様式2-4-1

日米科学技術協力事業「脳研究」分野
グループ共同研究実施報告書　　［研究分野：　③　］

１．グループ共同研究代表者

所属機関・部局　財団法人　東京都医学研究機構　東京都精神医学総合研究所
職名　分子精神医学研究ディレクター（副参事研究員）
(ﾌﾘｶﾞﾅ)　イケダ　カズタカ
氏名　池　田　和　隆
所属機関所在地　〒１５６－８５８５　東京都世田谷区上北沢２－１－８
連絡先　TEL:０３－３３０４－５７０１
E-mail:ikedak@prit.go.jp

２．研究課題名（和文）快・不快情動発現制御の神経回路機構

（英文）Neural mechanisms mediating reward and anhedonia

３．日本側グループ組織（代表者及び分担者の所属・職・氏名）

代表者
所属　財団法人　東京都医学研究機構　東京都精神医学総合研究所･分子精神医学研究チーム
職　分子精神医学研究ディレクター（副参事研究員）
氏名　池田　和隆

分担者
所属　財団法人　東京都医学研究機構　東京都精神医学総合研究所･分子精神医学研究チーム
職　主事研究員
氏名　山本　秀子

所属　財団法人　東京都医学研究機構　東京都精神医学総合研究所･分子精神医学研究チーム
職　流動研究員
氏名　笠井　慎也

所属　財団法人　東京都医学研究機構　東京都精神医学総合研究所･分子精神医学研究チーム
職　技術研究員
氏名　高松　幸雄

所属　財団法人　東京都医学研究機構　東京都精神医学総合研究所･分子精神医学研究チーム
職　技術研究員
氏名　萩野　洋子

所属　財団法人　東京都医学研究機構　東京都精神医学総合研究所･分子精神医学研究チーム
職　派遣研究員
氏名　大谷　保和

所属　財団法人　東京都医学研究機構　東京都精神医学総合研究所･分子精神医学研究チーム
職　非常勤職員
氏名　西澤　大輔

所属　　財団法人　東京都医学研究機構　東京都精神医学総合研究所･分子精神医学研究チーム
　職　　客員研究員
氏名　　小林　徹

所属　　財団法人　東京都医学研究機構　東京都精神医学総合研究所･分子精神医学研究チーム
　職　　研究生
氏名　　岩田　健

所属　　財団法人　東京都医学研究機構　東京都精神医学総合研究所･分子精神医学研究チーム
　職　　研究生
氏名　　繁田　悦宏

所属　　財団法人　東京都医学研究機構　東京都精神医学総合研究所･分子精神医学研究チーム
　職　　研究生
氏名　　塩月　寛美

４．米国側グループ組織（代表者及び分担者の所属・職・氏名）

代表者
所属　　Department of Psychiatry, School of Medicine, University of California, San Diego
　職　　Professor
氏名　　Athina Markou

分担者
所属　　Department of Psychiatry, School of Medicine, University of California, San Diego
　職　　Assistant Project Scientist
氏名　　Svetlana Semenova

５．研究期間（３年を限度）

平成１７年　４月　１日～平成２０年　３月３１日（　３年間）

６．研究の概要，成果及び意義（1000 字）

快・不快の情動は、人間や動物の行動を決定する最も重要な脳機能の一つである。快・不快に密接に関わることが知られているドーパミンシステムとオピオイドシステムについて、ドーパミン欠乏マウスやミューオピオイド受容体ノックアウトマウスなどの遺伝子改変マウスを準備し､行動薬理学的試験を行った｡また、ドーパミン神経伝達を亢進させるメタンフェタミンに関して、その嗜好性をマウス薬物条件付け場所嗜好性試験によって解析し、嗜好性を抑制する薬物を探索した。

ドーパミン欠乏マウスは、生後数週間以内に死亡するが、Ｌドーパを投与することで長期間飼育することに成功した｡Ｌドーパ投与を中断して脳内ドーパミンを欠乏させた後に行動薬理学的な解析を行ったところ、抗精神病薬で抑制されない異常な多動が観察された。ドーパミン欠乏下での異常な興奮は従来まったく知られておらず、その機序の解明は興奮を惹起する薬物の作用機序の解明にも繋がると期待できる。

薬物条件付け場所嗜好性試験において、メタンフェタミンに対する嗜好性は、選択的セロトニン取り込み阻害剤(SSRI)の１つであるフルオキセチンを条件付けと嗜好性試験の前に投与することで、消失する事が明らかになった(Takamatsu Y, Yamamoto H, Ogai Y, Hagino Y, Markou A, Ikeda K (2006) Fluoxetine as a potential pharmacotherapy for methamphetamine dependence: studies in mice. **Ann N Y Acad Sci** 1074:295-302)｡また､この研究成果を基に､他の SSRI であるパロキセチンとフルボキサミンについて、覚せい剤嗜好性に与える影響を検討し、パロキセチンは抑制効果を持つが、フルボキサミンは抑制効果

を持たないことを見出した(Takamatsu Y, Yamamoto H, Hagino Y, Markou A, Ikeda K, The selective serotonin reuptake inhibitor paroxetine, but not fluvoxamine, decreases methamphetamine conditioned place preference in mice. **Ann N Y Acad Sci** in press)。メタンフェタミンは日米において広く乱用されている薬物であり、精神病症状を惹起する極めて危険な薬物である。メタンフェタミンへの嗜好性を減弱、消失させる薬物の発見は、快・不快の情動の理解に繋がるとともに、薬物依存問題への有効な対策に結びつくと考えられる。

７．その他（実施上の問題点，特記事項等）

米国側研究者の Dr. Athina Markou は、本共同研究の実施期間中に、スクリップス研究所（准教授）からカリフォルニア大学サンディエゴ校(UCSD)医学部精神科（教授）に異動した。研究スタッフのほぼ全員も移籍し、研究環境もさらによくなったので、共同研究の推進にはむしろプラスとなった。また、本事業による支援終了後も、共同研究を継続している。

◎参考資料があれば，添付ください。

1. Takamatsu Y, Yamamoto H, Ogai Y, Hagino Y, Markou A, Ikeda K (2006) Fluoxetine as a potential pharmacotherapy for methamphetamine dependence: studies in mice. **Ann N Y Acad Sci** 1074:295-302.
2. Takamatsu Y, Yamamoto H, Hagino Y, Markou A, Ikeda K, The selective serotonin reuptake inhibitor paroxetine, but not fluvoxamine, decreases methamphetamine conditioned place preference in mice. **Ann N Y Acad Sci** in press

