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Contribution of NMDA NR2B Subunit to Methamphetamine Conditioned Place Preference

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Abstract

Psychostimulant abuse has become a major issue in the United States. Methamphetamine, which has high abuse potential, has become especially problematic. With no current FDA certified pharmacotherapies for psychostimulant drugs, it is difficult for individuals to decrease their methamphetamine use. The goal of the current experiments was to determine if Ro 63-1908, a highly selective N-methyl-D-aspartate (NMDA) NR2B subunit antagonist, is capable of decreasing the conditioned rewarding effects of methamphetamine. Male Sprague-Dawley rats ($N = 36$) were tested in two conditioned place preference (CPP) procedures. The effects of Ro 63-1908 (0, 1.0, 3.0 mg/kg; s.c.) were tested on the acquisition (Experiment 1) or the expression (Experiment 2) of methamphetamine CPP. Ro 63-1908 (3.0 mg/kg) blocked the acquisition of methamphetamine CPP, as evidenced by a decrease in preference ratios. Although rats treated with Ro 63-1908 (3.0 mg/kg) did not show expression of methamphetamine CPP, preference ratios did not significantly differ from rats treated with vehicle; therefore, caution is needed when interpreting the effects of Ro 63-1908 on the expression of methamphetamine reward. Together, these findings suggest that the NR2B subunit is an important mediator of the acquisition, but not necessarily the expression, of the conditioned rewarding effects of methamphetamine.

Keywords: methamphetamine, NMDA receptor, NR2B subunit, conditioned place preference, acquisition, expression

Understanding the neurobiology of substance use disorders is important for developing novel pharmacotherapies that can block the rewarding effects of drugs. In 2016 alone, more than 64,000 individuals died from drug overdoses, an increase of over 25% from the previous year (Katz, 2017). Kentucky ranks third out of all states in rates of drug overdose death at 29.9 overdose deaths for every 100,000 people (Hauser, 2017). Specifically, in Northern Kentucky, heroin has become an epidemic and has claimed hundreds of lives with one overdose death occurring approximately every 40 hours (Warren, 2016). There are multiple options available to individuals in order to combat heroin use disorder. Methadone has been one such option, as well as Suboxone, which is a combination of buprenorphine and naloxone (Sontag, 2013). However, for psychostimulant (e.g., cocaine, methamphetamine, ADHD medications) abuse, despite extensive research, no FDA-approved pharmacotherapies have been found that are effective in attenuating the reinforcing/rewarding effects of psychostimulants (Shearer & Gowing, 2004). More research is needed to discover such pharmacotherapies, which in turn, can decrease psychostimulant dependence and abuse.

Many psychostimulants are used for medicinal purposes that are often prescribed in order to treat adolescents and adults diagnosed with attention-deficit/hyperactivity disorder (ADHD), but concerns have been raised in regard to their reinforcing and potentially addictive properties (Biederman & Faraone, 2005). One common psychostimulant used to treat ADHD is Adderall, which is a combination of L-amphetamine and D-amphetamine. Another common ADHD medication is methylphenidate (Ritalin® and Concerta®). One out of every ten children in the United States is diagnosed with ADHD, and this number is predicted to increase (Lane, 2015). With this growth, the possibility that these types of drugs are abused may ultimately increase. While these psychostimulants are commonly abused, methamphetamine has become problematic

in the United States and other parts of the world. In the United States the number of meth labs that have been found has decreased due to increased efforts by law enforcement. However, methamphetamine trafficking has been on the rise, and methamphetamine is being imported from areas such as Mexico, where it can then be sold cheaply on the streets (Huus, 2006). While the amount being made domestically has dwindled as a result of more methamphetamine being imported (Madras, 2016), there are still people who synthesize this drug and can do so readily due to many of the ingredients needed being readily available and not geographically specific. Pseudoephedrine and ephedrine are inexpensive and widely used in allergy medications, so those who make methamphetamine can easily do so. It has become increasingly difficult for law enforcement to track down those who synthesize this drug since those two "precursor chemicals" are not difficult to obtain (Mazerolle, Mcguffog, Ferris, & Chamlin, 2016). Moreover, methamphetamine is relatively inexpensive and can be purchased for as little as $30-$100 per gram on the black market (Dobkin & Nicosia, 2009). Together, these reasons account for the continuing increase of methamphetamine use, which can lead to addiction.

