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Research Article

Additive Effects of Coadministration of A_{2A} Receptor Agonist CGS-21680 and mGluR5 Antagonist MPEP on the Development and Expression of Methamphetamine-Induced Locomotor Sensitization in Rats

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Abstract *Background.* Accumulating evidence suggests that A_{2A} adenosine receptors and metabotropic glutamate receptors 5 (mGluR5) may modulate the nucleus accumbens (nAcc) dopamine neurotransmission. This study examined the effects of coadministration of the A_{2A} receptor agonist CGS-21680 and the mGluR5 antagonist MPEP on the development and expression of methamphetamine (METH)-induced locomotor sensitization. *Methods.* Rats were treated with different doses of CGS (0.1 mg/kg, 0.2 mg/kg or 0.4 mg/kg) or MPEP (1.0 mg/kg, 3.0 mg/kg or 10.0 mg/kg) or coadministration of CGS (0.1 mg/kg, 0.2 mg/kg or 0.4 mg/kg) with MPEP (3.0 mg/kg) plus METH (1.0 mg/kg). For the challenge test, rats received a single injection of METH (1.0 mg/kg). In experiments to assess the expression of METH-induced locomotor sensitization, rats were treated with a daily dose of METH (1.0 mg/kg), and the challenge test was preceded by injection of CGS (0.1 mg/kg, 0.2 mg/kg or 0.4 mg/kg), MPEP (1.0 mg/kg, 3.0 mg/kg or 10.0 mg/kg) or coadministration of CGS (0.1 mg/kg, 0.2 mg/kg or 0.4 mg/kg) with MPEP (3.0 mg/kg) plus METH (1.0 mg/kg). *Results.* The data showed that CGS and MPEP treatment prevented both the development and expression of METH-induced locomotor sensitization. Furthermore, CGS increased the effects of a low MPEP dose on METH-induced locomotor sensitization under both conditions. *Conclusion.* These findings suggest the potential utility of adenosine A_{2A} receptors and mGluR5 ligands to treat psychostimulant addiction.

Keywords methamphetamine; A_{2A} receptors; mGluR5s; locomotor sensitization

1. Introduction

Behavioral sensitization is defined as the progressive and persistent enhancement of the locomotor effects of psychostimulant drugs after their repeated and intermittent administration [1]. It is thought to reflect the neuroadaptations contributing to drug addiction and model some aspects of addictive behaviors such as drug craving and relapse [2]. This process can be separated into two distinct phases, development (or initiation) and expression. Development is the immediate molecular and cellular alterations that induce behavioral sensitization, and expression is the long-term consequences of these initial events. It is well known that the development and expression of psychostimulant-induced locomotor sensitization result in adaptive changes in dopamine (DA) neurotransmission in the ventral tegmental area (VTA) and ventral striatum, also known as the nucleus accumbens (nAcc) [2,3].

Although DA neurotransmission plays a critical role in psychostimulant-induced locomotor sensitization, recent evidence suggests that adenosinergic and glutamatergic transmission are also involved in this sensitization. Adenosine is an endogenous nucleoside that acts as a neuromodulator in the central nervous system (CNS). To date, four adenosine receptors have been identified (A_1R, $A_{2A}R$, $A_{2B}R$, and A_3R); they are part of a large family of seven transmembrane-spanning G protein-coupled receptors [4]. Recent data indicate that $A_{2A}Rs$ are involved in psychostimulant-induced locomotor sensitization and earlier studies reported that CGS-21680, an $A_{2A}R$ agonist, inhibits the development of cocaine and methamphetamine (METH) sensitization to locomotor activity [5,6]. Conversely, a low, but not a high, dose of MSX-3, an antagonist of adenosine $A_{2A}Rs$, has been shown to increase cocaine-induced locomotor hyperactivity [5]. In contrast, another study found that administration of the $A_{2A}R$ antagonist SCH-58261 reduced the development but not the expression of amphetamine-induced locomotor sensitization [7]. The reason for this discrepancy is not clear; it may be related to drug type, dosage regimen or procedural variables.

There is also evidence that glutamate neurotransmission plays important roles in several processes underlying drug addiction [8]. Recent studies have identified metabotropic glutamate receptors (mGluRs) as potential targets for

treating drug addiction. Eight different subtypes of this receptor have been described, and the subtype mGluR5, in particular, is most strongly implicated in addictive behaviors [9, 10, 11]. Previous studies have shown that the mGluR5 antagonists MPEP and MTEP attenuate the expression of morphine-, cocaine-, and nicotine-induced psychomotor sensitization [12, 13, 14]. On the other hand, Veeneman et al. [15] showed that MTEP suppressed the development of cocaine- but not morphine-induced locomotor sensitization. Furthermore, it has been reported that the psychomotor stimulant effect of cocaine is absent in mGluR5-knockout mice [16] and MPEP administration decreased the acute psychomotor effects of cocaine, amphetamine, and nicotine [14, 17, 18]. Collectively, these studies suggest that mGluR5 is critically involved in locomotor sensitization.

Although it has been reported that both A_{2A} and mGluR5 receptors are involved in some psychostimulant-induced behavioral effects, it remains to be demonstrated whether their simultaneous activation and blockade, respectively, can reduce psychostimulant-induced locomotor sensitization. In the present study, we examined the effect of a low dose of the mGluR5 antagonist MPEP in combination with different doses of the A_{2A}R agonist CGS on the development and expression of METH-induced locomotor sensitization in rats.

2. Methods

2.1. Subjects

Male Wistar rats (FES-Iztacala-UNAM, Mexico) weighing 220–250 g at the beginning of the experiment were used in the present study. The rats were individually housed in standard plastic rodent cages in a colony room maintained at a temperature of 21 °C (±1 °C) under a 12-hour light/dark cycle (lights on at 06:00 AM) and had a continuous access to water and food (Teklad LM485 Rat Diet; Harlan, Mexico City, Mexico). All experiments were conducted during the light phase (between 11:00 AM and 1:00 PM). Animal care and handling procedures were conducted in accordance with the Official Mexican Norm (NOM-062-ZOO-1999) entitled "Technical Specifications for the Production, Care, and Use of Laboratory Animals".