Fluoxetine as a Potential Pharmacotherapy for Methamphetamine Dependence

Studies in Mice

YUKIO TAKAMATSU,[a] HIDEKO YAMAMOTO,[a] YASUKAZU OGAI,[a] YOKO HAGINO,[a] ATHINA MARKOU,[b] AND KAZUTAKA IKEDA[a]

[a]*Division of Psychobiology, Tokyo Institute of Psychiatry, Tokyo 156-8585, Japan*

[b]*Department of Molecular and Integrative Neuroscience, The Scripps Research Institute, La Jolla, California 92037, USA*

ABSTRACT: The monoamine transporters are the main targets of psychostimulant drugs, including methamphetamine (METH) and cocaine. Interestingly, the rewarding effects of cocaine are retained in dopamine transporter (DAT) knockout (KO) mice, while serotonin transporter (SERT) and DAT double KO mice do not exhibit conditioned place preference (CPP) to cocaine. These data suggest that SERT inhibition decreases the rewarding effects of psychostimulants. To further test this hypothesis, in the present study, we investigated the effects of intraperitoneal (i.p.) injections of 20 mg/kg fluoxetine, a selective serotonin reuptake inhibitor (SSRI), on 2 mg/kg METH (i.p.) CPP and locomotor sensitization to 1 mg/kg METH (i.p.) in C57BL/6J mice. Fluoxetine treatment before both the conditioning and preference tests abolished METH CPP. A two-way analysis of variance (ANOVA) revealed that METH CPP tended to be lower in mice pretreated with fluoxetine before the preference test than in control mice pretreated with saline before the preference test. Furthermore, pretreatment with fluoxetine had inhibitory effects on METH-induced locomotor sensitization. These results suggest that fluoxetine, a widely used medication for depression, may be also a useful tool for treating METH dependence.

KEYWORDS: methamphetamine; fluoxetine; conditioned place preference; locomotor activity; sensitization; mice

Address for correspondence: Kazutaka Ikeda, Division of Psychobiology, Tokyo Institute of Psychiatry, 2-1-8 Kamikitazawa, Setagaya-ku, Tokyo 156-8585, Japan. Voice: +81-3-3304-5701, ext. 508; fax: +81-3-3329-8035.
e-mail: ikedak@prit.go.jp

Ann. N.Y. Acad. Sci. 1074: 295–302 (2006).
doi: 10.1196/annals.1369.026

INTRODUCTION

Methamphetamine (METH) is widely abused in the world.[1] Especially in Japan, the number of people arrested for METH possession or use is approximately 100 times more than those arrested for cocaine or cannabis. Further, METH frequently induces psychotic states with symptoms similar to those seen in schizophrenia of the paranoid type.[2] Such psychotic states are mainly treated in hospitals resulting in large medical costs. Thus, there is great need for the discovery of new medications for METH abuse,[3] as the current treatments are primarily oriented toward the treatment of psychosis with no treatments available to prevent relapse to METH abuse.

Dopamine transporters (DAT) are the main targets for METH and cocaine. However, mice lacking the DAT show preference for cocaine[4] and self-administer cocaine.[5] Interestingly, heterozygous and homozygous serotonin transporter (SERT) knockout (KO) mice that also have a homozygous KO of the DAT do not exhibit cocaine place preference.[6] Further, findings indicated that extracellular dopamine concentration increases after cocaine administration in the striatum of DAT KO mice but not of DAT/SERT double KO mice.[7] Taken together, these reports suggest that SERT inhibition may decrease METH and cocaine place preference.

In the present study, we tested this hypothesis by assessing whether fluoxetine, a selective serotonin reuptake inhibitor (SSRI) with the trade name of Prozac, reduces METH place preference and sensitization to the locomotor activating effects of METH.

MATERIALS AND METHODS

Male C57BL/6J mice (8–10 weeks old) were purchased from CLEA Japan, Inc. (Tokyo, Japan) and were housed for 1–2 weeks before the experiments were begun in an animal facility maintained at 24 ± 1°C and 50% relative humidity under a 12/12 h light/dark cycle with lights on at 8:00 AM and off at 8:00 PM. Food and water were available *ad libitum*. The experimental procedures and housing conditions were approved by the Institutional Animal Care and Use Committee of the Tokyo Institute of Psychiatry, and all animals were cared for and treated humanely in accordance with our institutional animal experimentation guidelines.

Conditioned Place Preference (CPP) Test

The conditioned place preference (CPP) test was carried out according to the method of Hoffman and Beninger[8] with some modifications. For this test, we used a two-compartment Plexiglas chamber (Neuroscience Inc., Osaka, Japan), one compartment was black with a smooth floor and the other was of

the same dimensions, but white with a textured floor. This two-compartment chamber was placed in a sound- and light-attenuated box under conditions of dim illumination (about 40 lx). There was no significant difference between time spent in the black compartment and time spent in the white compartment on day 2 of testing (see below), indicating that there was no preference for either side under the present conditions before conditioning. As described previously,[9] we selected a counterbalanced protocol in order to nullify each mouse's initial compartment preference.

On day 1, the mice ($n = 15 \sim 16$ per group) were allowed to freely explore the two compartments for 15 min. On day 2, again the mice were allowed to explore the two compartments freely for 15 min, and the time spent in each compartment and the number of transitions between compartments were measured. Then, conditioning sessions were conducted once daily for 4 consecutive days (days 5–8). Mice were intraperitoneally (i.p.) injected with METH (2 mg/kg) and immediately confined to the black or white compartment for 50 min on day 5. On day 6, the mice were injected with saline and immediately confined to the opposite compartment for 50 min. On days 7 and 8, the same conditioning as on days 5 and 6 was repeated. Fluoxetine (20 mg/kg i.p.) or saline was injected 60 min before METH or saline treatment. On day 9, the mice were pretreated with saline or fluoxetine (20 mg/kg). Sixty minutes later, the mice were allowed to freely explore the two compartments for 15 min without METH injection, and the time spent in each compartment and the number of transitions between compartments were measured (see FIG. 1 for a diagram of the experimental design). The CPP score was designated as the time spent