Methamphetamine typically induces strong feelings of alertness, energy and euphoria at high doses when compared to other psychostimulants (Chiu & O'Schenk, 2012). It can also stimulate the cardiovascular system, making heart rate and blood pressure increase, potentially leading to a hypertensive crisis. One of the primary actions of methamphetamine is to elevate levels of extracellular monoamine neurotransmitters, which includes dopamine, serotonin, and norepinephrine (Rothman & Baumann, 2003). While it is not completely known how methamphetamine causes the release of these neurotransmitters, it appears that they are redistributed from synaptic vesicles to the neuronal cytoplasm via the vesicular monoamine transporter (VMAT2) (Kish, 2008). Typically, in the mesolimbic pathway of the brain, the

neurotransmitters dopamine and norepinephrine are released from nerve terminal vesicles where they will then go into the synapse and bind to receptors that cause the downstream neuron to fire. The elevated dopamine levels are associated with the euphoric feelings one experiences after taking methamphetamine (Kish, 2008). Moreover, methamphetamine blocks the breakdown of both dopamine and norepinephrine. It inhibits monoamine oxidase (MAO), the enzyme that can cleave monoamines, resulting in high concentrations of dopamine/norepinephrine in the synapse.

Methamphetamine is unique in that its half-life is longer relative to other psycho-stimulants, as it will be present in the neuron for up to 12 hours, so the user is constantly feeling the heightened euphoric effects during this duration (Harris et al., 2003). Also, the methamphetamine that is taken into the terminal will destroy some of the terminal after each use. While the terminals can regrow, it requires time (Ares-Santos, Granado, Espadas, Martinez-Murillo, & Moratalla, 2013). If these terminals are damaged, methamphetamine will not release as much dopamine, unless the individual takes more drug. Studies have also shown that long-term methamphetamine users have a decreased level of dopamine receptor availability in the brain, specifically D2 receptors (Volkow et al., 2001). Therefore, the user will continue using more in order to generate that same euphoric feeling. The mechanism of action of methamphetamine is quite complex and has many negative impacts on the dopamine rich areas of the brain such as the ventral tegmental area (VTA) and the ventral striatum, which includes the nucleus accumbens. Due to the high number of dopamine receptors in these areas, they are highly susceptible to the impacts of methamphetamine.

There are two major ways to examine the reinforcing/rewarding effects of drugs of abuse in animals. First, drug self-administration paradigms use operant conditioning techniques to measure the direct reinforcing effects of a drug. If a drug is reinforcing, an animal will respond

on some manipulandum (e.g., lever) to earn more of the drug. Because this technique requires surgical procedures, using drug self-administration can be difficult. Second, conditioned place preference (CPP) has become a popular alternative to drug self-administration for assessing the conditioned rewarding effects of drugs (Carr, Fibiger, & Phillips, 1989). According to Bardo and Bevins (2000), a typical CPP experiment includes the pairing of two distinctive environmental cues with a stimulus of interest. In much of the previous literature, this consists of two chambers of various floorings, colors, shapes, etc., with the stimulus being the drug of choice. Through Pavlovian conditioning, an animal learns to associate one environmental context with the stimulus of interest. If the stimulus is rewarding, the animal will spend more time in the chamber previously paired with that stimulus.

Past studies have looked at conditioned place preference (CPP) as a means of examining the conditioned rewarding effects of drugs and to screen potential pharmacotherapies for substance use disorders (see Bardo, Horton, & Yates, 2015). Specifically, methamphetamine has conditioned rewarding effects (Trazon, Suzuki, Misawa, & Watanabe, 1992). Furthermore, drugs such as mirtazapine (antidepressant; Herrold et al., 2009) and baclofen (GABA receptor agonist; Voigt, Herrold, & Napier, 2011) have been shown to decrease the rewarding effects of methamphetamine. Finally, the selective D3 receptor antagonist YQA14 did not attenuate the acquisition of methamphetamine CPP but blocked the expression of CPP in mice (Sun et al., 2015).

While there has been some evidence that dopamine receptor antagonists attenuate the reinforcing/rewarding effects of psychostimulants (Cervo & Samanin, 1995; Woolverton & Virus, 1989), these antagonists can have undesirable side effects, such as motor disturbances and weight gain (Li, Snyder, & Vanover, 2016). Considering that methamphetamine interacts

