ORIGINAL ARTICLE

Absence of methamphetamine-induced conditioned place preference in weaver mutant mice

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Funding information
Japan Agency for Medical Research and Development, Grant/Award Number: JP18mk0101076, JP19dk0307071 and JP19ek0610011; Japan Society for the Promotion of Science, Grant/Award Number: 17K08612, 16H06276 and 16K15565; Smoking Research Foundation

Abstract

Aims: G protein-activated inwardly rectifying potassium (GIRK) channels are related to rewarding effects of addictive drugs. The GIRK2 subunit is thought to play key roles in the reward system. Weaver mutant mice exhibit abnormal GIRK2 function and different behaviors that are caused by several addictive substances compared with wild-type mice. However, mechanisms of reward-related alterations in weaver mutant mice remain unclear. The present study investigated changes in the rewarding effects of methamphetamine (METH) in weaver mutant mice.

Methods: The rewarding effects of METH (4.0 mg/kg) were investigated using the conditioned place preference (CPP) paradigm. Extracellular dopamine level in the nucleus accumbens (NAc) was measured by in vivo microdialysis. To identify brain regions that were associated with these changes in rewarding effects, METH-induced alterations of Fos expression were investigated by immunohistochemical analysis.

Results: Weaver mutant mice exhibited a significant decrease in METH-induced CPP and dopamine release in the NAc. Methamphetamine significantly increased Fos expression in the posterior NAc (pNAc) shell in wild-type but not in weaver mutant mice.

Conclusions: Methamphetamine did not induce rewarding effects in weaver mutant mice. The pNAc shell exhibited a significant difference in neuronal activity between wild-type and weaver mutant mice. The present results suggest that the absence of METH-induced CPP in weaver mutant mice is probably related to an innate reduction of dopamine and decreased neural activity in the pNAc shell that is partially attributable to the change of GIRK channel function. GIRK channels, especially those containing the GIRK2 subunit, appear to be involved in METH dependence.

KEYWORDS
CPP, GIRK, methamphetamine, reward, weaver mutant mice

1 | INTRODUCTION

The substance addiction such as drug, alcohol, and nicotine, and the behavioral addiction such as gambling and Internet are caused by neurochemical changes within the brain.1,2 Although various brain regions and biomolecules have been reported to relate to the pathogenesis of addiction, it has not led to the development of effective addiction treatments. G protein-activated inwardly rectifying...
potassium (GIRK) channels are reportedly related to acute rewarding effects or adaptations that occur with chronic exposure to various addictive drugs, such as opioids, \( \gamma \)-hydroxybutyrate (a designer club drug), cannabinoids, and alcohol. They are activated by ligand-stimulated \( G_{\text{bg}} \)-linked G protein-coupled receptors, which include opioid receptors, cannabinoid CB\(_1\) receptors, serotonin 5-hydroxytryptamine-1A (5HT\(_{1A}\)) receptors, M2 muscarinic receptors, \( \gamma \)-aminobutyric acid-B (GABA\(_{\beta}\)) receptors, dopamine D\(_2\) receptors, and adenosine A\(_1\) receptors. In mammals, GIRK channels are expressed as four subunits (GIRK1-GIRK4). GIRK1, GIRK2, and GIRK3 subunits are distributed throughout the central nervous system, including in key regions that are involved in cognition, learning, memory, emotion, and motor function. The GIRK2 subunit plays a role in regulating addictive substance-induced behaviors. GIRK2 knockout mice exhibit alterations of behavioral effects of alcohol, including an increase in alcohol consumption, a decrease in alcohol dependence, the stimulation of motor activity, and a decrease in the anxiolytic action of alcohol. Moreover, cocaine-induced locomotor sensitization is enhanced in GIRK2 knockout mice, and intravenous cocaine self-administration is dramatically reduced.