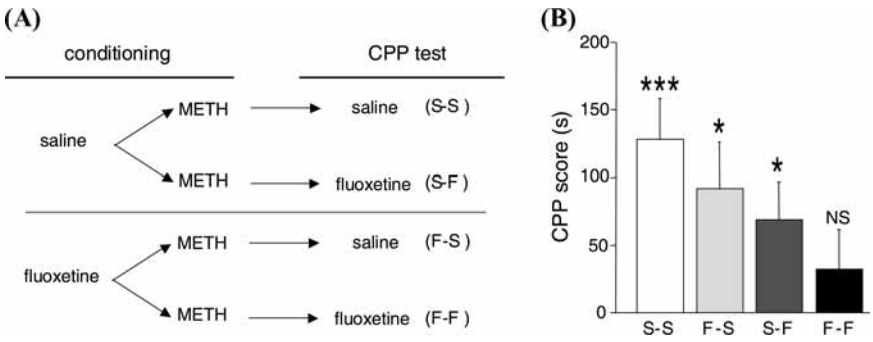


FIGURE 1. (A) Depiction of the experimental design. Four mouse groups were treated with saline or fluoxetine during the conditioning phase and the CPP test phase. **(B)** Abolishment of METH CPP by fluoxetine pretreatment. The CPP score was designated as the time spent in the METH-paired compartment minus the time spent in the same compartment in the preconditioning phase. There was significant CPP in the S-S, F-S, and S-F groups, but not when fluoxetine was administered before both the conditioning phase and the CPP test phase (F-F) (within-group paired *t*-tests, $^{***}P < 0.001$, $^{*}P < 0.05$, NS: not significant [$P > 0.05$]). The CPP scores were expressed as means ± the standard error of the mean (SEM).

in the METH-paired compartment on day 9 minus the time spent in the same compartment in the preconditioning phase on day 2.

Locomotor Activity

METH-induced locomotor activity was examined in mice ($n = 15\sim16$ per group) injected with METH (1 mg/kg i.p.) seven times every other day, for a total of seven injections over 13 consecutive days. Mice were placed at the center of the test chamber (250 mm in diameter and 270 mm in height; Muromachi Kikai Co., Tokyo, Japan) and allowed to freely explore the chamber for 120 min. Then, fluoxetine (20 mg/kg) or saline was injected i.p. and the mice were placed in the locomotor activity chamber immediately afterwards. Sixty minutes later, METH was injected i.p. and locomotor activity was assessed for 60 min after the METH administration by using an infrared activity monitor.

Drugs

Methamphetamine hydrochloride was purchased from Dainippon Pharmaceutical (Osaka, Japan), and fluoxetine hydrochloride was purchased from Sigma (St. Louis, MO, USA). All drugs were dissolved in saline. Drugs and vehicle were administered (i.p.) in a volume of 0.1 mL/10 g of body weight.

Statistical Analyses

For the CPP data, the time that the mice spent in the METH-paired compartment before and after conditioning were compared with within-group paired *t*-tests for each group. The CPP scores of mice pretreated with fluoxetine or saline in the conditioning phase and CPP test phase were subjected to a two-way analysis of variance (ANOVA). Locomotor activity was subjected to a two-way mixed-design ANOVA followed by *post hoc* comparisons with the Scheffe test.

RESULTS

Effects of Fluoxetine on the METH CPP

Mice pretreated with saline in the conditioning phase and the CPP test phase (S-S) spent significantly longer time in the METH-paired compartment after conditioning than before conditioning ($df = 14$, $t = -4.262$, $P = 0.0008$) (FIG. 1). The mice pretreated with fluoxetine (20 mg/kg) in the conditioning phase or the CPP test phase (F-S and S-F) also showed significant METH

CPP ($df = 14$, $t = -2.739$, $P = 0.0160$; $df = 14$, $t = -2.400$, $P = 0.0309$, respectively), although the CPP scores were lower than that of S-S group. Mice pretreated with fluoxetine (20 mg/kg) in the conditioning phase and the CPP test phase (F-F) did not show METH-CPP ($df = 14$, $t = -1.073$, $P = 0.3015$). Furthermore, the two-way ANOVA revealed that fluoxetine pretreatment in the CPP test phase resulted in a trend toward a decrease of the CPP score when compared to mice treated with saline before the CPP test phase ($F_{1,56} = 3.857$, $P = 0.0545$). There was no statistically significant interaction between the factors fluoxetine/saline for the conditioning phase and CPP test phase ($F_{1,56} < 0.0001$, $P = 0.9972$). Fluoxetine pretreatment had no significant effects on number of transitions between the compartments (data not shown).

Effects of Fluoxetine on METH-Induced Locomotor Sensitization

Fluoxetine (20 mg/kg) or saline was injected 60 min before METH (1 mg/kg) administration every other day when METH was administered. We selected this fluoxetine dose because the number of transitions between compartments in the CPP test was not altered by this dose of fluoxetine, indicating no significant influence of fluoxetine itself on locomotor activity at this dose. Locomotor activity during the 60-min period immediately after METH administration was analyzed using a two-way mixed-design ANOVA (FIG. 2). The administration of METH led to sensitization to the locomotor activating effects of METH as

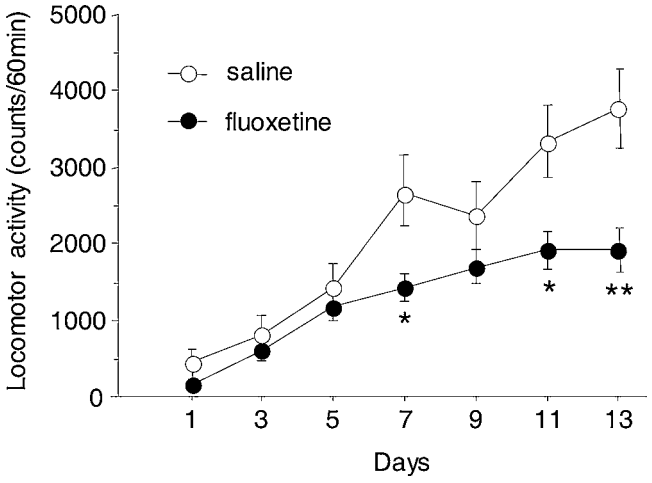


FIGURE 2. Locomotor activity after METH injection in mice pretreated with either fluoxetine or saline. Locomotor activity was measured by infrared activity counters. Locomotor activity of mice pretreated with fluoxetine was significantly lower than that of mice pretreated with saline. The locomotor activity counts were expressed as mean ± SEM
$^{*}P < 0.05$, $^{**}P < 0.01$.