directly with glutamate receptors, examining the contribution of the glutamatergic system to methamphetamine abuse may provide a novel molecular target for treating substance use disorders. The glutamatergic system is fast-signaling and is important in the processing of information in neuronal networks. Glutamate is heavily involved in this system and aids in the process of long-term potentiation, a neuronal model of memory (Riedel, Platt, & Micheau, 2003). Glutamate receptors can be divided into metabotropic receptors (mGluRs) and ionotropic receptors (iGluRs). iGluRs can be further subdivided into N-methyl-D-aspartate (NMDA) receptors and non-NMDA receptors (AMPA and kainate). NMDA receptors are composed of NR1 and NR2 subunits with NR2 being subdivided into NR2A, NR2B, NR2C, and NR2D (Monaghan & Larsen, 1997). Once methamphetamine is introduced, it will interact with the NR2B subunit, which increases the binding of glutamate (Loftis & Janowsky, 2003). Glutamate is responsible for mediating how much dopamine is released in areas such as the nucleus accumbens (Hjelmstad, 2004). Therefore, with this increase in glutamate, it can interact with the vesicles that contain both dopamine and norepinephrine and release them. Normally, the neurotransmitters would remain in the synapse for a few microseconds and then be taken back into the nerve terminal, but methamphetamine reverses the re-uptake mechanism and causes more dopamine to be released. This ultimately induces the euphoric effects with the influx of dopamine and norepinephrine and can lead to dependence.

In one previous study, rhynchophylline, which targets all NMDA receptors as a noncompetitive antagonist (Kang et al., 2002) was used to see if it could decrease the rewarding effects of methamphetamine in mice as assessed with CPP. Injections of rhynchophylline (40 mg/kg and 80 mg/kg) eliminated methamphetamine place preference. This study reported an increase in NR2B subunit expression in the hippocampus following methamphetamine

administration, which was reversed after administration of rhynchophylline (Li et al., 2014). Additionally, rhynchophylline can inhibit methamphetamine reward in zebrafish; similar to the report by Li et al. (2014), the NR2B subunit has been proposed to mediate the conditioned rewarding effects of methamphetamine in zebrafish (Chen et al., 2016). These studies ultimately revealed that rhynchophylline could potentially decrease the rewarding effects of methamphetamine. While the results of both of these studies were promising, one weakness of these studies is that they do not fully explain the precise mechanism by which rhynchophylline attenuates reward. Are the effects of rhynchophylline mediated by the NR1 or the NR2 subunit of NMDA receptors? Although Li et al. (2014) and Chen et al. (2016) observed differential NR2B subunit levels following methamphetamine administration, they did not measure levels of other subunit levels. Thus, we cannot ascertain if the ability of rhynchophylline to attenuate the rewarding effects of methamphetamine is due to its actions at the NR2B subunit or at another subunit (e.g., NR1, NR2A, etc.). A more selective ligand can be used in order to isolate the contribution of the NR2B to methamphetamine reward. Other studies have examined the role of ifenprodil, a selective NR2B subunit antagonist, on attenuating the rewarding effects of other drugs of abuse. Ifenprodil has been found to block the rewarding effects of heroin; however, ifenprodil is known to interact with other neurotransmitter systems, particularly norepinephrine (Chenard et al., 1991; Peters & De Vries, 2012). Thus, the ability of ifenprodil to decrease drug reward could be mediated by a different neurotransmitter system.

With this information, it is imperative that further research be done in order to find pharmacotherapies that can combat methamphetamine abuse. The goal of the present study was to determine if Ro 63-1908, a highly selective NR2B subunit antagonist (Gill et al., 2002), can attenuate the conditioned rewarding effects of methamphetamine.

Method

Subjects

Thirty-six male Sprague-Dawley rats (Envigo, Indianapolis, IN, USA) were used. Rats arrived weighing between 200-224 grams. They were housed individually immediately upon delivery to the laboratory. Rats were housed in a temperature- and humidity-controlled colony room that was maintained on a light–dark cycle in which lights were on from 7:00 a.m. to 7:00 p.m. Rats were allowed to acclimate to the colony for 6 days before the start of the experiment. Rats had ad libitum access to food and water in their home cage for the entire experiment. All procedures were in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 2011) and were approved by the Institutional Animal Care and Use Committee at Northern Kentucky University.

Apparatus

A 3-compartment chamber (68 × 21 × 21 cm; ENV-013; MED Associates; St. Albans, VT) located inside a sound-attenuating chamber (ENV-020M; MED Associates) was used to measure locomotor activity and CPP. The three compartments were separated by sliding guillotine doors. The middle compartment (12 × 21 × 21 cm) had gray walls with a smooth gray PVC floor. The end compartments (28 × 21 × 21 cm) provided different contexts, with one compartment having black walls with a stainless-steel grid rod floor and the other end compartment having white walls with a stainless-steel mesh floor. Recessed trays were located 2 cm below each compartment. A computer controlled the experimental session using Med-IV software. A series of infrared photobeams (6 beams in the black and white compartments and 3 beams in the gray compartment) were used to detect the rats' presence in a particular

compartment and record the amount of time spent in that compartment, as well as to record locomotor activity during conditioning sessions.

