



## Comparison of single *versus* repeated methamphetamine injection induced behavioral sensitization in mice



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### HIGHLIGHTS

- Single METH injection produced similar magnitude sensitization as repeated injection.
- Sensitized locomotion peaked 8 days after a single METH injection.
- Single METH-induced sensitization lasted at least 21 days.

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### ABSTRACT

Repeated exposure to drugs of abuse produces a persistent behavioral sensitization to stimulants, which is often used to study drug-associated behavioral plasticity. Interestingly, even a single exposure to some drugs of abuse is sufficient to elicit long-lasting behavioral sensitization. However, few studies have directly compared the magnitude of sensitization between single *versus* repeated drug treatments. This study examined the magnitude and duration of single methamphetamine (METH) injection-induced behavioral sensitization and compared it to the more typical repeated drug injection-induced sensitization in mice. Different groups of mice were injected with METH (0.5, 1.0, 2.0 mg/kg, i.p.) only once or daily for 7 consecutive days. A challenge dose of METH (1.0 mg/kg, i.p.) was tested 7 days later. The time-course of a single METH injection-induced behavioral sensitization was assessed where METH (2.0 mg/kg, i.p.) was injected and a challenge dose of METH (1.0 mg/kg, i.p.) was tested after different drug-free periods. Single METH injection produced similar magnitude of behavioral sensitization as compared to repeated injection. Such a sensitized locomotor response peaked 8 days after METH injection and lasted for at least 21 days. This long lasting behavioral alteration induced by single METH injection suggests the value of future studies to explore the underlying neural mechanisms, particularly in comparison to those underlying repeated METH-induced sensitization.

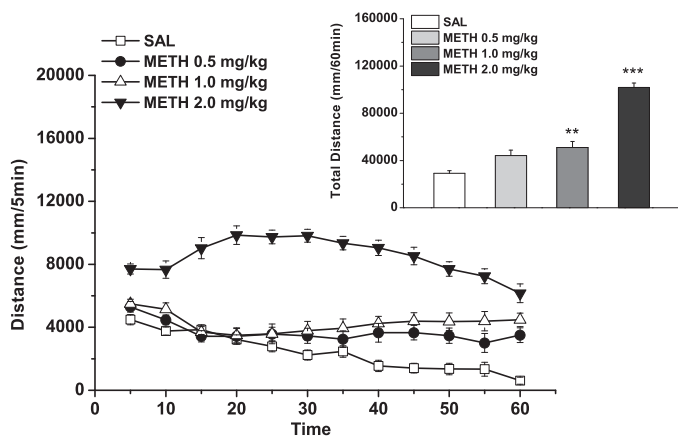
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Methamphetamine (METH) is a highly abused central nervous system stimulant with high reinforcing properties [16], and its prolonged use results in dependence and psychosis [12]. Repeated administration of METH leads to a progressive increase in drug response on re-exposure to the drug. Behavioral sensitization, the main characteristics of which are progressively intensifying, persistent and stimulant-inducible response during re-exposure,

is thought to play a key role in certain aspects of drug addiction such as compulsive drug-seeking behavior [2,17,19]. Thus, behavioral sensitization in rodents is widely used as a model for the study of behavioral plasticity associated with repeated drug treatment [4,17]. The degree of drug-induced behavioral sensitization depends on various factors such as dose, dosing regimen and environmental context. For instance, repeated drug exposure with long intervals is more effective to induce sensitization as compared to chronic exposure to either high and/or escalating dosage with short intervals [17,23]. Interestingly, even a single exposure to cocaine [5,7], amphetamine [18,22] and morphine [6,10,21] can produce long-lasting behavioral and neurochemical sensitization.

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**Fig. 1.** Dose–effect relationship of single METH injection-induced hyperactivity in mice. Mice were injected with saline or METH (0.5, 1.0, 2.0 mg/kg, i.p.) and then placed individually in the test chambers to record the locomotion for 60 min. Data are expressed as mean  $\pm$  S.E.M. \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs. saline group.  $n = 11$ –12 per group.