reflected by a main effect of Day ($F_{6,29} = 41.542$, $P < 0.0001$). Further, there was a main effect of fluoxetine indicating that fluoxetine inhibited locomotor activity ($F_{1,29} = 6.696$, $P = 0.0149$) at this test. Finally, there was also a statistically significant interaction effect reflecting the fact that pretreatment with fluoxetine had inhibitory effects on locomotor sensitization (interaction between the factor METH and the factor pretreatment; $F_{6,29} = 4.851$, $P = 0.0001$). The *post hoc* comparisons revealed that the locomotor activity of mice pretreated with fluoxetine was significantly lower than that of mice pretreated with saline on days 7, 11, and 13.

DISCUSSION

Fluoxetine abolished METH CPP and significantly reduced sensitization to the locomotor activating effects of METH in the present study. The abolishment of METH CPP by fluoxetine pretreatment suggests that fluoxetine reduces preference for contextual stimuli previously associated with METH, while the inhibition of sensitization to METH-induced locomotor activation by fluoxetine indicates that fluoxetine may be a useful tool for preventing sensitization to some of the effects of METH, such as psychosis that is often seen after repeated use of high METH doses.

There are three candidate mechanisms possibly mediating the inhibitory effects of fluoxetine on behaviors relating to METH dependence: (a) increase of basal extracellular serotonin concentration due to inhibition of SERT by fluoxetine, (b) blockade by fluoxetine of the actions of METH on SERT, and (c) actions of fluoxetine on molecules other than SERT. The first mechanism is supported by a report that inhibition of monoamine oxydase A, a major serotonin metabolizer, reduces METH-induced hyperlocomotion.[10] Secondly, the blockade of the actions of METH on the SERT by fluoxetine may reduce the toxicity induced by METH. METH induces reverse flow of dopamine and serotonin through DAT and SERT, respectively, and leads to toxicity after entering into the cytoplasm.[11] Thus, fluoxetine may protect the SERT from the actions of METH and thus reduces both the behavioral and toxic effects of METH. This second possibility is supported by reports that the METH-induced decrease in tryptophan hydroxylase activity is attenuated by fluoxetine.[12,13] Finally, recent studies have shown that fluoxetine modulates the function of several ion channels and receptors, such as G protein-activated inwardly rectifying K^+ (GIRK) channels,[14] voltage-gated Ca^{2+}, Na^+ and K^+ channels,[15–21] Cl^- channels,[22] 5-HT2C[23] and 5-HT3 receptors,[24] and nicotinic acetylcholine receptors.[25,26] Thus, the actions of fluoxetine on these molecules, besides the actions on the SERT, may mediate the inhibitory effects of fluoxetine on METH dependence. Reduced cocaine self-administration in mice lacking the GIRK2 or GIRK3 subunit[27] supports this third possibility that fluoxetine inhibits METH dependence through inhibition of the GIRK channels.

In conclusion, we found that fluoxetine, a widely used medication for depression, inhibited both METH CPP and sensitization to METH-induced locomotor activation in mice. Although further preclinical studies are needed to elucidate the mechanisms underlying these inhibitory effects of fluoxetine on processed relating to METH dependence, it appears worthwhile to investigate the clinical effects of fluoxetine on METH abuse.

ACKNOWLEDGMENTS

We are grateful to Dr. Keiko Matsuoka for animal care, and Kazuya Tawara and Akira Sato for technical assistance. This work was supported by a research grant (17025054) from the MEXT of Japan, and by grants (H17-pharmaco-001, H16-iyaku-029, H15-kou-3–03) from the MHLW of Japan. AM was supported by a USA National Institute on Drug Abuse grant (R01 DA11946).

REFERENCES

1. United Nations Office for Drug Control and Crime Prevention Ed. 2005. World Drug Report 2005. Oxford University Press. New York, NY.
2. Ujike, H. 2002. Stimulant-induced psychosis and schizophrenia: the role of sensitization. Curr. Psychiatry Rep. **4:** 177–184.
3. National Institute on Drug Abuse (NIDA) Ed. 2002. Research Report Series: Methamphetamine Abuse and Addiction. National Institutes of Health. Maryland, MA.
4. Sora, I. *et al*. 1998. Cocaine reward models: conditioned place preference can be established in dopamine- and in serotonin-transporter knockout mice. Proc. Natl. Acad. Sci. USA **95:** 7699–7704.
5. Rocha, B.A. *et al*. 1998. Cocaine self-administration in dopamine-transporter knockout mice. Nat. Neurosci. **1:** 132–137.
6. Sora, I. *et al*. 2001. Molecular mechanisms of cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference. Proc. Natl. Acad. Sci. USA **98:** 5300–5305.
7. Shen, H.-W. *et al*. 2004. Regional differences in extracellular dopamine and serotonin assessed by in vivo microdialysis in mice lacking dopamine and/or serotonin transporters. Neuropsychopharmacology **29:** 1790–1799.
8. Hoffman, D.C. & R.J. Beninger. 1989. Preferential stimulation of D1 or D2 receptors disrupts food-rewarded operant responding in rats. Pharmacol. Biochem. Behav. **34:** 923–925.
9. Ide, S. *et al*. 2004. Buprenorphine antinociception is abolished, but naloxone-sensitive reward is retained, in mu-opioid receptor knockout mice. Neuropsychopharmacology **29:** 1656–1663.
10. Kitanaka, N. *et al*. 2005. Inhibition of methamphetamine-induced hyperlocomotion in mice by clorgyline, a monoamine oxidase-A inhibitor, through alteration of the 5-hydroxytriptamine turnover in the striatum. Neuroscience **130:** 295–308.
11. Sulzer, D. *et al*. 1995. Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. J. Neurosci. **15:** 4102–4108.