Procedure

This study involved a CPP paradigm that lasted for a total of 10 days for all 36 rats. On the first day of the two experiments (acquisition and expression), the rats underwent a pre-test. They were able to travel freely in the CPP chamber for 15 min. Data were collected by determining how many photo beams were broken in each chamber during session. The chamber that the rat spent the least amount of time in during the pre-test was the one that was paired with methamphetamine during the conditioning phase (i.e., biased design).

Acquisition (n = 18). On days two through eight, rats were isolated to one end of the CPP chamber following treatment with either methamphetamine (1.0 mg/kg; s.c.) or saline (1.0 ml/kg; s.c.) for 30 min. Each treatment occurred on alternating days (e.g., day 2 = methamphetamine; day 3 = saline, etc.; note: the order in which rats received methamphetamine/saline injections was counterbalanced across rats). When injected with methamphetamine, rats were isolated to the initially non-preferred chamber. When injected with saline, rats were isolated to the initially preferred chamber. Three groups of rats (n = 6 per group) were used in the current experiment. Group 1 received a 30-min pretreatment of vehicle (saline mixed with 5% Tween 80; 1.0 ml/kg; s.c.) before each methamphetamine injection. Group 2 received a 30-min pretreatment of Ro 63-1908 (1.0 mg/kg; s.c.) before each methamphetamine injection. Group 3 received a 30-min pretreatment of Ro 63-1908 (3.0 mg/kg; s.c.) before each methamphetamine injection. Rats did not receive a pretreatment before each saline injection. The post-test was performed on the last day of testing (day 10) and was identical to the pre-test.

Expression (n = 18). The expression experiment was similar to the acquisition experiment described above, with the following exceptions. First, rats did not receive pretreatments of Ro 63-1908 during the conditioning sessions. Second, 30 min before the post-test, rats received treatments of either vehicle (saline mixed with 5% Tween 80; 1.0 ml/kg; s.c.), Ro 63-1908 (1.0 mg/kg; s.c.), or Ro 63-1908 (3.0 mg/kg; s.c.).

Statistical Analyses

To determine if Ro 63-1908 augmented the locomotor-stimulant effects of methamphetamine during the acquisition experiment, locomotor activity (measured in arbitrary units) was analyzed with a mixed-factor ANOVA, with methamphetamine treatment (methamphetamine vs. saline) and session as within-subjects factors and Ro 63-1908 treatment as a between-subjects factor. Significant interactions were probed with repeated-measures ANOVAs. When sphericity was violated, degrees of freedom were corrected using Greenhouse-Geisser estimates. A similar analysis was used to examine locomotor activity across each expression experiment (note: conditioning sessions were identical for rats tested in the expression experiments).

Preference ratios were calculated as the time spent in chamber paired with methamphetamine/(time spent in both methamphetamine and saline chambers). Ratios above 0.5 represent a preference for the chamber previously paired with methamphetamine, whereas ratios below 0.5 indicate an aversion. To determine if rats developed a significant preference to the chamber paired with methamphetamine, a one-sample t test was performed. A one-way ANOVA, with Tukey's post hoc tests, was also used for each experiment to determine if Ro 63-1908 altered preference ratios relative to vehicle. Statistical significance was defined as $p < .05$ in all cases.

Results

Locomotor Activity

For acquisition, the 2 × 4 × 3 mixed factor ANOVA revealed main effects of methamphetamine treatment, $F(1, 15) = 33.593$, $p < .001$, $\eta_p^2 = .691$, and session, $F(3, 45) = 3.804$, $p = .016$, $\eta_p^2 = .202$, as well as significant methamphetamine treatment × session interaction, $F(3, 45) = 5.001$, $p = .004$, $\eta_p^2 = .250$. There was a trend for a methamphetamine treatment × Ro 63-1908 treatment interaction, $F(2, 15) = 3.614$, $p = .052$, $\eta_p^2 = .325$. None of the other main effects/interactions were significant, all F's ≤ 1.839, all p's ≥ .193, all η_p^2's ≤ .197. Overall, locomotor activity was greater for rats when treated with methamphetamine compared to saline (Figure 1a, b, and c). To probe the significant drug × day interaction, separate one-way repeated measures ANOVAs were conducted for conditioning sessions in which saline was administered and in which methamphetamine was administered (note: each experiment was collapsed into these analyses; Figure 1d). Results indicated that locomotor activity did not change across saline sessions, $F(1.640, 27.885) = .624$, $p = .513$, $\eta_p^2 = .035$, but increased across methamphetamine sessions, $F(2.163, 36.766) = 7.225$, $\eta_p^2 = .298$; significant linear contrast: $F(1, 17) = 10.830$, $p = .004$, $\eta_p^2 = .389$.