Weaver mutant mice have a spontaneously occurring autosomal recessive mutation of the \( \text{Girk2} \) gene that is mapped to chromosome 16. The single G-to-A transition in the \( \text{Girk2} \) gene replaces glycine with serine in the pore-forming region of the GIRK2 subunit. This mutation leads to a reduction of GIRK2 function and causes abnormalities of dopamine signaling. Alcohol- and opioid-induced analgesia are reportedly reduced in weaver mutant mice. Furthermore, amphetamine caused less hyperlocomotion in weaver mutant mice, although direct dopamine receptor agonism, such as with apomorphine, increases motor activity. However, only few studies have investigated the rewarding effects of addictive substances in weaver mutant mice or the association between GIRK2 subunit functions and methamphetamine (METH) dependence. Therefore, the present study investigated changes in the rewarding effects of METH using weaver mutant mice.

## 2 | METHODS

### 2.1 | Animals

Heterozygous/heterozygous matings of weaver mutant mice on a C3H/HeJ JC1 background were used to produce wild-type, heterozygous, and homozygous weaver mutant mice. The mice were 16-20 weeks of age at the time of the experiments. The mice were housed 4-6 per cage in an environment at 23 ± 1°C and 50 ± 5% humidity with free access to food and water under a 12-h/12-h light/dark cycle.

### 2.2 | Drugs

Methamphetamine hydrochloride (Dainippon Sumitomo Pharma) was dissolved in saline and administered intraperitoneally (i.p.) in a volume of 10 mL/kg body weight. All experiments were conducted with the METH dose at 4 mg/kg that reportedly induces not abnormal behaviors, but place preference in wild-type mice. Methamphetamine was freshly prepared for all of the experiments.

## 2.3 | Conditioned place preference test

The conditioned place preference (CPP) test was performed using a two-compartment Plexiglas chamber. One compartment (175 mm width \( \times \) 150 mm length \( \times \) 175 mm height) was black with a smooth floor. The other compartment had the same dimensions but was white with a textured floor. For the preconditioning, postconditioning, extinction, and priming phases, a T-style division with double 60 mm \( \times \) 60 mm openings allowed the mice to access both compartments. During the conditioning phases, the openings were eliminated to restrict the mice to one of the compartments. Locomotion and the time spent in each compartment were recorded using an animal activity monitoring apparatus that was equipped with an infrared detector (Neuroscience). The CPP apparatus was placed in a sound-attenuated, light-controlled box (~3100 lux). Conditioned place preference was assessed in five phases (preconditioning, conditioning, postconditioning, extinction, and priming). On days 1 and 2, the mice were allowed to freely explore both compartments through the openings for 900 seconds and acclimate to the apparatus. On day 3 (preconditioning phase), the same trial was performed, and the time spent in each compartment was measured for 900 seconds. We selected a counterbalanced protocol to nullify each mouse’s initial preference as discussed previously. Biased mice that spent more than 80% of the time (ie, 720 seconds) on one side on day 3 or more than 600 seconds on one side on day 2 and more than 600 seconds on the other side on day 3 were excluded from further experiments. Conditioning was conducted once daily for 4 consecutive days (days 4-7). The mice were injected with either METH (4.0 mg/kg, i.p.) or saline and immediately confined to the black or white compartment for 50 minutes on day 4. On day 5, the mice were injected with alternate saline or METH injections and immediately confined to the opposite compartment for 50 minutes. On days 6 and 7, the same conditioning as on days 4 and 5 was repeated. The assignment of the mice to the conditioned compartment was performed randomly and counterbalanced across subjects. During the postconditioning phase on day 8, the time spent in each compartment was measured for 900 seconds without drug injection. After 28 days of the postconditioning phase (day 36), to confirm the extinction of CPP, the part of experimented mice was allowed to freely explore the two compartments through the openings for 900 seconds (extinction phase). The next day (day 37), 5 minutes after a low-dose METH priming injection (0.5 mg/kg, i.p.), the mice were free to explore the compartments for 900 seconds, and the time spent in each compartment was measured (priming phase). The CPP score and priming score were designated as the time spent in the drug-paired compartment on day 8 or day 37 minus the time spent in the same compartment in the preconditioning phase on day 3.
2.4 | Surgical implantation of microdialysis probes

The mice were stereotaxically implanted with microdialysis probes under sodium pentobarbital anesthesia (50 mg/kg, i.p.) in the nucleus accumbens (NAc; anterior, +0.8 to 1.54 mm; lateral, +1.0 mm; ventral, −5.0 mm from bregma) according to a mouse brain atlas.\(^{16}\) The probe tips were constructed with regenerated cellulose membranes that provided a 50 kDa molecular weight cutoff, 0.22 mm outer diameter, and 2 mm membrane length (Eicom). The dialysis probe placements were verified histologically at the end of the experiment. Data were excluded if the membrane portions of the dialysis probes were outside the NAc region.