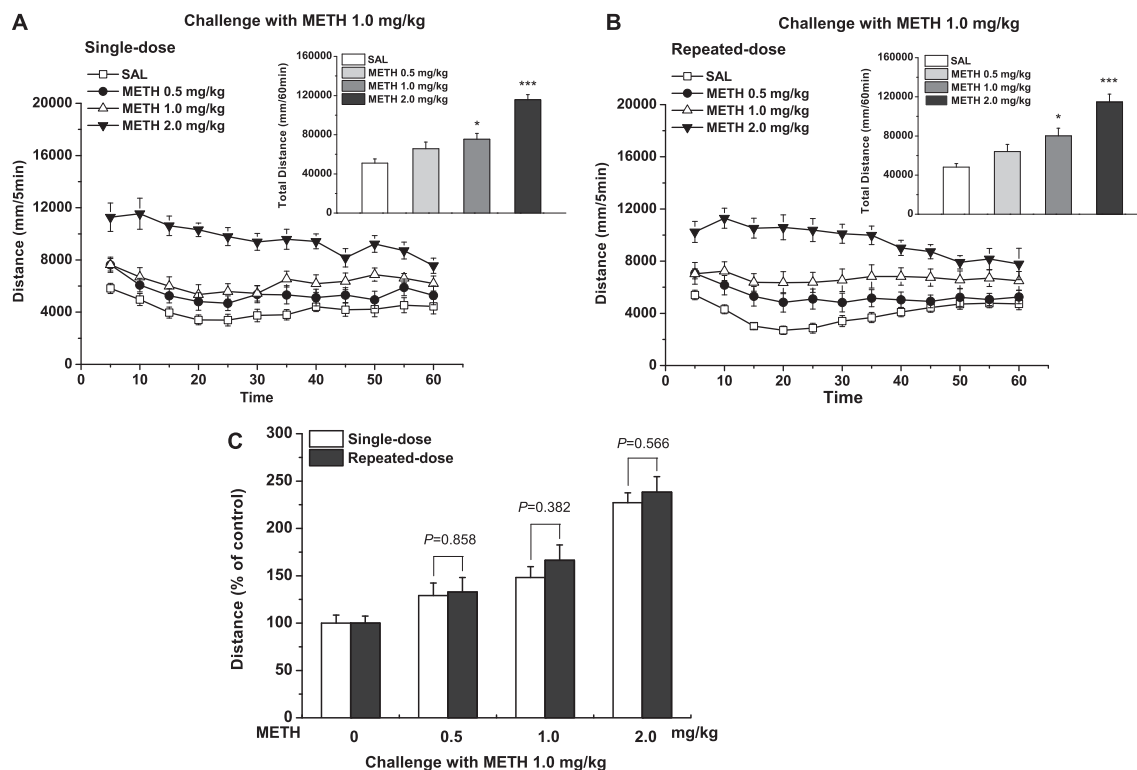
Although the majority of the literature utilizes repeated treatment regimen to induce behavioral sensitization, few studies have systematically compared the magnitude of sensitization induced by single or repeated drug treatment. This study attempted to address this issue by directly comparing two treatment regimens for their ability to induce behavioral sensitization.

Male C57BL/6J mice (initial body weight 18–20 g) were obtained from the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences and housed (5–6 per cage) in temperature- and humidity-controlled ( $22 \pm 1^\circ\text{C}$  and  $50 \pm 10\%$ ) environment with 12/12 h light/dark cycle (lights on at 08:00 AM) and free access to