For expression, the 2 × 4 × 3 mixed factor ANOVA revealed main effects of methamphetamine treatment, $F(1, 15) = 53.525$, $p < .001$, $\eta_p^2 = .781$, and session, $F(3, 45) = 7.095$, $p = .001$, $\eta_p^2 = .321$, as well as significant methamphetamine treatment × session interaction, $F(1.889, 28.332) = 5.851$, $p = .008$, $\eta_p^2 = .281$. None of the other main effects/interactions were significant, all F's ≤ 1.969, all p's ≥ .174, all η_p^2's ≤ .208. Overall, locomotor activity was greater for rats when treated with methamphetamine compared to saline (Figure 2). To probe the significant drug × day interaction, separate one-way repeated measures

ANOVAs were conducted for conditioning sessions in which saline was administered and in which methamphetamine was administered (note: each experiment was collapsed into these analyses). Results indicated that locomotor activity did not change across saline sessions, $F(1.775, 30.171) = 1.821$, $p = .155$, $\eta_p^2 = .097$, but increased across methamphetamine sessions, $F(1.828, 31.084) = 16.754$, $\eta_p^2 = .496$; significant linear contrast: $F(1, 17) = 24.717$, $p < .001$, $\eta_p^2 = .592$.

Acquisition of Methamphetamine CPP

A one-way ANOVA showed a significant effect of Ro 63-1908 treatment on acquisition of methamphetamine CPP, $F(2, 15) = 7.212$, $p = .006$, $\eta_p^2 = .490$. Tukey's post hoc test revealed that rats pretreated with Ro 63-1908 (3.0 mg/kg) had significantly lower preference ratios compared to rats pretreated with vehicle. One-sample t tests revealed a significant preference for the methamphetamine-conditioned chamber following pretreatment of vehicle, $t(5) = 6.247$, $p = .002$, $d = 2.550$, and Ro 63-1908 (1.0 mg/kg), $t(5) = 4.981$, $p = .004$, $d = 2.034$. Following Ro 63-1908 (3.0 mg/kg), there was no significant CPP, $t(5) = 0.560$, $p = .600$, $d = 0.228$ (Figure 3a).

Expression of Methamphetamine CPP

A one-way ANOVA showed no effect of Ro 63-1908 treatment, $F(2, 15) = 1.142$, $p = .345$, $\eta_p^2 = .132$. One-sample t tests revealed a significant preference for the methamphetamine-conditioned chamber following pretreatment of vehicle, $t(5) = 5.760$, $p = .002$, $d = 2.352$, and Ro 63-1908 (1.0 mg/kg), $t(5) = 4.474$, $p = .007$, $d = 1.826$. Following Ro 63-1908 (3.0 mg/kg), there was no significant CPP, $t(5) = 1.368$, $p = .230$, $d = 0.559$ (Figure 3b).

Discussion

The goal of the current experiments was to determine if blocking the NR2B subunit could attenuate the conditioned rewarding effects of methamphetamine using a CPP paradigm. Results

showed that Ro 63-1908 (3.0 mg/kg) blocked the acquisition of methamphetamine CPP. Although rats treated with Ro 63-1908 (3.0 mg/kg) did not show a statistically significant increase in preference ratios relative to indifference in the expression experiment, these results need to be interpreted with caution. There was no significant difference in preference ratios for rats treated with Ro 63-1908 (3.0 mg/kg) compared to rats treated with vehicle. Furthermore, the variability was higher for the Ro 63-1908 (3.0 mg/kg) condition relative to the other groups, which may be explained by one subject that showed a strong aversion to chamber paired with methamphetamine following treatment with Ro 63-1908 (preference ratio of .31). Three of the rats treated with Ro 63-1908 (3.0 mg/kg) had preference ratios above .70. Overall, these results suggest that the NMDA NR2B subunit is an important mediator of the acquisition of methamphetamine reward.

The acquisition of CPP determines how well an animal can consolidate information regarding the pairing of a stimulus (in this case, methamphetamine) to a specific environmental context. The finding that Ro 63-1908 (3.0 mg/kg) significantly decreased preference ratios is not surprising considering the NMDA receptor, particularly within the hippocampus, is important for learning and memory formation (Cercato et al., 2017). Moreover, a previous study showed that blocking intra-hippocampal NMDA receptors impaired performance of object location and recognition in rats, which shows that these receptors are vital for encoding, consolidation, and retention of object location memory (Yamada, Arai, Suenaga, & Ichitani, 2017). To determine if Ro 63-1908 is causing a general impairment in memory consolidation or if it is selectively decreasing the conditioned rewarding effects of methamphetamine, a control experiment needs to be conducted in which rats learn to associate a natural reinforcer (e.g., sucrose) to an environmental context. If Ro 63-1908 blocks the acquisition of CPP with natural reinforcers, one

would have to question the utility of using this drug as a potential pharmacotherapy for treating psychostimulant use disorders.