2.5 | Microdialysis and analytical procedure

Twenty-four hours after implantation, the dialysis experiments were performed in freely moving mice. Ringer’s solution (145 mmol/L Na\(^+\), 3 mmol/L K\(^+\), 1.26 mmol/L Ca\(^{2+}\), 1 mmol/L Mg\(^{2+}\), and 152.5 mmol/L Cl\(^-\), pH 6.5) was perfused at 1 µL/min for 180 minutes. Baselines of extracellular dopamine concentrations were obtained from average concentrations of three consecutive samples when they were stable. Perfusates were directly injected into the high-performance liquid chromatography system every 10 minutes using an autoinjector (EAS-20; Eicom). Dialysate dopamine was separated using a PP-ODS reverse-phase column (Eicom) and ECD-100 graphite electrode detector (HTEC-500; Eicom). The mobile phase consisted of 0.1 mol/L phosphate buffer (pH 5.5) that contained sodium decanesulfonate (500 mg/L), ethylenediaminetetraacetic acid (50 mg/L), and 1% methanol. The dopamine detection limit was 0.3 fmol/sample, with a signal-to-noise ratio of at least 2.

2.6 | Fos immunohistochemistry

The mice were injected with either METH (4.0 mg/kg, i.p.) or saline and immediately confined to the black or white compartment of the CPP apparatus for 50 minutes. Two hours after the drug injection (reached peak Fos protein expression), the mice were deeply anesthetized with an overdose of sodium pentobarbital and transcardially perfused with 50 mL phosphate-buffered saline (PBS), followed by ice-cold 4% paraformaldehyde in 0.1 mol/L phosphate buffer (PB; pH 7.4). The brains were quickly removed from the skull and postfixed overnight in the same fixative solution. After fixation, the brains were cryoprotected in 20% sucrose in 0.1 mol/L phosphate buffer (pH 5.5) was perfused at 1 µL/min for 28 days of the postconditioning phase and extinction phase after 28 days of the postconditioning phase (preconditioning: 442.6 ± 35.2 seconds; postconditioning: 562.2 ± 27.8 seconds; P = .004, paired t test; Figure 1A). No significant difference was found between the postconditioning phase and extinction phase after 28 days of the postconditioning phase (preconditioning: 442.6 ± 35.2 seconds; extinction: 504.2 ± 31.4 seconds; P = .21, paired t test; Figure 1A).

3 | RESULTS

3.1 | Loss of rewarding effects of METH in weaver mutant mice

To assess the relationship between functional changes in GIRK2 and the rewarding effects of METH, we conducted the METH-induced CPP test in wild-type mice, heterozygous weaver mutant mice, and homozygous weaver mutant mice. Methamphetamine significantly increased the time spent in the previously drug-paired compartment in wild-type mice (preconditioning: 442.6 ± 35.2 seconds; postconditioning: 562.2 ± 27.8 seconds; P = .004, paired t test; Figure 1A). No significant difference was found between the preconditioning phase and extinction phase after 28 days of the postconditioning phase (preconditioning: 442.6 ± 35.2 seconds; extinction: 504.2 ± 31.4 seconds; P = .21, paired t test; Figure 1A). On the following day, a significant increase in the time spent in the previously drug-paired compartment was found in wild-type mice between
the priming phase and preconditioning phase (preconditioning: 442.6 ± 35.2 seconds; priming: 638.9 ± 35.4 seconds; P < .001, paired t test; Figure 1A). Heterozygous weaver mutant mice exhibited a trend toward an increase in the time spent in the drug-paired compartment in the postconditioning phase compared with the preconditioning phase (preconditioning: 454.3 ± 36.4 seconds; postconditioning: 539.7 ± 48.9 seconds; P = .15, paired t test; Figure 1A) and a significant increase in the priming phase compared with the preconditioning phase (preconditioning: 454.3 ± 36.4 seconds; priming: 560.5 ± 62.4 seconds; P = .034, paired t test; Figure 1A). No significant difference in METH-induced CPP was observed in any of the tests in homozygous weaver mutant mice (preconditioning: 455.7 ± 26.0 seconds; postconditioning: 416.6 ± 49.8 seconds; priming: 484.5 ± 59.0 seconds; Figure 1A). The one-way ANOVA of CPP scores showed significant differences between homozygous weaver mutant mice and wild-type mice (F_{2,30} = 3.81, P = .034). Post hoc comparisons indicated that METH-induced CPP scores were significantly higher in wild-type mice than in homozygous weaver mutant mice (P = .040, Sidak’s multiple-comparison post hoc test; Figure 1B). Similarly, the one-way ANOVA of priming scores indicated significant differences between homozygous weaver mutant mice and wild-type mice (F_{2,26} = 3.58, P = .042). The post hoc comparisons indicated a significant difference between homozygous weaver mutant mice and wild-type mice (P = .041, Sidak’s multiple-comparison post hoc test; Figure 1C).