2.2. Locomotor activity measurement

Locomotor activity was recorded individually for each animal in an open-field activity monitoring system (ENV-515 model; Med Associates, St. Albans, VT, USA). Each Plexiglas cage ($40 \times 40 \times 30$ cm) was equipped with two sets of eight photobeams placed 2.5 cm above the floor surface on opposite walls to record x-y ambulatory movements. Photobeam interruptions were recorded and translated using a software to yield the horizontal distance traveled (in cm), which was the dependent measure used for the analyses. The timeline of the general procedure is shown in Figure 1. Each

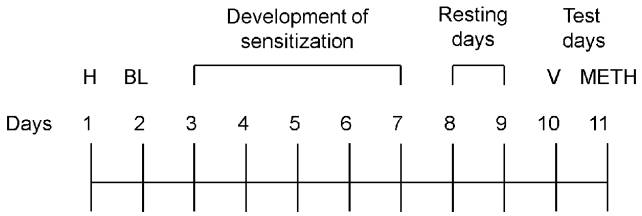

Figure 1: Schematic diagram illustrating the timeline of METH (1 mg/kg)-induced locomotor sensitization. H: habituation; BL: baseline locomotor activity; V: vehicle; METH: methamphetamine.

day or session started with a 10-minute period of habituation to the cages, followed by administration of drug(s) or vehicle. The rats ($n = 10$ per group) were returned to the cages, and their locomotor activity was recorded for 1 h. On day 1, the rats were habituated to the open-field cages and injection procedures (habituation session). On day 2, locomotor activity after vehicle administration (locomotor activity baseline) was evaluated. For the specific procedure of the development of locomotor sensitization and challenge days, see Sections 2.5, 2.6 and Table 1 (this protocol was performed as previously described by Cedillo and Miranda [19]).

2.3. Drugs

The following drugs were used: methamphetamine hydrochloride (Sigma, St. Louis, MO, USA), CGS-21680 (4-[2-[{6-amino-9-(N-ethyl-b-D-ribofuranuronamidosyl)-9H-purin-2-yl}amino]ethyl]benzene-propanoic acid hydrochloride; Tocris, Bristol, UK), and MPEP hydrochloride (2-methyl-6-[phenylethynyl]-pyridine hydrochloride, Tocris, Bristol, UK). All drugs were dissolved in saline and injected intraperitoneally (IP) at a volume of 1 mL/kg. CGS-21680 and MPEP were administrated 10 min before methamphetamine. The drug doses were chosen based upon their functional selectivity at A_{2A}Rs [5, 6] and mGluR5 [17, 20].

2.4. Acute effects of adenosine A_{2A}R agonist CGS and mGluR5 antagonist MPEP on locomotor activity

To investigate the acute effects of CGS and MPEP on locomotor activity, we conducted an initial experiment. Separated groups of rats were assessed once in response to the different doses of MPEP (0.0 mg/kg, 1.0 mg/kg, 3.0 mg/kg, and 10.0 mg/kg) and CGS (0.0 mg/kg, 0.1 mg/kg, 0.2 mg/kg, and 0.4 mg/kg) on day 3.

2.5. Development of METH-induced locomotor sensitization

During days 3–7 of the experiment, the animals received the following injections: vehicle + vehicle (groups 2V), vehicle + METH (1.0 mg/kg; groups VM), CGS (0.1–0.4 mg/kg) + METH (1.0 mg/kg; groups C1-M, C2-M, and C4-M),

Table 1: Experimental design for the development and expression of METH-induced locomotor sensitization.

Name of groups	Repeated treatment	Challenge	
	Days 3 to 7	Day 10	Day 11
	Development of locomotor sensitization		
2V	VEH+VEH	VEH	
VM	VEH+METH(1.0)	VEH	METH(1.0)
C1-M	CGS(0.1)+METH(1.0)	VEH	METH(1.0)
C2-M	CGS(0.2)+METH(1.0)	VEH	METH(1.0)
C4-M	CGS(0.4)+METH(1.0)	VEH	METH(1.0)
2V	VEH+VEH	VEH	METH(1.0)
VM	VEH+METH(1.0)	VEH	METH(1.0)
P1-M	MPEP(1.0)+METH(1.0)	VEH	METH(1.0)
P3-M	MPEP(3.0)+METH(1.0)	VEH	METH(1.0)
P10-M	MPEP(10.0)+METH(1.0)	VEH	METH(1.0)
2V	VEH+VEH	VEH	METH(1.0)
VM	VEH+METH(1.0)	VEH	METH(1.0)
C1-P3-M	CGS(0.1)+MPEP(3.0)+METH(1.0)	VEH	METH(1.0)
C2-P3-M	CGS(0.2)+MPEP(3.0)+METH(1.0)	VEH	METH(1.0)
C4-P3-M	CGS(0.4)+MPEP(3.0)+METH(1.0)	VEH	METH(1.0)
	Expression of locomotor sensitization		
V-VM	VEH+VEH	VEH	VEH+METH(1.0)
M-VM	VEH+METH(1.0)	VEH	VEH+METH(1.0)
C1-M	VEH+METH(1.0)	VEH	CGS(0.1)+METH(1.0)
C2-M	VEH+METH(1.0)	VEH	CGS(0.2)+METH(1.0)
C4-M	VEH+METH(1.0)	VEH	CGS(0.4)+METH(1.0)
V-VM	VEH+VEH	VEH	VEH+METH(1.0)
M-VM	VEH+METH(1.0)	VEH	VEH+METH(1.0)
P1-M	VEH+METH(1.0)	VEH	MPEP(1.0)+METH(1.0)
P3-M	VEH+METH(1.0)	VEH	MPEP(3.0)+METH(1.0)
P10-M	VEH+METH(1.0)	VEH	MPEP(10.0)+METH(1.0)
V-VM	VEH+VEH	VEH	VEH+METH(1.0)
M-VM	VEH+METH(1.0)	VEH	VEH+METH(1.0)
C1-P3-M	VEH+VEH+METH(1.0)	VEH	CGS(0.1)+MPEP(3.0)+METH(1.0)
C2-P3-M	VEH+VEH+METH(1.0)	VEH	CGS(0.2)+MPEP(3.0)+METH(1.0)
C4-P3-M	VEH+VEH+METH(1.0)	VEH	CGS(0.4)+MPEP(3.0)+METH(1.0)

VEH: appropriate vehicle; METH: methamphetamine; in parentheses doses (mg/kg; IP).