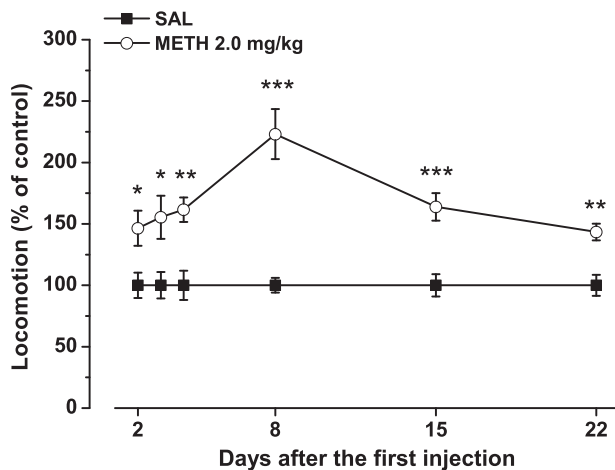
food and water. All mice were habituated to the housing conditions for 1 week before experiments. During test days, the animals were kept in the test room at least 30 min before recording their locomotor activity. All experiments were conducted in Dr. Jian-Hui Liang's lab in National Institute on Drug Dependence (China), according to the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996) and were approved by the Peking University Animal Care and Use Committee.

Locomotor activity was measured in four identical chambers ( $25\text{ cm} \times 25\text{ cm} \times 45\text{ cm}$ ) situated in a sound-attenuating cabinet and the total distance of horizontal locomotor activity was recorded with a video camera placed above the chamber and analyzed with the DigBehv software (DigBehv-LG Ver 2.0, Shanghai Jiliang Software Technology Co. Ltd., Shanghai, China). METH-HCl was provided by National Institute on Drug Dependence (China). The drug was dissolved in 0.9% saline and administered intraperitoneally in a volume of 10 ml/kg.

For acute studies, mice were injected with saline or METH (0.5, 1.0, 2.0 mg/kg, i.p.) and then immediately put into test chambers individually to monitor the locomotor activity for 60 min (Fig. 1). For studies that compared acute or repeated (7 daily injection) METH injection-induced sensitization, mice were injected with saline or METH (0.5, 1.0, 2.0 mg/kg, i.p.) only once or daily for 7 consecutive days, but the locomotion was only monitored on Day 1 or both Day 1 and Day 7 (data not shown). All mice were re-tested one week later after a challenge dose of 1.0 mg/kg METH (Fig. 2A and B). For studies that examined the duration of single METH injection-induced behavioral sensitization, mice were injected with saline or METH (2.0 mg/kg, i.p.) and then left for 60 min in the test chambers on Day 1 (data not shown). After various drug-free periods (housed in the home cages with no treatment or test), mice received the challenge injections of METH (1.0 mg/kg, i.p.) on Day 2, 3, 4, 8, 15, 22 and the locomotor activity was recorded for 60 min (Fig. 3).



**Fig. 2.** Locomotor response to 1 mg/kg METH challenge in mice injected with a single or repeated dose of METH. (A) Single-dose METH injection-induced behavioral sensitization; (B) repeated-dose METH injection-induced behavioral sensitization; (C) comparison of the locomotor sensitization induced by single and repeated dose injection of METH. The data were normalized to the corresponding saline group (SAL + METH 1.0 mg/kg, taken as 100%) and were expressed as mean  $\pm$  S.E.M.  $n = 11$ –12 per group. See Fig. 1 for other details.



**Fig. 3.** Time-course of the locomotor sensitization to a single METH injection in mice. Different groups of mice ( $n = 10$  per group) were injected with saline or METH (2.0 mg/kg, i.p.) on Day 1 and then tested with 1.0 mg/kg METH injection on Days 2, 3, 4, 8, 15, and 22. See Figs. 1 and 2 for other details.

The time course data of locomotor activity were analyzed by two-way repeated measures ANOVA with the between-subjects factors of treatment and the within-subjects factors of time. The cumulative locomotion data were analyzed by one-way ANOVA followed by *post hoc* Bonferroni's test. In Figs. 2C and 3, METH-induced sensitization was calculated as a percentage of the saline-injected control mice (100%) and comparisons between two treatments were analyzed by independent-sample *t*-test. Results are expressed as mean  $\pm$  S.E.M. and  $P < 0.05$  was considered statistically significant.