12. HOTCHKISS, A.J. & J.W. GIBB. 1980. Long-term effects of multiple doses of methamphetamine on tryptophan hydroxylase and tyrosine hydroxylase activity in rat brain. J. Pharmacol. Exp. Ther. **214:** 257–262.
13. FLECKENSTEIN, A.E. *et al*. 1997. Methamphetamine-induced decrease in tryptophan hydroxylase activity: role of 5-hydroxytryptaminergic transporters. Eur. J. Pharmacol. **324:** 179–186.
14. KOBAYASHI, T. *et al*. 2003. Inhibition of G protein-activated inwardly rectifying K^+ channels by fluoxetine (Prozac). Br. J. Pharmacol. **138:** 1119–1128.
15. PANCRAZIO, J.J. *et al*. 1998. Inhibition of neuronal Na^+ channels by antidepressant drugs. J. Pharmacol. Exp. Ther. **284:** 208–214.
16. CHOI, J.-S. *et al*. 1999. Mechanism of fluoxetine block of cloned voltage-activated potassium channel Kv1.3. J. Pharmacol. Exp. Ther. **291:** 1–6.
17. YEUNG, S.Y. *et al*. 1999. Inhibition of neuronal KV potassium currents by the antidepressant drug, fluoxetine. Br. J. Pharmacol. **128:** 1609–1615.
18. DEÁK, F.B. *et al*. 2000. Inhibition of voltage-gated calcium channels by fluoxetine in rat hippocampal pyramidal cells. Neuropharmacology **39:** 1029–1036.
19. CHOI, B.H. *et al*. 2001. Effects of norfluoxetine, the major metabolite of fluoxetine, on the cloned neuronal potassium channel Kv3.1. Neuropharmacology **41:** 443–453.
20. PERCHENET, L. *et al*. 2001. Effects of anorexinogen agents on cloned voltage-gated K^+ channel hKv1.5. J. Pharmacol. Exp. Ther. **298:** 1108–1119.
21. THOMAS, D. *et al*. 2002. The antidepressant drug fluoxetine is an inhibitor of human ether-a-go-go-related gene (HERG) potassium channels. J. Pharmacol. Exp. Ther. **300:** 543–548.
22. MAERTENS, C. *et al*. 1999. Block by fluoxetine of volume-regulated anion channels. Br. J. Pharmacol. **126:** 508–514.
23. NI, Y.G. & R. MILEDI. 1997. Blockage of 5HT2C serotonin receptors by fluoxetine (Prozac). Proc. Natl. Acad. Sci. USA **94:** 2036–2040.
24. FAN, P. 1994. Effects of antidepressants on the inward current mediated by 5-HT3 receptors in rat nodose ganglion neurones. Br. J. Pharmacol. **112:** 741–744.
25. GARCÍA-COLUNGA *et al*. 1997. Blockage of muscle and neuronal nicotinic acetylcholine receptors by fluoxetine (Prozac). Proc. Natl. Acad. Sci. USA **94:** 2041–2044.
26. MAGGI, L. *et al*. 1998. Effects of fluoxetine on wild and mutant neuronal alpha 7 nicotinic receptors. Mol. Psychiatry **3:** 350–355.
27. MORGAN, A.D. *et al*. 2003. Decreased cocaine self-administration in Kir3 potassium channel subunit knockout mice. Neuropsychopharmacology **28:** 932–938.

The Selective Serotonin Reuptake Inhibitor Paroxetine, but not Fluvoxamine, Decreases Methamphetamine Conditioned Place Preference in Mice

Running title: Paroxetine, but not fluvoxamine, decreases methamphetamine CPP

YUKIO TAKAMATSU,[a] HIDEKO YAMAMOTO,[a] YOKO HAGINO,[a] ATHINA MARKOU,[b] KAZUTAKA IKEDA[a]

[a]*Division of Psychobiology, Tokyo Institute of Psychiatry, Tokyo 156-8585, Japan.*

[b]*Department of Psychiatry, School of Medicine, University of California San Diego, La Jolla, California 92093-0603, USA*

Address for correspondence: Kazutaka Ikeda, Division of Psychobiology, Tokyo Institute of Psychiatry, 2-1-8 Kamikitazawa, Setagaya-ku, Tokyo 156-8585, Japan. Voice: +81-3-3304-5701, ext. 508; fax: +81-3-3329-8035; e-mail: ikedak@prit.go.jp

ABSTRACT: Monoamine transporters are the main targets of methamphetamine (METH). Recently, we showed that fluoxetine, a selective serotonin reuptake inhibitor (SSRI), decreased METH conditioned place preference (CPP), suggesting that serotonin transporter (SERT) inhibition reduces the rewarding effects of METH. To further test this hypothesis, in the present study we investigated the effects of additional SSRIs, paroxetine and fluvoxamine, on METH CPP in C57BL/6J mice. In the CPP test, pretreatment with 20 mg/kg paroxetine abolished the CPP for METH, whereas pretreatment with 100 mg/kg fluvoxamine prior to administration of METH failed to inhibit METH CPP. These results suggest that paroxetine, a medication widely used to treat depression, may be a useful tool for treating METH dependence. Further, these data suggest that molecules other than the SERT [such as G protein-activated inwardly rectifying K^+ (GIRK) channels] whose activities are modulated by paroxetine and fluoxetine, but not by fluvoxamine, are involved in reducing METH CPP by paroxetine and fluoxetine.

KEYWORDS: methamphetamine; paroxetine; fluvoxamine; serotonin transporter; conditioned place preference; mice

INTRODUCTION

Methamphetamine (METH) is abused in worldwide.[1] In Japan, the number of people arrested for METH possession or use is approximately 100 times higher than those arrested for cocaine, opioids, or cannabis. Further, METH frequently induces psychotic states with symptoms similar to those seen in paranoid schizophrenia.[2] Such psychotic states are treated primarily in hospitals resulting in high medical costs. Thus, there is great need for the discovery of new medications for METH abuse[3] because the current treatments are mostly oriented toward the treatment of psychosis with no treatments available to prevent relapse to METH abuse.

The dopamine transporter (DAT) is the main target for METH and cocaine. However, mice lacking the DAT show conditioned place preference (CPP) to cocaine[4] and self-administer cocaine.[5] Interestingly, heterozygous and homozygous serotonin transporter (SERT) knockout mice that also have a homozygous knockout of the DAT do not exhibit cocaine CPP.[6] Cocaine administration leads to increases in extracellular dopamine concentration in the striatum of DAT knockout mice but not of DAT/SERT double knockout mice.[7] Taken together, these reports suggest that SERT inhibition may decrease METH and cocaine CPP.