MPEP (1.0–10.0 mg/kg) + METH (1.0 mg/kg; groups P1-M, P3-M, and P10-M) or CGS (0.1–0.4 mg/kg) + MPEP (3.0 mg/kg) + METH (1.0 mg/kg; groups C1-P3-M, C2-P3-M, and C4-P3-M). On day 10, the animals received a challenge with vehicle, and on day 11 (a test for locomotor sensitization), the animals received a challenge dose of METH (1.0 mg/kg). Locomotor activity was recorded. On days 8 and 9, the animals remained drug-free in their home cages. Each rat underwent only one test session (see Table 1 for protocol details).

2.6. Expression of METH-induced locomotor sensitization

During experiment days 3–7, the animals received the following injections: vehicle(s) or vehicle(s) and METH (1.0 mg/kg). On days 8 and 9, the animals remained drug-free in their home cages. On day 10, they received a challenge with vehicle, and on day 11, the animals received vehicle + METH (1.0 mg/kg; groups V-VM, M-VM), CGS (0.1–0.4 mg/kg) + METH (1.0 mg/kg; groups C1-M, C2-M, and C4-M), MPEP (1.0–10.0 mg/kg) + METH (1.0 mg/kg; groups P1-M, P3-M, and P10-M) or CGS (0.1–0.4 mg/kg) + MPEP (3.0 mg/kg) + METH (1.0 mg/kg; groups C1-P3-M, C2-P3-M, and C4-P3-M). Locomotor activity was recorded and each rat underwent only one test session (see Table 1 for further details).

2.7. Statistical analyses

The data are expressed as mean horizontal locomotor activity (±SEM) for the 60-minute observation period. The data obtained during the development of locomotor sensitization were analyzed using two-way analysis of variance (ANOVA) for repeated measures, with day (3 to 7) as the repeated measure factor and groups as the between factor. The data obtained during the challenge days