The expression of CPP is a better animal analog to substance use disorders in humans compared to the acquisition of CPP. In real-life, people seek treatment for substance use disorders after they have acquired the association between the drug and environmental/social contexts. Therefore, trying to find a pharmacotherapy that blocks the expression of CPP is important, as this has more clinical relevance compared to blocking the acquisition of CPP. In the current experiment, Ro 63-1908 did not significantly decrease preference ratios relative to vehicle. At first glance, these results would suggest that the NR2B is an important mediator of the acquisition, but not the expression, of methamphetamine CPP. However, considering Ro 63-1908 tended to produce a linear decrease in preference ratios with increasing doses, future research should examine a higher dose of Ro 63-1908 (10.0 mg/kg) on the expression of methamphetamine CPP.

Not surprisingly, methamphetamine significantly increased locomotor activity across each session for both the acquisition and expression experiments, which is consistent with previous studies (e.g., Miller, O'Callaghan, & Johnson, 2000; Yoo et al., 2006). Although Ro 63-1908 increases locomotor activity at the doses tested in the current study (Higgins et al., 2016), combining methamphetamine and Ro 63-1908 did not have an additive effect on locomotor activity in the acquisition experiment. In fact, the highest dose of Ro 63-1908 (3.0 mg/kg) tended to attenuate the locomotor-stimulant effects of methamphetamine. One potential explanation for this effect is that combining Ro 63-1908 and methamphetamine increased stereotypies that interfered with locomotor activity. Because behavior was not videotaped during each conditioning session, this possibility cannot be ruled out. One important consideration is that the

locomotor activity measured during conditioning sessions does not provide a true account of locomotion, as the dependent variable does not provide any information about how far the animal has traveled. Future work can further explore the combined effects of psychostimulants and NR2B subunit blockade by using locomotor chambers that measure distance traveled.

One future direction would be to gain further insight into the possibility of sex differences in the acquisition and expression of methamphetamine CPP, as well as to determine if Ro 63-1908 differentially affects males and females. In a previous study, female and male rats have been compared in terms of NMDA receptor expression by using NMDA competitive antagonists (Bryant, Eitan, Sinchak, Fanselow, & Evans, 2006). Yet, this study failed to look specifically at the NR2B subunit between both females and males. Moreover, it did not utilize CPP. There have been very few studies that have examined sex differences in the expression of the NR2B subunit and that utilize a CPP paradigm. Therefore, collecting data among sets of female rats will provide benefit and data that other studies have not previously examined.

Although further work is needed to gain more insight and understanding of psychostimulant, specifically methamphetamine, reward, our findings show that Ro 63-1908 is able to attenuate the acquisition of psychostimulant reward. These results suggest that the NR2B subunit is a critical mediator of the acquisition of methamphetamine CPP. To further validate the role of the NR2B subunit to the conditioned rewarding effects of drugs, techniques that selectively reduce the number of NR2B-containing NMDA receptors in distinct regions of the brain can be used. Research has demonstrated that rats injected with siRNAs that reduce NR2B expression in nucleus accumbens show decreased CPP for morphine (Kao, Huang, & Tao, 2011). These knockdown models can be useful for determining the precise mechanism by which NR2B-selective antagonists attenuate drug reward.