3.2 Methamphetamine-induced increase in dopamine release in the NAc in weaver mutant mice decreased compared with wild-type mice

We next examined the effects of METH on dopamine release in the NAc in wild-type mice and homozygous weaver mutant mice using in vivo microdialysis. Baseline extracellular dopamine levels in the NAc were significantly different between wild-type and weaver mutant mice (P = .018, Student’s t test). Mean baseline dopamine levels in the NAc were 0.187 ± 0.030 ng/10 μL in wild-type mice (n = 19) and 0.091 ± 0.017 ng/10 μL in homozygous weaver mutant mice (n = 14). Methamphetamine significantly increased dopamine release in the NAc in wild-type mice (one-way ANOVA: F_{1,19;15.92} = 23.25, P < .001; Figure 2A). Although weaver mutant mice exhibited a METH-induced increase in dopamine release, the extent of the increase was milder than in wild-type mice (one-way ANOVA, F_{2,05;16.36} = 15.62, P < .001; Figure 2A). The one-way ANOVA of the temporal course of the effects of METH on changes in dopamine release during a 180-minute interval after the injection, reflected by a cumulative response (ie, area under the curve [AUC]), showed significant differences in dopamine release in the NAc between groups (F_{2,25} = 24.94, P < .001). The post hoc comparisons indicated that METH significantly increased dopamine release in both homozygous weaver mutant mice and wild-type mice compared with saline treatment (wild-type mice, P < .001; homozygous weaver mutant mice, P = .002, Sidak’s multiple-comparison post hoc test). The extent of the increase in dopamine levels in METH-treated homozygous weaver mutant mice was significantly lower than in METH-treated wild-type mice (P = .047, Sidak’s multiple-comparison post hoc test; Figure 2B).

3.3 Methamphetamine-induced Fos expression in the pNAc shell was suppressed in weaver mutant mice

To evaluate the possible relationship between neuronal activation and our in vivo results, we analyzed Fos expression in the aNAc core, aNAc shell, pNAc core, pNAc shell, and VTA using immunohistochemistry. Representative photomicrographs of Fos-positive nuclei
the number of Fos-positive nuclei in the pNAc shell (drug interaction, (drug drug, tWEEN the METH-treated and saline-treated groups. hoc treated wild-type mice (P in wild-type mice (P < .001, Sidak’s multiple-comparison post hoc test). Methamphetamine-induced Fos expression was significantly lower in homozygous weaver mutant mice compared with METH-treated wild-type mice (P < .001, Sidak’s multiple-comparison post hoc test). No differences were found in the other brain regions between the METH-treated and saline-treated groups.

4 | DISCUSSION

We investigated changes in the rewarding effects of METH in weaver mutant mice in the CPP test. Methamphetamine induced both CPP and priming effects in wild-type mice but not in weaver mutant mice. Although METH-induced CPP and priming effects increased in heterozygous weaver mutant mice, they were decreased compared with wild-type mice. Weaver mutant mice are known to exhibit ataxia, characterized by mild locomotor hyperactivity. Thus, the abolishment of METH-induced rewarding effects in the present study does not appear to be attributable to abnormal gait-related locomotor activity. The present results suggest that decreases in METH-induced CPP and priming effects are attributable to the copy number of the weaver mutant gene.