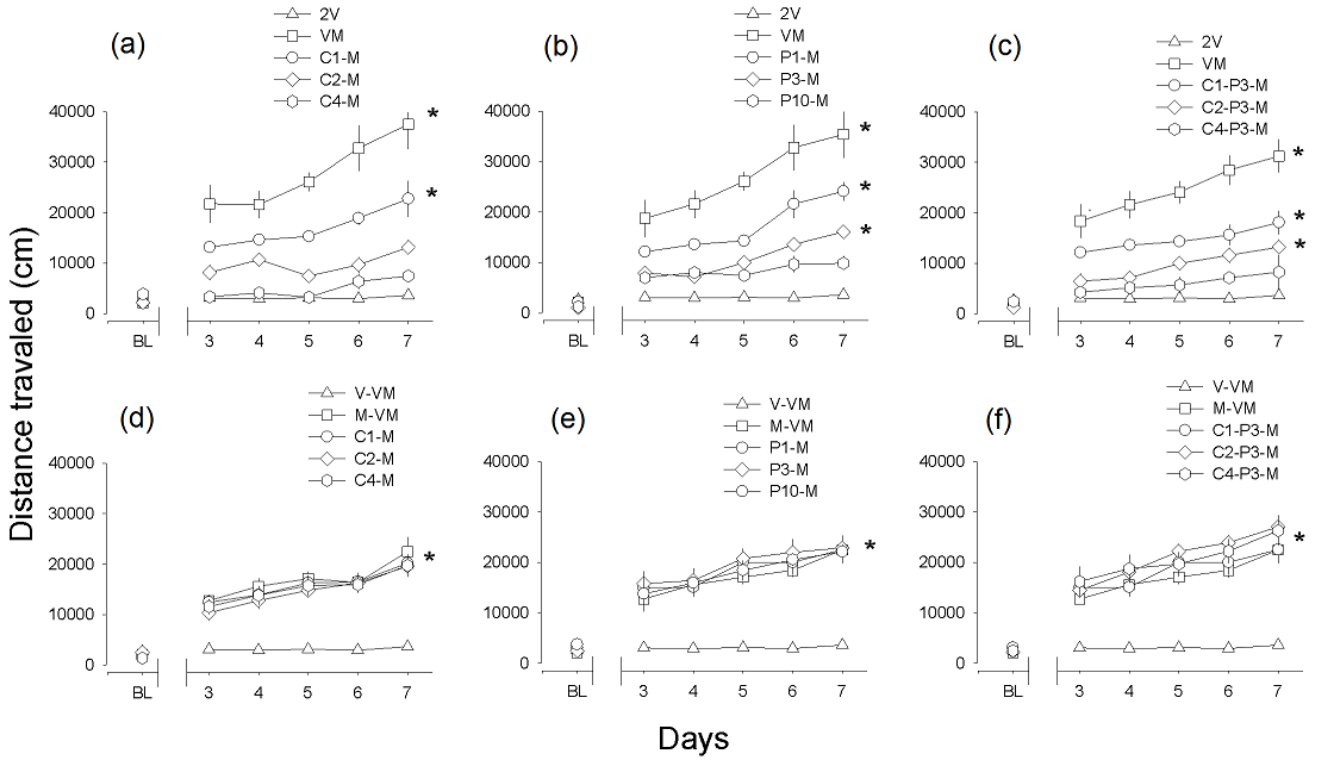

Figure 2: Development of METH-induced locomotor sensitization. Symbols are mean ±SEM from 10 rats. Repeated treatment of METH in (d), (e), and (f) developed locomotor sensitization in rats. Coadministration of (a) CGS, (b) MPEP or (c) CGS + MPEP with METH reduced the development of locomotor sensitization. BL: baseline of locomotor activity. In (a), (b), and (c), 2V: two vehicle injections; VM: vehicle and METH injections; C1, C2, and C4-M: CGS-21680 (0.1 mg/kg, 0.2 mg/kg, and 0.4 mg/kg) and METH, respectively; P1, P3, and P10-M: MPEP (1.0 mg/kg, 3.0 mg/kg, and 10.0 mg/kg) and METH, respectively; C1, C2, and C4-P3-M: CGS-21680 (0.1 mg/kg, 0.2 mg/kg, and 0.4 mg/kg), MPEP (3.0 mg/kg), and METH, respectively. In (d), (e), and (f), V-VM: vehicle injections and METH; M-VM: METH, vehicle, and METH; C1, C2, and C4-M: CGS-21680 (0.1 mg/kg, 0.2 mg/kg, and 0.4 mg/kg) and METH, respectively; P1, P3, and P10-M: MPEP (1.0 mg/kg, 3.0 mg/kg, and 10.0 mg/kg) and METH, respectively; C1, C2, and C4-P3-M: CGS-21680 (0.1 mg/kg, 0.2 mg/kg, and 0.4 mg/kg), MPEP (3.0 mg/kg), and METH, respectively (see Table 1 for further details).

were analyzed with one-way ANOVAs. When the ANOVA results were significant, Tukey's test ($P < .05$) was used to perform a posteriori comparisons.

3. Results

3.1. Acute effects of CGS and MPEP on locomotor activity

The results of this experiment revealed that neither CGS ($F[3,36] = 0.048$, $P > .05$) nor MPEP ($F[3,36] = 0.91$, $P > .05$) altered rat locomotor activity (data not shown).

3.2. Development of METH-induced locomotor sensitization

Administration of a single dose of METH (1.0 mg/kg) induced approximately a five-fold increase in horizontal locomotor activity. Treatment with CGS (0.1–0.4 mg/kg) attenuated METH-induced hyperlocomotion (Figure 2(a)). Two-way ANOVA for repeated measures indicated significant effects of the group ($F[4,45] = 92.360$, $P < .05$), day ($F[5,225] = 39.86$, $P < .05$), and the group × day interaction ($F[20,225] = 7.07$, $P < .05$); and Tukey's test revealed that the VM group was different from all of the other groups and also revealed significant differences between day 7 and day 3 on VM and C1-M groups. When the animals were treated with MPEP (1.0–10.0 mg/kg), the increase in the horizontal locomotor activity was also attenuated (Figure 2(b)). We also observed significant effects of the group ($F[4,45] = 71.358$, $P < .05$), day ($F[5,225] = 52.855$, $P < .05$), and group × day interaction ($F[20,225] = 6.892$, $P < .05$); and Tukey's test revealed that the VM group was different from all of the other groups and also revealed significant differences between day 7 and day 3 on VM, P1-M, and P3-M groups. Finally, the coadministration of CGS (0.1–0.4 mg/kg) + MPEP (3.0 mg/kg) significantly attenuated the effects of METH-induced locomotor sensitization (Figure 2(c)). Again, we noted significant effects of the group ($F[4,45] = 100.038$,

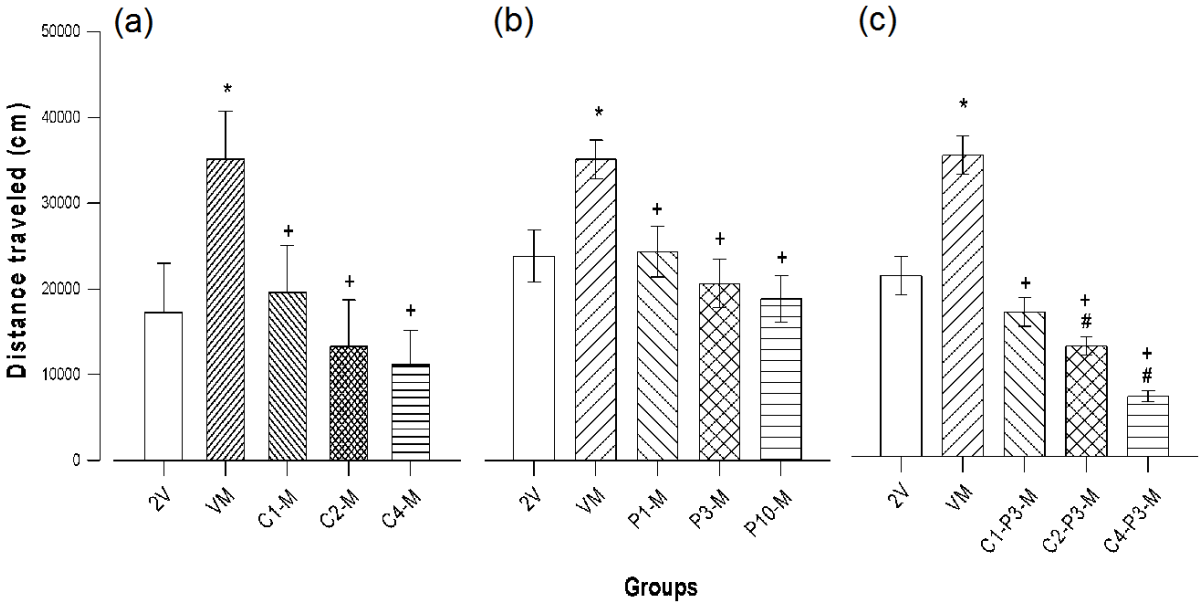

Figure 3: Results for challenge days after the development of METH-induced locomotor sensitization. The bars represent the mean ±SEM from 10 rats. Differences were compared with one-way ANOVA followed by Tukey's post hoc test: $^{*}P < .05$ compared to the 2V group, $^{+}P < .05$ compared to the VM group, $^{\#}P < .05$ compared to the 2V group. The group names refer to the treatment received during the development of METH-induced locomotor sensitization (see Table 1 for additional details). In (a), (b), and (c), 2V: two vehicle injections; VM: vehicle and METH injections; C1, C2, and C4-M: CGS-21680 (0.1 mg/kg, 0.2 mg/kg, and 0.4 mg/kg) and METH, respectively; P1, P3, and P10-M: MPEP (1.0 mg/kg, 3.0 mg/kg, and 10.0 mg/kg) and METH, respectively; C1, C2, and C4-P3-M: CGS-21680 (0.1 mg/kg, 0.2 mg/kg, and 0.4 mg/kg); MPEP (3.0 mg/kg), and METH, respectively.