As shown in Fig. 1, acute METH injection produced a dose-dependent hyperactivity in mice. Two-way repeated measures ANOVA revealed significant main effects of treatment, time and treatment  $\times$  time interaction ( $F_{(\text{treatment})(3,43)} = 57.058$ ,  $P < 0.001$ ;  $F_{(\text{time})(11,473)} = 12.399$ ,  $P < 0.001$ ;  $F_{(\text{time} \times \text{treatment})(33,473)} = 7.654$ ,  $P < 0.001$ ). One-way ANOVA and *post hoc* Bonferroni's test demonstrated that METH (0.5, 1.0, 2.0 mg/kg i.p.) induced a dose-dependent hyperlocomotion ( $F_{(\text{treatment})(3,43)} = 57.058$ ,  $P < 0.001$ ) with 1.0 and 2.0 mg/kg reaching statistical significance.

As shown in Fig. 2A and B, both single and repeated METH injection induced a dose-dependent and significant behavioral sensitization. Two way ANOVA revealed a significant main effect of METH treatment under both conditions [Fig. 2A:  $F_{(\text{treatment})(3,41)} = 24.969$ ,  $P < 0.001$  and Fig. 2B:  $F_{(\text{treatment})(3,43)} = 16.645$ ,  $P < 0.001$ ]. One-way ANOVA and *post hoc* Bonferroni's test was used to further analyze the cumulative locomotion data (insets) and it was found that both single and repeated 1.0 or 2.0 ( $P < 0.05$ ,  $P < 0.001$ , respectively) but not 0.5 mg/kg METH injection led to significant behavioral sensitization. A further look at the cumulative data by normalizing the data as the percentage of locomotion in saline-injected mice revealed no significant difference between the single *versus* repeated METH injected groups across all doses as analyzed by independent *t*-test ( $P > 0.05$  for all three dose conditions).

Repeated stimulant exposure induced sensitization is well known to be long lasting. The sustained hyperactive response could last several months to even more than one year in rats. To further determine the duration of the sensitization effects after single METH injection, different groups of mice were injected with single dose of METH (2.0 mg/kg, i.p.), and then challenged with a small dose of METH (1.0 mg/kg, i.p.) after varying off days. As shown in Fig. 3, 1.0 mg/kg METH challenge displayed a significant behavioral sensitization at all time points. When compared to saline group (no prior 2 mg/kg METH injection history but only received 1 mg/kg

METH on test day), independent-sample *t*-test revealed that the locomotion response to 1 mg/kg METH challenge was significantly increased by 46.4% 1 day (Day 2:  $t = -2.634$ ,  $P = 0.017$ ), 55.4% 2 days (Day 3:  $t = -2.688$ ,  $P = 0.017$ ), 61.6% 3 days (Day 4:  $t = -3.956$ ,  $P = 0.001$ ), 123.1% 7 days (Day 8:  $t = -5.739$ ,  $P = 0.000$ ), 63.9% 14 days (Day 15:  $t = -4.424$ ,  $P = 0.000$ ) and 43.4% 21 days (Day 22:  $t = -3.973$ ,  $P = 0.000$ ) after single METH injection. The sensitized hyperactive response to METH progressively increased during the first week, reaching the maximum on day 8. This sensitization effect lasted for at least 21 days.

The primary findings of the current study was that single injection to METH produced a highly significant behavioral sensitization effect that was comparable to the more traditionally used repeated (7 daily) treatment regimen and the effect was also long lasting. Although long-term behavioral sensitization induced by a single psychostimulant exposure has previously been reported [14], the present study represents the first systematic evidence that a single dose of METH pre-injection regimen produces essentially the same magnitude of sensitization as repeated-injection in the same strain of mice under the same conditions.