Based on these results, we measured changes in dopamine release in the NAc after METH administration in wild-type and homozygous weaver mutant mice. The baseline release of dopamine in weaver mutant mice was lower than in wild-type mice. Although METH increased dopamine release in both weaver mutant mice and wild-type mice, the extent of the increase in weaver mutant mice was milder than in wild-type mice. Several studies have reported that the rewarding effects of addictive substances are associated with dopamine release in the NAc. Thus, the disappearance of METH-induced CPP in weaver mutant mice may be at least partially attributable to alterations of dopamine signaling in the NAc.

Supporting the in vivo results, we conducted immunohistochemical analyses to reveal brain regions where neuronal activation by METH was altered in weaver mutant mice. We analyzed alterations of Fos protein expression, a well-known marker of neuronal activation, in five brain regions that are related to the reward system: aNAc core, pNAc shell, and VTA. Methamphetamine treatment increased the number of Fos-positive nuclei in the pNAc shell wild-type mice. In weaver mutant mice, no difference in Fos expression was found between saline and METH treatment in any of the brain regions. These results suggest that the pNAc shell plays an important role in the absence of METH-induced CPP in weaver mutant mice. The absence of METH-induced CPP in weaver mutant mice may have several explanations as discussed below.

4.1 | Influence of dopamine reduction in weaver mutant mice on METH-induced CPP

Dopamine depletion was observed in the brain in weaver mutant mice. In the NAc, dopamine levels decreased by 43% in heterozygous weaver mutant mice and by 24% in homozygous weaver mutant mice...
The expression of both dopamine D1 receptors and dopamine D2 receptors was previously reported to not be different between wild-type and weaver mutant mice. In the present study, we found that baseline extracellular dopamine levels were lower in weaver mutant mice than in wild-type mice. The METH-induced increase in dopamine levels was less in weaver mutant mice than in wild-type mice. These results, together with previous reports, suggest that total levels of dopamine or changes in dopamine release in the NAc may be involved in the abolishment of METH-induced CPP in weaver mutant mice.

4.2 Influence of abnormal GIRK2 subunit activation on METH-induced CPP

The activation of GIRK channels plays an important role in various brain functions, such as pain perception, analgesia, rewarding effects, and memory modulation. Ifenprodil, which inhibits the function of not only N-methyl-D-aspartate receptors but also GIRK channels, suppressed morphine-induced CPP. Additionally, paroxetine and fluoxetine but not fluvoxamine inhibited METH-induced CPP. Although these drugs are selective serotonin reuptake inhibitors, paroxetine and fluoxetine but not fluvoxamine inhibit GIRK channels. Thus, the inhibition of GIRK channels may be partially involved in the inhibition of METH-induced CPP. Furthermore, the METH- and cocaine-induced dysfunction of inhibitory GABAergic-GIRK signaling in VTA GABAergic neurons were shown to enhance excitatory VTA dopaminergic neurons, resulting in an increase in dopamine release in the NAc and medial prefrontal cortex. These previous findings suggest that the absence of METH-induced CPP in weaver mutant mice in the present study may have been caused by alterations of neurotransmission in the reward system that resulted from impairments in GIRK channel function.
4.3 | Attenuation of reward-related learning and memory

