in Figure 3A, compare Day 1 for saline-conditioned rats and Day 1 for morphine-conditioned rats), and sensitization assessments can also reflect a tolerance to the cataleptic effects (e.g., Figure 3B, the difference in morphine+sham in saline-conditioned rats and morphine+sham in morphine-conditioned rats) (Mickiewicz, Dallimore, and Napier, 2009). The ability of mirtazapine to antagonize the expression of this phenomenon would be in keeping with prior reports of mirtazapine inhibiting tolerance to morphine-induced analgesia (Milne, Sutak, Cahill, and Jhamandas, 2008).

Mirtazapine embodies a complex pharmacological profile including multiple targets, which likely contribute to its effects. This atypical antidepressant antagonizes $NE_{\alpha 2}$, $5-HT_{2A/C}$, $5-HT_3$, and H_1 receptors, in addition to emulating multiple $5-HT_{1A}$ behavioral and neurochemical actions (Berendsen and Broekkamp, 1997; Kakui, Yokoyama, Yamauchi, Kitamura, Imanishi, Inoue, and Koyama, 2009; Nakayama, Sakurai, and Katsu, 2004) *via* indirect agonist actions. We have shown that ligands acting at $5-HT_2$ receptors exhibit a similar behavioral profile as mirtazapine in psychostimulant-seeking (Nic Dhonnchadha, Fox, Stutz, Rice, and Cunningham, 2009)(unpublished data). The ability of mirtazapine to abrogate behaviors induced by both methamphetamine (Graves and Napier, 2011; Herrold, Shen, Graham, Harper, Specio, Tedford, and Napier, 2009; McDaid, Tedford, Mackie, Dallimore, Mickiewicz, Shen, Angle, and Napier, 2007; Voigt, Mickiewicz, and Napier, 2011) and opioids (Kang, Wang, Li, Hu, Zhang, and Li, 2008) suggests the mechanism of action engaged by mirtazapine $5-HT_2$ receptors. However, opioid receptors are clearly involved in the actions of morphine, and it has recently been shown that some effects of mirtazapine (i.e., antinociceptive actions) indirectly involve mu opioid receptors (Sikka, Kaushik, Kumar, Kapoor, Bindra, and Saxena, 2011). Additional morphine-reward studies focusing on the pharmacological profile of mirtazapine are needed to definitively ascertain which receptor(s) are responsible for the current observations.

While our previous studies have focused on mirtazapine as substance abuse pharmacotherapy for methamphetamine (Graves and Napier, 2011; Herrold, Shen, Graham, Harper, Specio, Tedford, and Napier, 2009; McDaid, Tedford, Mackie, Dallimore, Mickiewicz, Shen, Angle, and Napier, 2007; Voigt, Mickiewicz, and Napier, 2011), we have expanded these findings to demonstrate the ability of mirtazapine to abrogate morphine-induced behaviors. Our findings complement and extend current literature demonstrating the ability of mirtazapine to attenuate morphine CPP (Kang, Wang, Li, Hu, Zhang, and Li, 2008), as well as the expression of morphine-induced MSn, thus providing converging evidence using multiple models that mirtazapine may be a suitable addiction pharmacotherapy for multiple substances of abuse. The intermediate dose tested in the current study (5mg/kg) shows similar preclinical success at attenuating methamphetamine-induced behaviors (Graves and Napier, 2011; Herrold, Shen, Graham, Harper, Specio, Tedford, and Napier, 2009; McDaid, Tedford, Mackie, Dallimore, Mickiewicz, Shen, Angle, and Napier, 2007; Voigt, Mickiewicz, and Napier, 2011). Our preclinical assessments are further validated by case reports in two patients with opioid addictions wherein administration of mirtazapine maintained abstinence (Rafeyan and Napier, 2008).

These preclinical and clinical findings suggest that mirtazapine may effectively serve as pharmacotherapy across a number of abused drugs. Given the complex pharmacological

profile of mirtazapine, there are likely multiple mechanisms of action contributing to the observed effects and further studies are needed to fully understand and improve upon the intramolecular polypharmacy of mirtazapine.