Recently, we showed that fluoxetine, a selective serotonin reuptake inhibitor (SSRI), abolished METH CPP when METH was administered during both the development and expression phases of the CPP procedure, supporting the hypothesis that SERT inhibition decreased the rewarding effects of METH.[8] To further test this hypothesis, in the present study we investigated the effects of the SSRIs paroxetine (Paxil®) and fluvoxamine (Lubox® or Depromel®) on METH CPP.

MATERIALS AND METHODS

Mice

Male C57BL/6J mice (8-10 weeks old) were purchased from CLEA Japan, Inc. (Tokyo, Japan) and were housed for 1-2 weeks before the experiments began in an animal facility maintained at 22 ± 2˚C and 55 ± 5% relative humidity under a 12/12 h light/dark cycle with lights on at 8:00 am. Food and water were available *ad libitum*. All behavioral testing was conducted during the light phase. The experimental procedures and housing conditions were approved by the Institutional Animal Care and Use Committee of the Tokyo Institute of Psychiatry, and all animals were cared for and treated humanely in accordance with our institutional animal experimentation guidelines.

Conditioned Place Preference (CPP) Test

The CPP test was performed according to the method of Hoffman and Beninger[9] with some modifications. We used a two-compartment Plexiglas chamber (Neuroscience Inc., Osaka, Japan). One compartment (17.5 × 15 × 17.5 cm: width × length × height) was black with a smooth floor, and the other compartment was of the same dimensions, but with a white textured floor. This two-compartment chamber was located in a sound- and light-attenuated box under conditions of dim illumination (approximately 40 lux) to reduce bias toward either compartment.[10] Mice were assigned randomly to the treatment groups (see below).

On Day 1, the mice (n = 14-26 per group) were allowed to freely explore the two

compartments for 15 min. On Day 2, the mice again were allowed to explore the two compartments freely for 15 min, and the time spent in each compartment and the number of transitions between compartments were measured. Conditioning sessions then were conducted once daily for 4 consecutive days (Days 5-8). For the Day 5 conditioning session, mice were intraperitoneally (i.p.) injected with saline or SSRI (20 mg/kg paroxetine or 100 mg/kg fluvoxamine) 60 min before injection with METH (2 mg/kg, i.p.). Immediately after METH administration, mice were confined to the black or white compartment for 50 min. On Day 6, the mice were pretreated with the same solution (saline or SSRI, i.p.) 60 min before a saline injection. Immediately after the saline injection, mice were confined to the opposite compartment for 50 min. On Days 7 and 8, the same conditioning as on Days 5 and 6 was repeated. On Day 9, the mice were pretreated with saline or SSRI (20 mg/kg paroxetine or 100 mg/kg fluvoxamine, i.p.), and 60 min later were allowed to freely explore the two compartments for 15 min without METH injection. The time spent in each compartment and the number of transitions between compartments were measured. In summary, there were a total of eight groups in this experiment corresponding to the four pretreatments (paroxetine, fluvoxamine, saline; there were two saline groups that were run concurrently with the paroxetine and fluvoxamine groups) and the two phases of the experiment during which they were pretreated with the drug (conditioning days 5-8 or test day 9). The CPP score was defined as the time spent in the drug-paired compartment during the CPP test phase (Day 9) minus the time spent in the same compartment during the preconditioning exploratory phase (Day 2). The transition score was defined as the number of transitions during the CPP test phase (Day 9) minus the number of transitions during the preconditioning exploratory phase (Day 2).

Drugs

Methamphetamine hydrochloride was purchased from Dainippon Pharmaceutical (Osaka, Japan). Paroxetine maleate and fluvoxamine maleate were purchased from Sigma (St. Louis, MO, USA) and TOCRIS (Hung Road, Bristol, UK), respectively. All drugs were dissolved in saline. Drugs and vehicle were administered i.p. in a volume of 0.1 ml/10 g body weight. All drug doses are reported as salt.

Statistical Analyses

The CPP and transition scores of mice pretreated with saline or SSRI during the conditioning and CPP test phases were subjected to a two-way analysis of variance (ANOVA). The ANOVA had two between-subjects factors, each with two levels (saline/SSRI pretreatment in the conditioning phase and saline/SSRI pretreatment in the CPP test phase). Two separate ANOVAs were conducted on the paroxetine and fluvoxamine data. Similar ANOVAs were conducted on the transition scores. The CPP scores from the paroxetine experiment were subjected to a one-way ANOVA followed by *post hoc* comparisons with the Scheffe test. In this ANOVA, there were four levels corresponding to the four treatment conditions (saline in both the conditioning and the CPP test phases, pretreatment with paroxetine only in the conditioning phase, pretreatment with paroxetine only in the CPP test phase, pretreatment with paroxetine in both the conditioning and the CPP test phases). For the CPP data, the durations of time that the mice spent in the METH-paired compartment before and after conditioning were compared using paired *t*-tests for each group. For the transition data, the number of transitions

between the METH-paired compartment and the saline-paired compartment before and after conditioning were compared using paired *t*-tests for each group. The level of significance was set at 0.05.

RESULTS

Effects of Paroxetine on METH CPP

The two-way ANOVA revealed that mice treated with paroxetine during the test phase exhibited decreased CPP scores compared to mice treated with saline during the test phase ($F_{1,72}$ = 7.888, $P < 0.01$), whereas mice treated with paroxetine during the conditioning phase did not differ significantly from mice treated with saline during the test phase in the CPP score [$F_{1,72}$ = 1.704, not significant (n.s.); FIG. 1A]. There was no statistically significant interaction between the factor saline/paroxetine during the conditioning phase and the factor saline/paroxetine during the CPP test phase ($F_{1,72}$ = 0.1690, n.s.), indicating that the important factor was treatment with paroxetine during the expression phase of the experiment. In addition, a one-way ANOVA on the CPP scores was conducted on data for all four groups. The ANOVA showed a significant difference in the CPP scores among these four groups ($F_{3,72}$ = 3.940, $P < 0.05$). The Scheffe *post hoc* test showed that the CPP score of the paroxetine/paroxetine group was significantly lower than that of the saline/saline group ($P < 0.05$). Paired *t*-tests were conducted to compare the duration of time before and after conditioning for each of the four groups (FIG. 1B). Whereas the saline/saline and paroxetine/saline groups spent significantly more time in the METH-paired compartment after conditioning than before conditioning (saline/saline: $n = 23$, *df*

= 22, t = -6.050, $P < 0.001$; paroxetine/saline: n = 15, df = 14, t = -2.884, $P < 0.05$), the saline/paroxetine and paroxetine/paroxetine groups did not show METH CPP (saline/paroxetine: n = 15, df = 14, t = -2.033, n.s.; paroxetine/paroxetine: n = 23, df = 22, t = -0.908, n.s.). Paroxetine pretreatment had no significant effects on the transition scores compared to the saline/saline treatment group (data not shown).