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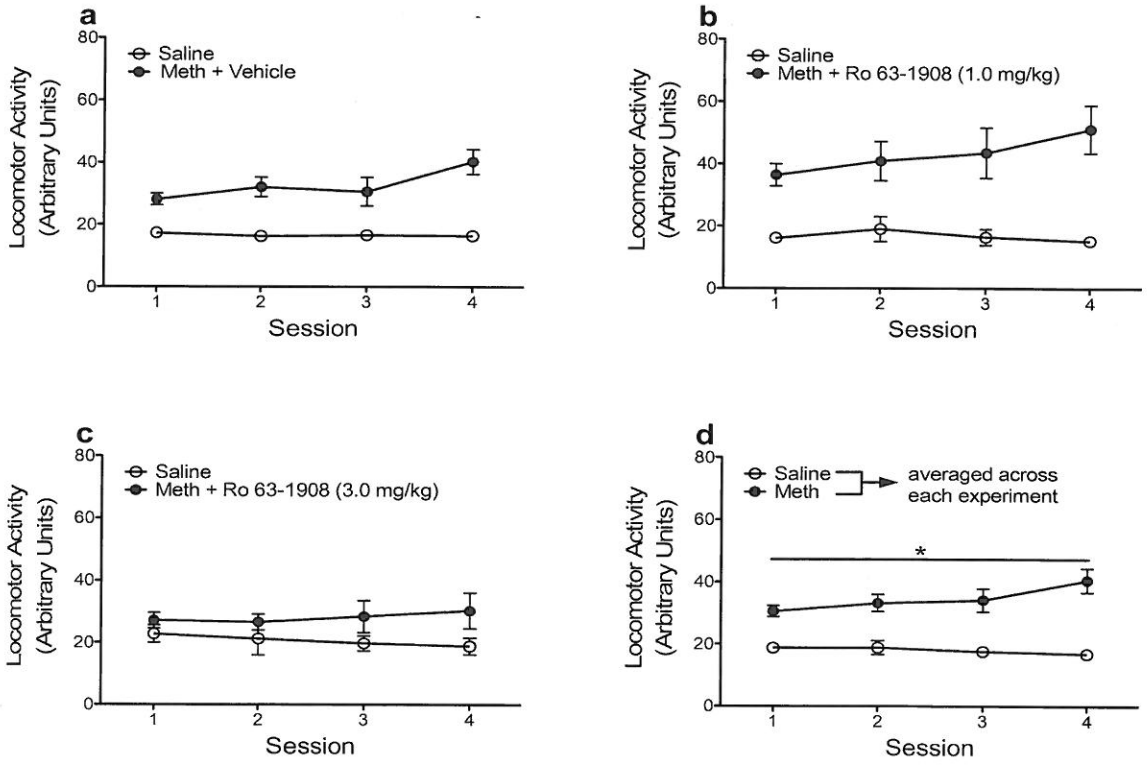

Figure 1. Mean (± SEM) locomotor activity (in arbitrary units) across each acquisition experiment. Rats were pretreated with vehicle (**a**), Ro 63-1908 (1.0 mg/kg; **b**), or Ro 63-1908 (3.0 mg/kg; **c**) 30 minutes before each methamphetamine injection. Panel **d** shows the average locomotor activity across each experiment. $*p < .05$, relative to saline.