4. Experimental procedures

4.1 Animals and housing

One hundred seventy two male Sprague Dawley rats (Harlan, Indianapolis, IN) weighing 225–250g at the start of the study, were housed in pairs in 12hr light/dark cycle, climate controlled environment and allowed *ad libitum* access to food, and water. Rats were allowed to acclimate to the *vivarium* and handled for at least five days prior to the onset of the experiments. Cage mates were given identical pharmacological treatments. All experiments were carried out with the approval of the Institutional Animal Care and Use Committee, and in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (National Research Council, Washington DC).

4.2 Drugs

Morphine sulfate (Sigma, St Louis, MO), calculated as the salt, was dissolved in 0.9% saline and administered as 10mg/kg for acquisition of CPP; this dose has been shown to produce robust CPP (Bardo, Rowlett, and Harris, 1995) and MSn (Johnson and Napier, 2000; McDaid, Dallimore, Mackie, and Napier, 2006; Mickiewicz, Dallimore, and Napier, 2009). Acute challenge for MSn studies were performed with a 5mg/kg challenge of morphine. 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 as 5mg/kg. This dose of mirtazapine is sufficient to antagonize methamphetamine-induced MSn when administered chronically (for 15 days) (McDaid, Tedford, Mackie, Dallimore, Mickiewicz, Shen, Angle, and Napier, 2007), CPP when administered 24hr prior to testing for expression (Herrold, Shen, Graham, Harper, Specio, Tedford, and Napier, 2009), as well as when administered chronically for 10 days (Voigt, Mickiewicz, and Napier, 2011). Cue-associated seeking-like behavior is also attenuated by 5mg/kg mirtazapine when administered as an acute pretreatment without altering motor function (Graves and Napier, 2011). All solutions were injected as 1ml/kg intraperitoneally (ip).

4.3 Test Apparatus

Testing occurred in a dimly lit room with white noise present (white noise generator, San Diego Instruments, San Diego, CA). As with our prior studies with morphine-induced CPP (Dallimore, Mickiewicz, and Napier, 2006), the behavioral apparatus (AccuScan Instruments, Inc., Columbus, OH) (63cm × 30cm × 30cm) consisted of three chambers; two large end conditioning chambers (25cm × 30cm × 30cm) were separated by a smaller center chamber (13cm × 30cm × 30cm). Each chamber had distinct visual and tactile cues. The two large conditioning chambers had vertical or horizontal white striped walls, and either a patterned floor with a raised platform in the middle or a grid floor. The smaller center chamber had solid white walls and a smooth raised platform floor. Chambers were separated by removable Plexiglas sliding doors. Time spent in each chamber and motor activity was monitored *via* two sets of photobeams (24 in the horizontal plane and 12 in the vertical plane). Our prior studies with this chamber configuration verified that the chambers were unbiased for group evaluations (Voigt, Mickiewicz, and Napier, 2011), and randomizing the drug-paired chamber among the rats (described below) helped account for any potential individual bias.

4.4 Conditioning Procedures

On conditioning days (days 1-8), rats were moved from the housing room across a hall into the testing room, weighed, and allowed to habituate to the room for at least 30min. After habituation, rats were injected with either 10mg/kg morphine on days 1, 3, 5, and 7 or saline on days 2, 4, 6, and 8 (morphine-conditioned rats). Controls were treated with saline prior to all conditioning sessions (days 1-8). Immediately after injections, rats were confined to the appropriate side of the box for 45min. Rats were randomly assigned to the morphine-paired chamber with half being drug-paired in one of the two large end chambers and half in the opposite end. Expression of CPP (day 10) is described below for each experiment.

4.5 Experimental Design for post-Conditioning Treatments

This study consisted of three separate experiments using separate groups of rats, each with different post-conditioning protocols for mirtazapine treatment during days 9 -11.

4.5.1 Experiment 1: Effect of mirtazapine administered 30min prior to CPP test

—On day 9, rats were left undisturbed in the home cage. On day 10, rats were transported to the testing room, weighed and administered sham injection (either mirtazapine vehicle or a “mock injection” with no fluid administered (Riddle, Rokosik, and Napier, 2012); $n=32$) or mirtazapine (5mg/kg; $n=24$). Thirty minutes later, rats were placed into the center chamber of the CPP apparatus and the two Plexiglas doors were removed, allowing the rats to explore the entire apparatus. Time spent in each chamber was recorded for 30min.