$P < .05$), day ($F[5,225] = 38.033$, $P < .05$), and group × day interaction ($F[20,225] = 5.384$, $P < .05$); and Tukey's test revealed that the VM group was different from all of the other groups and also revealed significant differences between day 7 and day 3 on VM, C1-P3-M, and C2-P3-M groups.

3.3. METH administration on challenge day after the development of METH-induced locomotor sensitization

Rats repeatedly treated with METH (days 3–7) showed a two-fold increase in horizontal locomotor activity when rats were challenged with METH (1.0 mg/kg) 3 days after the last treatment injection (group VM) compared to the effect of acute METH in vehicle-treatment animals during days 3–7 (group 2V). Pretreatment with CGS (0.1–0.4 mg/kg) dose-dependently attenuated METH-induced locomotor sensitization ($F[4,45] = 42.5$, $P < .05$). Tukey's post hoc test revealed that the VM group was significantly different from the 2V group, and the C1-M, C2-M, and C4-M groups were different from the VM group (see Figure 3(a)).

3.4. Effects of MPEP on the development of METH-induced locomotor sensitization

After five days of repeated METH (1.0 mg/kg) administration, a challenge dose of METH (1.0 mg/kg) induced marked behavioral sensitization that was observed as an increase in horizontal locomotor activity (group VM) compared with the response to acute METH injection (day 11) in animals treated with repeated vehicle treatment (group 2V). Pretreatment with MPEP (1.0–10.0 mg/kg) reduced METH-induced locomotor sensitization in a dose-dependent manner when METH was administered on day 11 ($F[4,45] = 5.15$, $P < .05$). Tukey's test revealed that the VM group was different from the 2V group, and the P1-M, P3-M, and P10-M groups were different from the VM group (see Figure 3(b)).

3.5. Effects of CGS and MPEP coadministration on the development of METH-induced locomotor sensitization

Rats repeatedly (days 3–7) treated with METH (1.0 mg/kg) showed an almost two-fold increase in horizontal locomotor activity when challenged with METH (1.0 mg/kg) 3 days after the last treatment injection (VM) compared to the acute effects of METH in saline-treatment animals (group 2V). Pretreatment with CGS (0.1–0.4 mg/kg) + MPEP (3.0 mg/kg) dose-dependently decreased locomotor activity when METH was administered on day 11 ($F[4,45] = 38.05$, $P < .05$). Tukey's test revealed that the VM group was significantly different from the 2V group, the C1-P3-M, C2-P3-M, and C4-P3-M groups were different from the

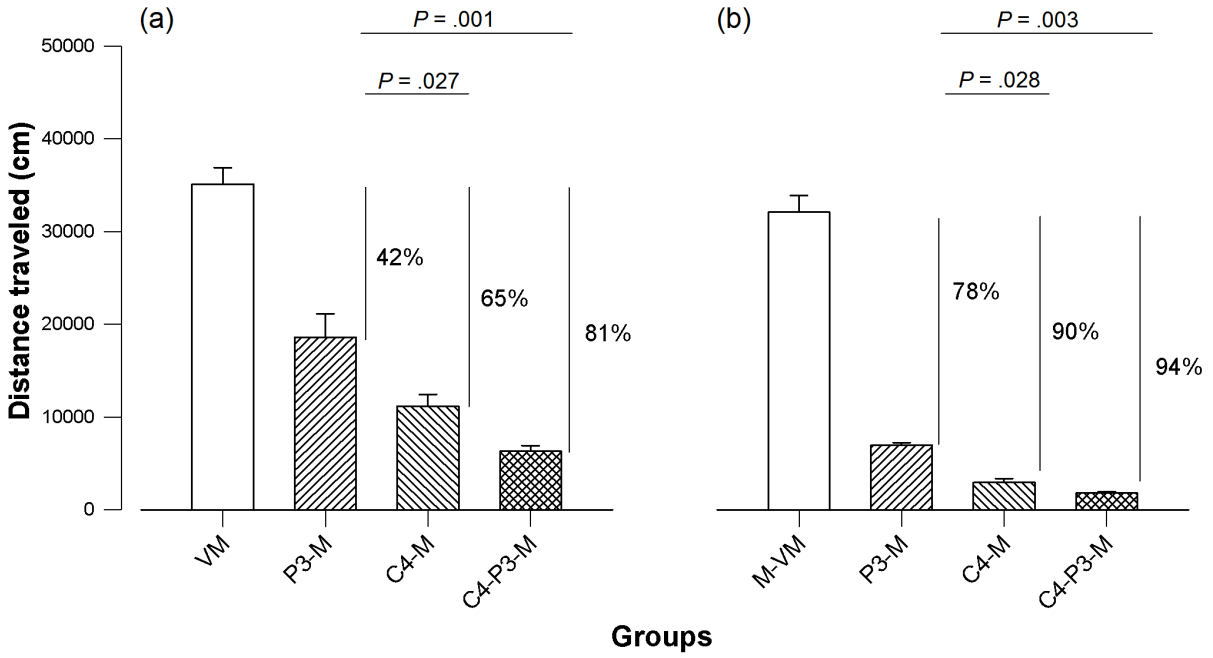

Figure 4: Coadministration of CGS-21680 and MPEP produced a greater effect than each drug administered separately. The bars represent the mean ±SEM from 10 rats. Panel (a) shows the percentage of reduction when P3-M, C4-M, and C4-P3-M groups were compared with the VM group. The data from P3-M, C4-M, and C4-P3-M groups were taken from Figures 3(b), 3(a), and 3(c), respectively. Panel (b) shows the percentage of reduction when P3-M, C4-M, and C4-P3-M groups were compared with the M-VM group. The data from P3-M, C4-M, and C4-P3-M groups were taken from Figures 5(b), 5(a), and 5(c), respectively. P: statistical significance value after ANOVA (panel (a): $F[3,36] = 38.536$, $P < .05$, panel (b): $F[3,36] = 226.615, P < .05$) and Tukey's test.