It is well known that the development and expression of behavioral sensitization depend on the nature of the pretreatment regimens, including doses of drugs, the duration of drug administration, and the interval after pretreatment [13,19]. Abundant evidence suggest that increasing the dose and/or duration of drugs of abuse can facilitate the induction of long-lasting behavioral sensitization [14,15]. The present study suggests that the dosage seems to play a more prominent role than the frequency of drug treatment for determining the magnitude of METH-induced behavioral sensitization. Regardless of whether the mice were injected once or daily for 7 days, a smaller dose of METH (0.5 mg/kg) did not produce significant behavioral sensitization. However, 1 mg/kg compared to 0.5 mg/kg METH produced significant and comparable behavioral sensitization in mice that received either single or repeated-injection. Not surprisingly, when the METH dose was further increased to 2 mg/kg, a higher sensitization was observed under both conditions. Previously, it has also been suggested that the magnitude of sensitization was linearly related to the pretreatment dose in both single [10] or repeated treatment schedules [1] for morphine-induced behavioral sensitization. Kitanaka et al. explored the functional alterations of monoaminergic neuronal systems in mice after single and repeated injection of METH, which showed that the degree and direction of modification of the monoaminergic metabolism depend on the treatment (single or repeated) and on the brain regions [8]. Based on results of the present study, we presented a hypothesis that a threshold dose of METH exists that is independent of the frequency of drug injection for inducing behavioral sensitization. When such a threshold is reached, drug-induced neurobehavioral plasticity will lead to a phenotype of sensitization, while the frequency of drug injection is less critical.

Previous studies showed that the conditioned locomotion, or context-dependent sensitization, is induced by a stimulus of the drug injection under the particular environment associated with drug experience [3,8]. In the present study, on the challenge days of both single and repeated METH regimens, the locomotion of METH pretreated mice in the first 5-min was higher than those of saline pretreated, even if they all received the same dose of METH 1.0 mg/kg (Fig. 2A and B), which was consistent with the previous study [8]. This initial (*i.e.*, within first 5 min) hyperlocomotor response to the challenge injection might be a consequence of context-dependent sensitization or the contribution of the drug history but not the presence of the drug itself.

Behavioral sensitization is thought to be the phenotypic demonstration of drug-induced neural adaptation [12]. Incubation or transfer, a period that is thought to be critical from the

development to the full expression of sensitization, has been implicated in the process of sensitization [9,11,21]. Previous studies have shown that the maximal effects of behavioral sensitization are not seen immediately but some time (days) after the last exposure to the drug [20,22]. In the present study, we examined the effects of a wide range of time intervals after a single injection to METH (2.0 mg/kg) on the intensity of behavioral sensitization in mice. It was found that 24 h after a single METH injection is sufficient to induce an apparent behavioral sensitization. More importantly, debating the idea that the longer the withdrawal period the greater the degree of behavioral sensitization [14], we found the intensity of sensitization was enhanced over time during the first week, peaked 8 days after METH injection, and then the effect gradually decreased, but lasted for at least 21 days, clearly showing a time-dependent sensitization.

Together, the current study significantly extended previous findings by showing that single injection to METH can lead to significant sensitization to a magnitude that is strikingly similar to repeated drug injection. It has already been shown that single exposure to amphetamine also leads to marked behavioral and neurochemical sensitization [11]. Given that our findings suggest a similar behavioral phenotype due to single or repeated drug injection, it waits to be seen whether (or not) similar molecular mechanisms underlie the apparently similar behavioral changes due to METH injection. By examining single versus repeated drug administration in parallel can offer a fresh avenue to better understand the neurobehavioral consequences of exposure to drugs of abuse.

### Conflicts of interest

None.

### Authors contribution

Li Jing was responsible for the overall design, the statistical analysis and execution of the study. Jian-Hui Liang contributed to the experimental design, the statistical analysis and the manuscript preparation. Jun-Xu Li participated in manuscript development. Min Zhang, Ping Huang, Qing Liu, Yu-Ling Li and Hui Liang assisted with revision of the manuscript. All authors critically reviewed the content of the manuscript and approved its final version for publication.

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