Effects of Fluvoxamine on the METH CPP

The two-way ANOVA revealed that both the factor saline/fluvoxamine pretreatment during the conditioning phase and the factor saline/fluvoxamine pretreatment during the CPP test phase had no effects on CPP scores (conditioning phase: $F_{1,68}$ = 0.045, n.s.; CPP test phase: $F_{1,68}$ = 3.016, n.s.; FIG. 2A). There was no statistically significant interaction between the two factors ($F_{1,68}$ = 0.066, n.s.). Paired t-tests were conducted to compare the duration of time before and after conditioning for each of the four groups. All four groups spent significantly more time in the METH-paired compartment after conditioning than before conditioning (saline/saline: n = 26, df = 25, t = -4.541, $P < 0.001$; saline/fluvoxamine: n = 14, df = 13, t = -2.983, $P < 0.05$; fluvoxamine/saline: n = 18, df = 17, t = -3.949, $P < 0.01$; fluvoxamine/fluvoxamine: n = 14, df = 13, t = -2.757, $P < 0.05$).

The two-way ANOVA revealed that both fluvoxamine pretreatment during the conditioning phase and during the CPP test phase significantly decreased transition scores (conditioning phase: $F_{1,68}$ = 24.321, $P < 0.001$; CPP test phase: $F_{1,68}$ = 10.292, $P < 0.01$; FIG. 2B). There was no statistically significant interaction between the two factors ($F_{1,68}$ = 0.007, n.s.). Paired t-tests were conducted to compare the number of transitions before and after conditioning

for each of the four groups. The S-S group showed no significant differences in the number of transitions before and after conditioning ($n = 26$, $df = 25$, $t = -1.213$, n.s.). However, mice pretreated with fluvoxamine (saline/fluvoxamine, fluvoxamine/saline, fluvoxamine/fluvoxamine) showed significant decreases in the number of transitions after conditioning (saline/fluvoxamine: $n = 14$, $df = 13$, $t = 3.829$, $P < 0.01$; fluvoxamine/saline: $n = 18$, $df = 17$, $t = 5.520$, $P < 0.001$; fluvoxamine/fluvoxamine: $n = 14$, $df = 13$, $t = 6.025$, $P < 0.001$).

DISCUSSION

In the present study, we showed that paroxetine, a widely used medication for treating depression, inhibited METH CPP in mice, similar to the results we reported previously with fluoxetine.[8] No significant effects of paroxetine on transition scores suggest that the effects of paroxetine on METH CPP are not due to changes in locomotor activity but due to reduction of METH reward and conditioned reward by paroxetine. Based on these findings, it appears worthwhile to investigate the clinical effects of paroxetine on METH abuse. By contrast, the other SSRI tested here, fluvoxamine, did not affect METH CPP. These data demonstrate that there are differences in the effects of SSRIs on METH CPP, suggesting the possibility that molecules other than the SERT are involved in the inhibition of METH CPP by paroxetine and fluoxetine reported here and in our previous study.[8]

In addition to SERT inhibition, paroxetine inhibits the function of muscarinic cholinergic receptors,[11] nicotinic acetylcholine receptors,[12] volume-related anion channels,[13] membrane steroid transporters,[14] and nitric oxide synthase.[15] Recently, Kobayashi and colleagues[16] reported that paroxetine also inhibits the function of G protein-activated inwardly

rectifying K^+ (GIRK) channels. It is intriguing that paroxetine and fluoxetine, but not fluvoxamine, inhibit GIRK channels.[16, 17, 18] Various G protein-coupled receptors (such as M2 muscarinic, α2 adrenergic, D_2 dopaminergic, 5-HT_{1A}, opioid, nociceptin/orphanin FQ, and A_1 adenosine) activate GIRK channels[19, 20, 21, 22] through the direct action of G protein subunits.[23] In addition, GIRK channels are activated by ethanol independently of G protein-coupled signaling pathways.[24, 25] Activation of GIRK channels leads to membrane hyperpolarization.[19] These channels play an important role in the inhibitory regulation of neuronal excitability. Thus, modulators of GIRK channel activity may affect many brain functions. Kobayashi and colleagues[26] also have reported that ifenprodil, a cerebral vasodilator which inhibits morphine CPP,[27] also inhibits the function of GIRK channels. Morgan and colleagues[28] demonstrated that GIRK channel knockout mice exhibited dramatically reduced intravenous self-administration of cocaine. In the present study, we found that paroxetine and fluoxetine, but not fluvoxamine, inhibited METH CPP. These findings, together with the previous findings, suggest that the inhibition of GIRK channels by paroxetine or fluoxetine may be involved in the inhibition of METH CPP by these drugs.

Fluvoxamine administration (60 mg/kg) leads to a significant decrease in spontaneous locomotor activity.[29] Consistent with this observation, significant decreases in transition scores were observed in all of the 100 mg/kg fluvoxamine-treated groups compared to the saline/saline-treated group in the present study. The number of transitions of the fluvoxamine/fluvoxamine treated group during the CPP test phase (101.4 ± 85.3, mean ± SEM) was the smallest among the four groups in this experiment, but more than 100 transitions indicated adequate locomotion to reveal potential differences in CPP. The lack of effect of fluvoxamine on CPP for methamphetamine is likely to reflect a lack of effect of fluvoxamine on

the rewarding effects of METH rather than being a nonspecific effect of fluvoxamine.