VM group, and C2-P3-M and C4-P3-M groups were significantly different from 2V group (see Figure 3(c)). These data could represent an additive effect because the percentage of reduction of the locomotor activity in the C4-P3-M group (81%) was greater than the reduction in P3-M (42%) and C4-M (65%) groups when they were compared with the VM group (see Figure 4(a) for further details). As it can also be noted in Figure 4(a), the statistical difference between P3-M and C4-P3-M groups was greater than the P3-M and C4-M groups.

Challenge with vehicle (day 10) did not affect basal horizontal locomotor activities of the animals in any of the experiments (data not shown).

3.6. Expression of METH-induced locomotor sensitization

Administration of a single dose of METH (1.0 mg/kg) induced approximately a five-fold increase in the horizontal locomotor activity, and repeated METH administration resulted in the development of sensitization to locomotor activity in all groups except those treated with vehicle (Figures 2(d), 2(e), 2(f)). In the case of the V-VM, M-VM, C1-M, C2-M, and C4-M groups (Figure 2(d)), the names of the groups refer only to the treatment received during the expression test of METH-induced locomotor sensitization (see Table 1 for further details). A two-way repeated measures ANOVA test indicated significant effects of group ($F[4,45] = 21.549$, $P < .05$), day ($F[5,225] = 131.769$, $P < .05$), and group × day interaction ($F[20,225] = 7.678$, $P < .05$); and Tukey's test revealed that the V-VM group was different from all of the other groups and also revealed significant differences between day 7 and day 3 in all other groups, except the V-VM group. For the V-VM, M-VM, P1-M, P3-M, and P10-M groups (Figure 2(e)), two-way repeated measures ANOVA revealed significant effects of group ($F[4,45] = 30.326$, $P < .05$), day ($F[5,225] = 94.88$, $P < .05$), and group × day interaction ($F[20,225] = 5.719$, $P < .05$), and Tukey's test revealed that the V-VM group was different from all other groups and also revealed significant differences between day 7 and day 3 in all other groups, except the V-VM group. In the case of the V-VM, M-VM, C1-P3-M, C2-P3-M, and C4-P3-M groups (Figure 2(f)), we observed significant effects of the group ($F[4,45] = 23.104$, $P < .05$), day ($F[5,225] = 156.704$, $P < .05$), and the group × day interaction ($F[20,225] = 10.273$, $P < .05$); and Tukey's test revealed that the V-VM group was significantly different from all of the other groups and also revealed significant differences between day 7 and day 3 in all other groups, except the V-VM group.

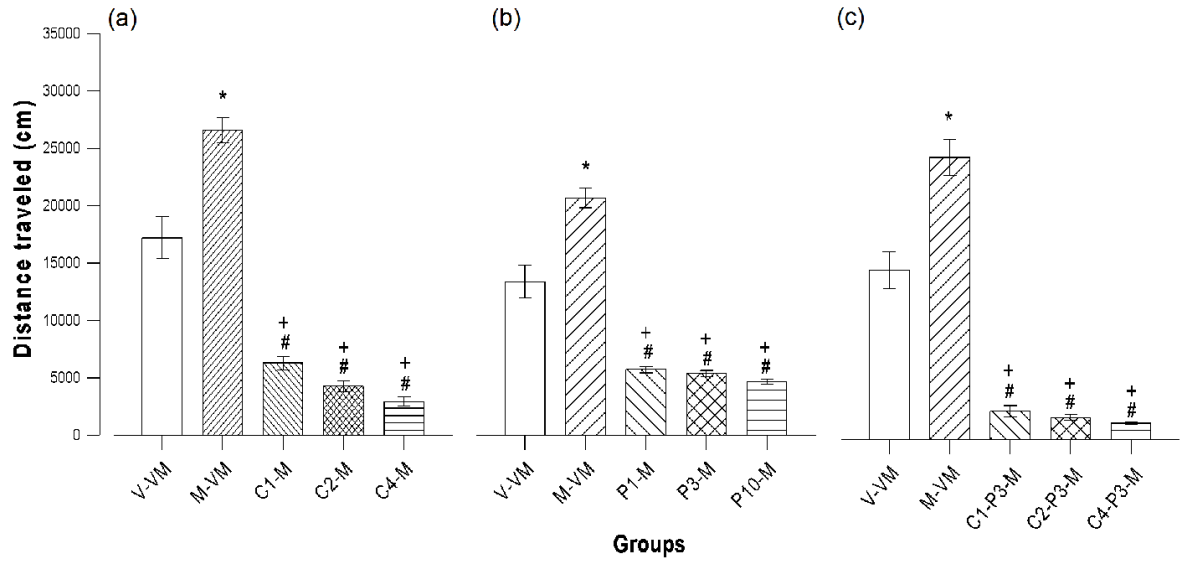

Figure 5: Results for challenge days during the expression of METH-induced locomotor sensitization. Bars represent the mean ±SEM from 10 rats. Differences were compared with one-way ANOVA followed by Tukey's post hoc test: $^{*}P < .05$ compared to the V-VM group, $^{+}P < .05$ compared to the M-VM group, $^{\#}P < .05$ compared to the V-VM group. The group names refer to the treatment received during the expression of METH-induced locomotor sensitization (see Table 1 for additional details). In (a), (b), and (c), V-VM: vehicle injections and METH; M-VM: METH, vehicle, and METH: C1, C2, and C4-M: CGS-21680 (0.1 mg/kg, 0.2 mg/kg, and 0.4 mg/kg) and METH, respectively; P1, P3, and P10-M: MPEP (1.0 mg/kg, 3.0 mg/kg, and 10.0 mg/kg) and METH, respectively; C1, C2, and C4-P3-M: CGS-21680 (0.1 mg/kg, 0.2 mg/kg, and 0.4 mg/kg), MPEP (3.0 mg/kg), and METH, respectively.