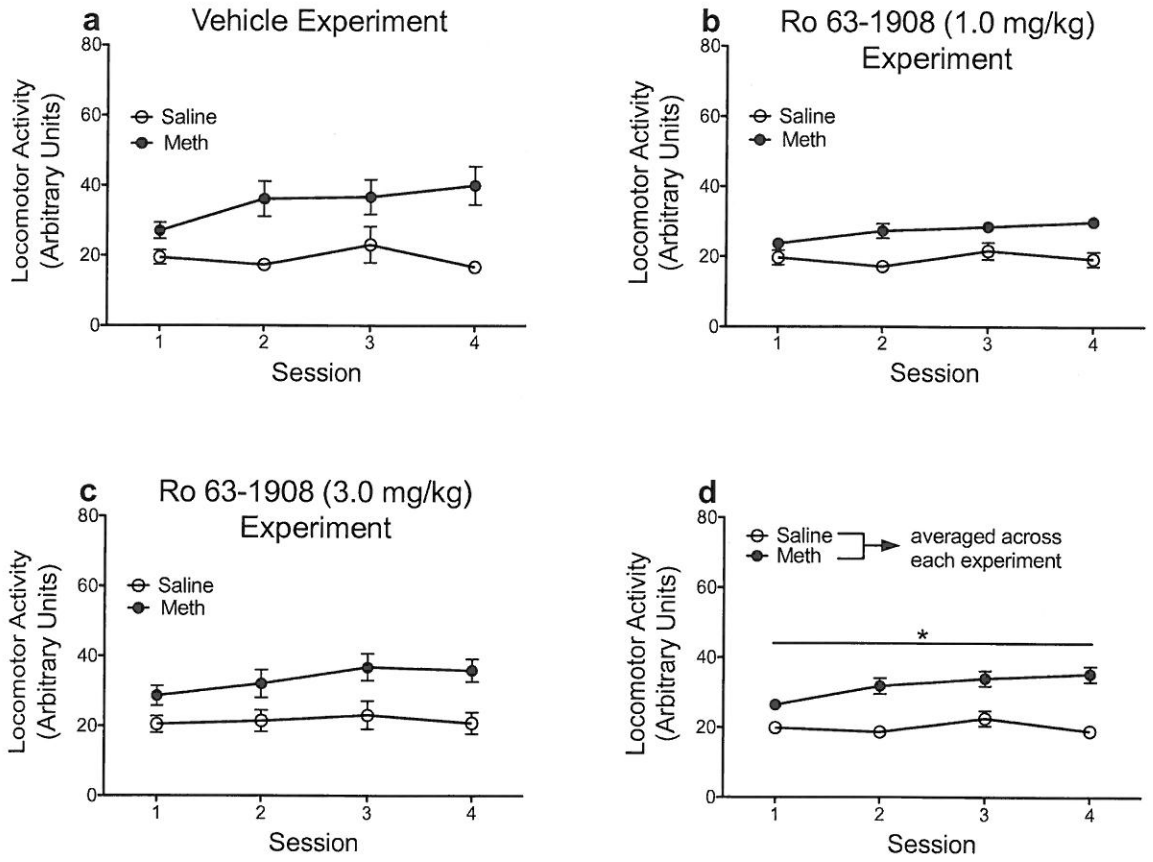

Figure 2. Mean (± SEM) locomotor activity (in arbitrary units) across each expression experiment. Rats were pretreated with vehicle (**a**), Ro 63-1908 (1.0 mg/kg; **b**), or Ro 63-1908 (3.0 mg/kg; **c**) 30 minutes before the post-test. Each rat (across all each experiment), received either methamphetamine or saline on a given conditioning session. Panel **d** shows the average locomotor activity across each experiment. *$p < .05$, relative to saline.

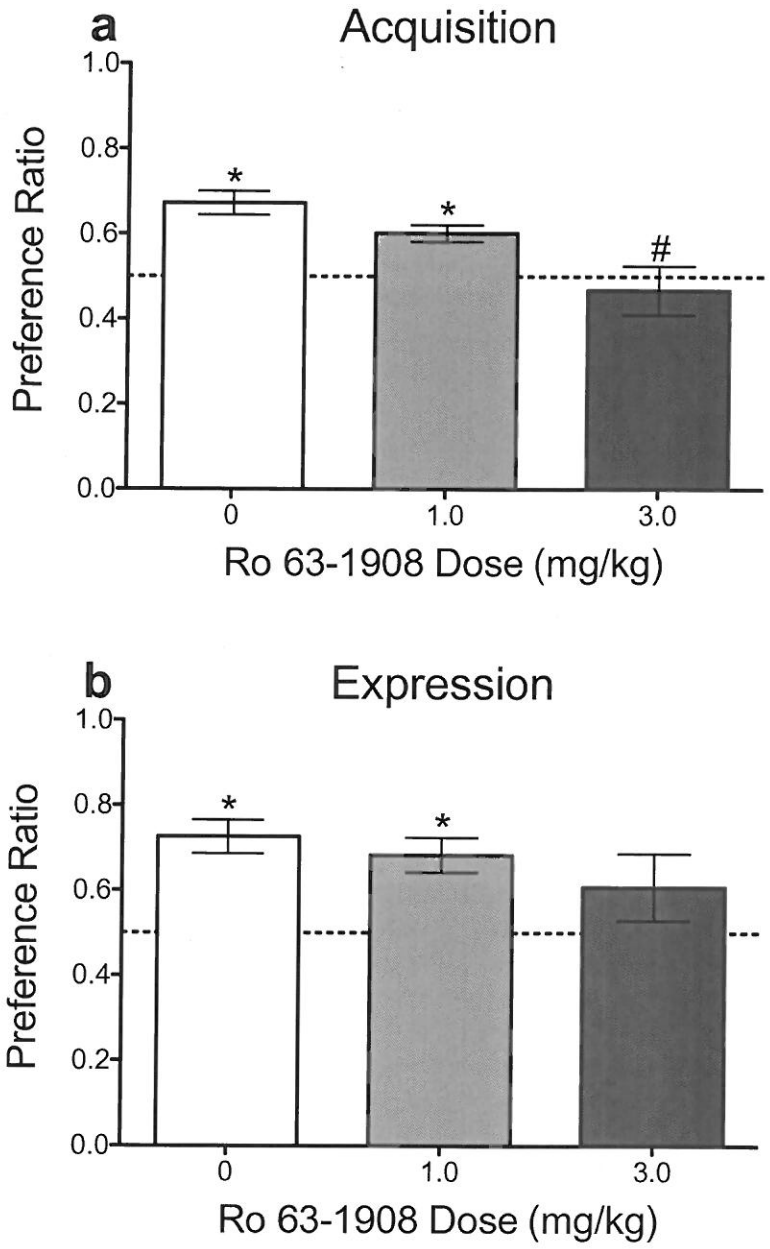

Figure 3. Mean (± SEM) preference ratios for the acquisition (**a**) and expression (**b**) experiments. *$p < .05$, relative to indifference (preference ratio of 0.5). #$p < .05$, relative to Ro 63-1908 (0.0 mg/kg).