The NAc plays an important role in reward processing. Reward-related information, such as reward seeking and reward consumption, is integrated by the NAc in cooperation with various brain regions, such as the hippocampus (HPC; memory), basolateral amygdala (drive and motivation), VTA (reward prediction), and ventral pallidum (goal-directed motor response).23 Anatomical studies have demonstrated that the NAc is divided into two functionally distinct areas, the core and shell. The NAc shell and HPC pathway are related to reward- or drug-seeking behavior, followed by spatial and contextual conditioning information. The NAc core and dopaminergic circuit are related to reward- and drug-seeking behavior that is elicited by discrete cues that are associated with reward-related substances.24 The CPP procedure is a widely used model that reveals associations between rewarding effects of drugs and environmental information, such as visual and olfactory cues. Spatial or contextual memory consolidation is very important in the acquisition of CPP. Nicotine-induced CPP was decreased by dopamine ablation in the NAc shell, whereas nicotine-induced CPP was enhanced by dopamine ablation in the NAc core.25 Similarly, the repeated amphetamine administration-induced increase in dopamine levels in the NAc shell enhances spatial conditioning, but this increase attenuates cue conditioning during the acquisition of CPP. These studies indicate that hyperactivity of the dopamine system from the NAc shell to HPC facilitates signal transduction during new associative learning.26 In the present study, METH-induced neuronal activation was observed in the NAc shell in wild-type mice. These results, together with previous studies, suggest that the METH-induced increase in Fos-positive nuclei in the NAc shell represents a hyperdopaminergic state that is involved in reward-related learning and memory consolidation in the CPP paradigm. In contrast, weaver mutant mice, which did not exhibit METH-induced CPP, exhibited a decrease in Fos expression in the NAc shell compared with METH-treated wild-type mice. GIRK-regulated signaling also plays an important role in learning and memory processes. Several studies reported GIRK-mediated currents in brain regions that are related to spatial and contextual learning, cognitive function, and the establishment of reward-related memory. Furthermore, dopamine signaling in the VTA is involved in the association between learning and addiction. The activity of GIRK channels is also partially involved in this process.7 Based on the present results and previous findings, weaver mutant mice may exhibit impairments in reward-related learning and memory processes that are attributable to abnormal GIRK2 subunit function. Consequently, the absence of METH-induced CPP in weaver mutant mice might result from a failure to remember the drug-paired compartment. However, although CPP can be tested in weaver mutant mice, other forms of cognition (eg, learning and memory) are impossible to test in weaver mutant mice because of their gross motor impairments. Thus, proving the above hypothesis based solely on the present results is difficult.

In the present study, we observed the absence of METH-induced CPP and a METH-induced decrease in extracellular dopamine release in weaver mutant mice. We also found significant differences in neuronal activity, reflected by Fos expression, in the pNAc shell after METH treatment between wild-type mice and weaver mutant mice. Our results suggest that defective neuronal activity in the pNAc shell that occurs through a direct or indirect influence of abnormal GIRK2 subunit function is involved in the absence of METH-induced CPP in weaver mutant mice.

G protein-activated inwardly rectifying potassium channels are key factors in the development and maintenance of drug dependence. Our group previously reported that the GIRK channel inhibitor ifenprodil inhibited alcohol use in patients with alcohol dependence.27 Although medications exist for the treatment of opioid and alcohol dependence, no effective pharmacological treatments have been discovered for psychostimulant dependence. Our group is currently conducting a clinical trial to examine the effects of ifenprodil in patients with METH dependence.28 Further animal and clinical studies will determine whether GIRK inhibitors are effective for the treatment of addiction.

ACKNOWLEDGMENTS

The authors are grateful to M. Arends for English editing and also thank E. Kamegaya and Y. Matsushima for technical assistance. This work was supported by JSPS KAKENHI (no. 16K15565 and 16H06276 to KI; no. 17K08612 to SI), AMED (no. JP19dk0307071, JP18mk0101076, and JP19ek0610011 to KI), and the Smoking Research Foundation (KI).

CONFLICT OF INTEREST

The authors declare no conflicts of interest for this article.

AUTHOR CONTRIBUTION

YI, SI, and KI were involved in the conception and design of the experiments. YI, SI, and YH performed the experiments and statistical analyses and wrote the manuscript. YI, SI, and KI finalized the manuscript. All of the authors read and approved the final manuscript.

ETHICAL APPROVAL

The experimental procedures and housing conditions were approved by the Institutional Animal Care and Use Committee (permission number: 20-019), and all of the animals were cared for and treated humanely in accordance with our institutional animal experimentation guidelines.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this article.

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REFERENCES

1. Kuhn BN, Kalivas PW, Bobadilla AC. Understanding addiction using animal models. Front Behav Neurosci. 2019;13:262.