3.7. Effects of CGS + METH after the development of METH-induced locomotor sensitization

The results of treatment with METH or different doses of CGS and METH on day 11 are shown in Figure 5(a). CGS reduced the expression of METH-induced locomotor activity ($F[4,45] = 97.176$, $P < .05$). Tukey's test revealed that locomotor activity in the M-VM group was significantly different from that measured in the V-VM and C1-M, C2-M, and C4-M groups; and C1-M, C2-M, and C4-M groups were significantly different from the V-VM group.

3.8. Effects of MPEP + METH after development of METH-induced locomotor sensitization

The results obtained following the administration of METH or different doses of MPEP and METH on day 11 (see Figure 5(b)) showed that MPEP reduced the expression of METH-induced locomotor activity ($F[4,45] = 82.517$, $P < .05$). Tukey's test revealed that the M-VM group was significantly different from the V-VM, P1-M, P3-M, and P10-M groups; and P1-M, P3-M, and P10-M groups were significantly different from V-VM group.

3.9. Effects of CGS + MPEP + METH after the development of METH-induced locomotor sensitization

The effects of different doses of CGS, MPEP (3.0 mg/kg), and METH on day 11 are shown in Figure 5(c). CGS administration increased the effects of MPEP on the expression of METH-induced locomotor sensitization ($F[4,45] = 100.201$, $P < .05$). Tukey's test revealed that the M-VM group was different from the V-VM, C1-P3-M, C2-P3-M, and C4-P3-M groups; and C1-P3-M, C2-P3-M, and C4-P3-M groups were significantly different from V-VM group. These data could represent an additive effect because the percentage of reduction of the locomotor activity in the C4-P3-M group (94%) was greater than the reduction in P3-M (78%) and C4-M (90%) groups when they were compared with the M-VM group (see Figure 4(b) for further information). As it can also be noted in the Figure 4(b), the statistical difference between P3-M and C4-P3-M groups was greater than the P3-M and C4-M groups.

Challenge with vehicle on day 10 did not affect basal horizontal locomotor activities in any of the experiments (data not shown).

4. Discussion

The purpose of the present study was to examine the effects of the simultaneous activation of adenosine A_{2A}Rs and blockade of glutamate mGluR5s on the development and expression of METH-induced locomotor sensitization in rats. We found that METH increased locomotor activity, but treatment with either the adenosine A_{2A}R agonist CGS or

the glutamate mGluR5 antagonist MPEP prevented both the development and expression of METH-induced locomotor sensitization. Moreover, coadministration of CGS and a low dose of MPEP produced an additive effect.

The behavioral results described above are consistent with those of existing studies demonstrating that the CGS attenuates psychostimulant-induced locomotor sensitization. For example, CGS was previously reported to prevent both the development and expression of sensitization to locomotor effects of METH [6], attenuate sensitization to locomotor stimulant effects of cocaine [5, 21], and reduce the acute effects of cocaine on motor activity in a dose-dependent manner [21].

Furthermore, it has been found that CGS attenuates psychostimulant-mediated behaviors related to drug addiction. For example, systemic administration of CGS blocks the initiation of cocaine self-administration in a dose-dependent manner [22], attenuates cocaine-seeking behavior and cue-induced reinstatement to cocaine seeking [23], and reduces the expression of cocaine-conditioned place preference [21]. Intra-nAcc CGS administration also alters cocaine-seeking behavior in rats [24]. In line with these findings, CGS attenuates stimuli- and drug-associated behaviors related to drug addiction. For example, CGS reduces the development of hypersensitivity to an acute morphine injection given during opiate withdrawal in rats [25] and attenuates different opiate withdrawal signs such as teeth chattering and forepaw treading in mice [26]. CGS also blocks cocaine self-administration in a dose-dependent manner [22]. Additionally, systemic administration and intra-nAcc infusion of CGS elevates thresholds of brain stimulation reward without increasing response latencies [27]. $A_{2A}R$ blockage, on the other hand, produces opposite effects. $A_{2A}R$ antagonist administration enhances both acute and sensitized cocaine-mediated locomotor activity [5], increases amphetamine- and METH-induced discriminative cue [28], and enhances ethanol intake in a voluntary drinking paradigm with alcohol-preferring rats [29]. In contrast to the present results and the findings reviewed above, some studies have reported that the rewarding effects of morphine are completely abolished in mice lacking the $A_{2A}R$ although the acute effects of morphine administration on locomotion were similar to those in wild-type mice [30]. It has also been reported that daily amphetamine administration does not induce locomotor sensitization in $A_{2A}R$-knockout mice [31]. The reason for this discrepancy is not clear; it may be related to drug type, dosage regimen, behavioral model or compensatory changes in knockout animals, such as $A_{2A}R$ reorganization or a lack of neuroanatomical specificity of $A_{2A}R$ knockout in the neural circuits regulating these behaviors [5,23].

The mechanism underlying the observed effects of CGS on METH-induced locomotor sensitization may involve $A_{2A}R$ modulation of DAergic neurotransmission in the nAcc. Previously published data suggested that DA D1 and D2 receptors are both required during the acquisition of METH-induced locomotor sensitization but with different underlying mechanisms [32]. Although both types are necessary, D2 receptors are more involved than D1 receptors in METH-induced locomotor sensitization [33]. D1 and D2 receptors are differentially expressed in distinct subpopulations of nAcc gamma-aminobutyric acid (GABA)ergic medium spiny neurons: D1 receptors are found mainly on neurons expressing substance P and dynorphin, while D2 receptors are mostly on neurons expressing enkephalin and neurotensin [34]. On the other hand, $A_{2A}Rs$ and D2 receptors are highly expressed and colocalize in dorsal striatum and nAcc neurons [35,36]. More specifically, $A_{2A}Rs$ are found in the dendritic spines of GABAergic enkephalinergic neurons [37], where they form heterodimers with D2 receptors to inhibit DA D2 receptor signaling. Thus, $A_{2A}R$ stimulation decreases DA binding at D2 receptors [38]. In support of this hypothesis, systemic administration of the $A_{2A}R$ agonist CGS inhibits the initiation of cocaine self-administration [22], whereas the $A_{2A}R$ antagonist MSX-3 enhances locomotor responses to cocaine [5]. The results of the present study provide further indirect evidence that $A_{2A}Rs$ could regulate the effects of CGS on METH-induced locomotor sensitization through inhibitory modulation of D2 receptors.