4.5.2 Experiment 2: Effect of mirtazapine administered 24hr prior to CPP test

—On day 9, rats were administered either sham injection ($n=50$) or mirtazapine (5mg/kg; $n=14$) in the home cage. Rats were tested for expression of CPP 24hr later (day 10). To do so, rats were given a mock injection (and immediately placed into the box for 30min; time spent in each chamber was recorded).

4.5.3. Experiment 3: Effect of mirtazapine on the expression of MSn—The expression of MSn was tested after an acute pretreatment of mirtazapine. Rats were conditioned as described above and left undisturbed in the home cage on days 9-10. On day 11, rats were transported to the testing room, weighed, and administered sham injection ($n=24$) or mirtazapine (5mg/kg; $n=28$). Thirty minutes later, all rats received an acute challenge of morphine (5mg/kg) and were immediately confined to the day 1/morphine-paired chamber for 45min. Motor activity was monitored *via* photos beam breaks in both the upper and lower registers. The motor parameters recorded include horizontal activity (number of beam interruptions in the horizontal plane), total distance (centimeters traversed; a measure of ambulatory activity), vertical activity (number of beam interruptions in the vertical plane), movement number (total number of discrete horizontal movements), stereotypy number (number of episodes or ‘bouts’ of stereotypic behavior) and stereotypy count (number of beam breaks within an episode or ‘bout’ of stereotypic behavior) to provide a thorough assessment of morphine-induced behaviors as previously reported (Dallimore, Mickiewicz, and Napier, 2006; Mickiewicz and Napier, 2011).

4.6 Statistical analyses

Each CPP treatment group experiment was analyzed using paired *t*-tests comparing the time spent in the morphine paired chamber *vs.* saline paired chamber (or day 1 chamber *vs.* day 2 chamber for saline-conditioned controls). The α level was adjusted to reflect the number of repeated tests to reduce the chance for Type II errors (see (Bozarth, 1988) for discussion of this approach in opioid-induced CPP protocols); α was divided by 4 and significance for CPP was set at $p<0.0125$. The development of MSn was assessed using paired *t*-tests

comparing motor activity on day 1 vs. day 7 for each parameter ($p<0.05$). The expression of MSn was analyzed using a one-way ANOVA with Newman-Keuls *post hoc* analysis between groups for motor data collected on day 11 ($p<0.05$). Data are presented as mean $\pm$ S.E.M. with data from individual rats also graphed to clearly illustrate each animal's place preference.

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Abbreviations

CPP	Conditioned place preference
MSn	Motor sensitization
5-HT	serotonin
NE	Noradrenergic

Highlights

- Mirtazapine, an atypical antidepressant, decreases psychostimulant-mediated behaviors
- We now show that mirtazapine also attenuates opioid-mediated behaviors
- Rats were tested for conditioned place preference and motor sensitization
- Mirtazapine pretreatment attenuated expression of place preference and sensitization

Fig. 1.

Saline conditioning did not induce place preference. Rats were administered a saline (1ml/kg) injection and restricted to one of two conditioning compartments and to the other chamber on alternate days. Day 9 rats remained in their homecages, and expression for CPP was tested on day 10. Shown are data for individual rats and the group mean $\pm$ S.E.M. collected during the test for expression of place preference. “Day 1” chamber refers to the conditioning chamber paired on days 1, 3, 5, and 7 whereas “Day 2” chamber refers to the chamber paired on days 2, 4, 6, and 8. All analyses were performed using a paired *t*-test comparing time spent in the designated chambers (i.e. Day 1 chamber *vs.* Day 2 chamber). (A) On day 10, saline-conditioned rats ($n=10$) were given either a 30min sham pretreatment. Rats did not develop a significant place preference ($p=0.11$). (B) Saline-conditioned rats ($n=28$) administered sham injection 24hr prior to CPP test (pretreatment on day 9 and CPP test on day 10) and also did not develop a place preference ($p=0.36$). (C) Saline-conditioned rats administered a 30min mirtazapine (5mg/kg) pretreatment prior to CPP testing ($n=12$) did not show a place preference ($p=0.2$). (D) Similarly saline-conditioned rats administered a 24hr mirtazapine (5mg/kg) pretreatment ($n=7$) showed no preference ($p=0.11$).

Fig. 2.

Morphine conditioning resulted in place preference. Rats were administered a morphine (10mg/kg) injection and restricted to one of two conditioning compartments (morphine-paired chamber) and to the other chamber on alternate days and administered saline 1ml/kg (saline-paired chamber). Day 9 rats remained in their home cages and tested for place preference on day 10. Shown are data for individual rats and the group mean that were collected during the test for expression of place preference. All analyses were performed using a paired *t*-test comparing time spent in the designated chambers (i.e. morphine-paired chamber vs. saline-paired chamber) with α divided by four to correct for multiple *t*-tests as described in the methods. (A) On day 10, rats ($n=22$) were given either a 30min sham pretreatment or mock injection and expressed a significant preference for the morphine paired chamber (** $p<0.01$). (B) Morphine-conditioned rats ($n=22$) were administered a 24hr sham pretreatment and tested for expression on day 10. Rats administered a 24hr sham pretreatment demonstrated a significant preference for the morphine-paired chamber (** $p<0.01$). (C) Morphine-conditioned rats ($n=12$) were administered a 30min mirtazapine (5mg/kg) pretreatment prior to testing for expression on day 10. With a 30min mirtazapine pretreatment morphine-conditioned rats did not express a significant place preference ($p=0.20$). (D) Morphine-conditioned rats ($n=7$) were administered 5mg/kg mirtazapine on day 9, 24hrs prior to testing for place preference. A 24hr mirtazapine pretreatment did not affect expression of CPP and morphine-conditioned rats displayed a significant preference for the morphine-paired chamber (** $p<0.01$).

Fig. 3. Mirtazapine blocked the expression of morphine-induced motor sensitization. (A) Repeated, intermittent administration resulted in development of morphine sensitization comparing total distance in cm traversed on day 1 vs. day 7 ($t_{(26)}=2.36$, $p=0.03$) whereas saline-conditioned rats habituated ($t_{(20)}=5.50$, $p<0.001$). (B) Rats were subsequently tested for the expression of motor sensitization on day 11 after an acute challenge of 5mg/kg morphine, following a pretreatment with mirtazapine (Mirt) or Sham. Rats conditioned with morphine displayed a greater level of motor responding to the morphine acute challenge (as measured by total distance traversed) compared to responding morphine acute challenge in saline-conditioned rats (* $p<0.05$). This verifies the expression of morphine-induced motor sensitization. Pretreatment with 5.0mg/kg mirtazapine blocked the expression of morphine sensitization (acute challenge in morphine-conditioned rats with pretreatment of sham vs. mirtazapine; * $p<0.05$). Data analyzed using a one-way ANOVA ($F_{(3,47)}=5.60$) with Newman-Keuls *post-hoc* analysis.

Table 1

Development of motor sensitization.

	Saline-Conditioned		Morphine-Conditioned	
	Day 1	Day 7	Day 1	Day 7
Horizontal Activity	2408 ± 76.7	1914 ± 98.9**	1620 ± 130.5	2627 ± 111.8**
Vertical Activity	807 ± 62.9	546 ± 41.5**	416 ± 71.8	479 ± 63.2
Movement Number	160 ± 8.3	105 ± 5.6**	109 ± 12.4	125 ± 8.8
Stereotypy Number	438 ± 82.1	359 ± 69.8**	217 ± 18.6	302 ± 32.5*
Stereotypy Count	1425 ± 59.9	1125 ± 69.5**	1079 ± 110.5	1492 ± 77.7**

Table 2

Expression of motor sensitization

	Saline-Conditioned	Morphine-Conditioned
Horizontal Activity	2373 ± 185.0	2704 ± 239.0
Vertical Activity	944 ± 71.1	1153 ± 172.4
Movement Number	134 ± 63.8	114 ± 12.1
Stereotypy Number	243 ± 14.0	244 ± 24.8
Stereotypy Count	1317 ± 108.9	1158 ± 80.8