We found that the mGluR5 antagonist MPEP decreased METH-induced locomotor sensitization. These results are consistent with those of previous studies demonstrating that MPEP attenuates psychostimulant-related behaviors. For example, MPEP can abolish the acute locomotor-stimulant effects of cocaine and amphetamine in mice [18] and rats [17]. The contribution of mGluR5s to psychostimulant locomotor effects was confirmed by demonstrating a lack of motor effects in mGluR5 knockout mice [16]. Furthermore, MPEP attenuates drug-related behaviors associated with drug addiction; MPEP pretreatment reduces the behavioral effects of nicotine [14], alcohol [39], and morphine [40]. Conversely, one study reported that MPEP administration did not affect the development of amphetamine-induced locomotor sensitization in mice [41]. The mechanism underlying the behavioral effects of MPEP on drug abuse-related behaviors could involve the modulation of mesolimbic dopaminergic neurotransmission by mGluR5s. Several lines of evidence support this hypothesis. First, most abused drugs increase extracellular DA concentrations in the nAcc [42], which receives DAergic input from the VTA [43] and glutamatergic inputs from the prefrontal cortex (PFC) [44] hippocampus [45], and amygdala [46]. Second, mGluR5 is highly expressed in the nAcc [47,48,49]. Specifically, the receptors are expressed postsynaptically and modulate the activity of medium spiny GABAergic

output neurons [50]. Thus, it can be speculated that MPEP counteracts glutamatergic activation of DAergic neurons by blocking mGluR5s on medium spiny GABAergic projection neurons. Indeed, several studies have reported the effects of MPEP on drug abuse-related behaviors (see Section 1).

The major finding of this study was the additive effects of coadministration of CGS and a low dose of MPEP on the development and expression of METH-induced locomotor sensitization. As the CGS dose was increased, the effects of MPEP on locomotor activity were larger, suggesting that the observed additive effects may be related to the combination of $A_{2A}R$ activation and mGluR5 blockade. These results are consistent with the findings that $A_{2A}R$ stimulation and mGluR5 antagonism separately reduced drug abuse-related behavior (see Section 1). The additive interaction between $A_{2A}Rs$ and mGluR5s could be attributed to several conditions. First, the mesolimbic DA system, particularly the projection from the VTA to the nAcc, is an important brain region involved in the locomotion, reinforcement, and reward effects of psychostimulants such as cocaine, amphetamine, and METH [51,52,53]. This system also plays an important role in both the development and expression of psychostimulant-induced locomotor sensitization [54]. Second, it has been reported that the nAcc is rich in both mGluR5s [48] and $A_{2A}Rs$ [55] localized on the dendritic spines of the GABAergic medium spiny neurons that also express encephalin (see [56]) and D2 receptors [57]. Third, the nAcc receives DAergic input from the VTA [43] and glutamatergic inputs from the PFC [44], hippocampus [45], and amygdala [46]; these inputs come in close opposition to each other and also form synaptic contacts in GABAergic medium spiny neurons [58,59]. In addition, it has been also suggested that glutamatergic and DAergic inputs make synaptic contacts on the heads and necks of dendritic spines, respectively [56,60]. These conditions would provide an anatomical basis for functional interactions between mGluR5s and $A_{2A}Rs$ and explain their effects on DA neurotransmission in the nAcc. There is a strong antagonistic interaction between $A_{2A}Rs$ and D2 receptors in the GABAergic medium spiny neurons in the nAcc [61], and $A_{2A}R$ activation can offset excessive D2 receptor stimulation due to repeated psychostimulant treatment, thus reducing METH responses. These effects could have increased the effects of a low dose of MPEP on the development and expression of METH-induced locomotor sensitization.

It should be noted that the administration of CGS or MPEP, alone or in combination, produced a greater reduction in locomotor activity during the challenge test of the expression of the METH-induced locomotor sensitization (see Figure 5) than during the challenge test of development of the METH-induced locomotor sensitization (see Figure 3). This might be due to the fact that acute effects of the CGS and MPEP were larger and the repeated treatment with these drugs produced a reduction of their effects. The previous idea could represent a tolerance effect. However, there is no evidence of the development of tolerance to central effects of CGS or MPEP. Another possibility is that the procedure used during the challenge tests of the development and the expression of METH-induced locomotor sensitization was different: while in the challenge test of the development of METH-induced locomotor sensitization METH was administered after five sessions of CGS and/or MPEP administrations with METH, yet in the challenge test of the expression of METH-induced locomotor sensitization, CGS and/or MPEP were administrated after only five sessions of METH administrations. These procedural differences could explain the results of this study.

In contrast to the present results and the some findings reviewed above, Wright et al. [62] reported that the combined administration of subthreshold doses of $A_{2A}R$ antagonist SCH-58261 and mGluR5 antagonist MTEP decreased METH-induced hyperactivity and METH-induced conditioned place preference. It is noteworthy to mention that, although we reported a reduction of METH-induced locomotor sensitization and the Wright's study also reported a prevention of METH-induced hyperactivity, there are some differences between both studies. In the present study, the $A_{2A}R$ agonist CGS-21680 was used instead of $A_{2A}R$ antagonist SCH-58261, the doses of the drugs were different, we used rats instead of mice, and the locomotor sensitization was used as a behavioral model instead of drug-induced hyperactivity. Due to these differences, more research is needed before coming to a final conclusion regarding the interaction between A2A and mGluR5 receptors.

In conclusion, our findings suggest that the $A_{2A}R$ agonist CGS facilitate the effects of the mGluR5 antagonist MPEP on the development and expression of METH-induced locomotor sensitization. The compounds exerted an additive effect on METH-induced behavioral responses, indicating the potential utility of adenosine $A_{2A}Rs$ and mGluR5 ligands to treat psychostimulant addiction.

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Conflict of interest The authors declare that they have no conflict of interest.

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