CONCLUSION

We found that paroxetine, but not fluvoxamine, inhibited METH CPP in mice. Although further preclinical studies are needed to elucidate the mechanisms underlying these inhibitory effects of paroxetine on processes relating to METH dependence, it appears worthwhile to investigate the clinical effects of paroxetine on METH abuse. The present results suggest that molecules other than the SERT (such as GIRK channels) are involved in the inhibition of METH CPP by paroxetine and fluoxetine.

ACKNOWLEDGEMENTS

We are grateful to Dr. Yasukazu Ogai for statistics instruction, Dr. Keiko Matsuoka for animal care, Yukiko Sakaki and Akira Sato for technical assistance, and Michael Arends for editorial assistance. This work was supported by a research grant (17025054) from the MEXT of Japan, by grants (H17-pharmaco-001, H16-iyaku-029, H18-shitei-3) from the MHLW of Japan, and by Japan-U.S. Brain Research Cooperative Program grant from JSPS. AM was supported by a USA National Institute on Drug Abuse grant (R01 DA11946).

REFERENCES

1. United Nations International Drug Control Programme 2005. World drug report 2005. New York: Oxford University Press.

2. Ujike, H. 2002. Stimulant-induced psychosis and schizophrenia: the role of sensitization. Curr Psychiatry Rep. **4**: 177-184.

3. National Institute on Drug Abuse 2002. Methamphetamine abuse and addiction (Research report series, NIH pub. no. 98-4210). Bethesda MD: National Institute on Drug Abuse.

4. Sora, I., C. Wichems, N. Takahashi, *et al*. 1998. Cocaine reward models: conditioned place preference can be established in dopamine- and in serotonin-transporter knockout mice. Proc. Natl. Acad. Sci. USA **95**: 7699-7704.

5. Rocha, B. A., F. Fumagalli, R. R. Gainetdinov, *et al*. 1998. Cocaine self-administration in dopamine-transporter knockout mice. Nat Neurosci. **1**: 132-137.

6. Sora, I., F. S. Hall, A. M. Andrews, *et al*. 2001. Molecular mechanisms of cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference. Proc. Natl. Acad. Sci. USA **98**: 5300-5305.

7. Shen, H. –W., Y. Hagino, H. Kobayashi, *et al*. 2004. Regional differences in extracellular dopamine and serotonin assessed by in vivo microdialysis in mice lacking dopamine and/or serotonin transporters. Neuropsychopharmacology **29**: 1790-1799.

8. Takamatsu, Y., H. Yamamoto, Y. Ogai, *et al*. 2006. Fluoxetine as a potential pharmacotherapy for methamphetamine dependence: studies in mice. Ann. N. Y. Acad. Sci. **1074**: 295-302.

9. Hoffman, D. C. & R. J. Beninger. 1989. Preferential stimulation of D1 or D2 receptors disrupts food-rewarded operant responding in rats. Pharmacol. Biochem. Behav. **34**: 923-925.

10. Ide, S., M. Minami, M. Satoh, *et al*. 2004. Buprenorphine antinociception is abolished, but naloxone-sensitive reward is retained, in mu-opioid receptor knockout mice. Neuropsychopharmacology **29**: 1656-1663.

11. Stanton, T., C. Bolden-Watson, B. Cusack, *et al*. 1993. Antagonism of the five cloned human muscarinic cholinergic receptors expressed in CHO-K1 cells by antidepressants and antihistaminics. Biochem. Pharmacol. **45**: 2352-2354.

12. Fryer, J. D. & R. J. Lukas. 1999. Antidepressants noncompetitively inhibit nicotinic acetylcholine receptor function. J. Neurochem. **72**: 1117-1124.

13. Maertens, C., G. Droogmans, R. Verbesselt, *et al*. 2002. Block of volume-regulated anion channels by selective serotonin reuptake inhibitors. Naunyn. Schmiedebergs Arch. Pharmacol. **366**: 158-165.

14. Pariante, C. M., A. Makoff, S. Lovestone, *et al* 2001. Antidepressants enhance glucocorticoid receptor function in vitro by modulating the membrane steroid transporters Br. J. Pharmacol. **134**: 1335-1343.

15. Finkel, M. S., F. Laghriss-Thode, B. G. Pollock, *et al*. 1996. Paroxetine is a novel nitric oxide synthase inhibitor. Psychopharmacol. Bull. **32**: 653-658.

16. Kobayashi, T., K. Washiyama & K. Ikeda K. 2006. Inhibition of G protein-activated inwardly rectifying K^+ channels by the antidepressant paroxetine. J. Pharmacol. Sci. **102**: 278-287.

17. Kobayashi, T., K. Washiyama & K. Ikeda. 2003. Inhibition of G protein-activated inwardly rectifying K^+ channels by fluoxetine (Prozac). Br. J. Pharmacol. **139**: 1119-1128.

18. Kobayashi, T., K. Washiyama & K. Ikeda. 2004. Inhibition of G protein-activated inwardly

rectifying K^+ channels by various antidepressant drugs. Neuropsychopharacology **29**: 1841-1851.

19. North, R. A. 1989. Drug receptors and the inhibition of nerve cells. Br. J. Pharmacol. **98**: 13-28.

20. Ikeda, K., T. Kobayashi, T. Ichikawa, *et al*. 1995. Functional couplings of the δ- and the κ-opioid receptors with the G-protein-activated K^+ channel. Biochem. Biophys. Res. Commun. **208**: 302-308.

21. Ikeda, K., T. Kobayashi, T. Ichikawa, *et al*. 1996. Comparison of the three mouse G-protein-activated K^+ (GIRK) channels and functional couplings of the opioid receptors with the GIRK1 channel. Ann. N. Y. Acad. Sci. **801**: 95-109.

22. Ikeda, K., K. Kobayashi, T. Kobayashi, *et al*. 1997. Functional coupling of the nociceptin /orphanin FQ receptor with the G-protein-activated K^+ (GIRK) channel. Mol. Brain. Res. **45**: 117-126.

23. Reuveny, E., P. A. Slesinger, J. Inglese, *et al*. 1994. Activation of the cloned muscarinic potassium channel by G protein βγ subunits. Nature **370**: 143-146.

24. Kobayashi, T., K. Ikeda, H. Kojima, *et al*. 1999. Ethanol opens G-protein-activated inwardly rectifying K^+ channels. Nat. Neurosci. **2**: 1091-1097.

25. Lewohl, J. M., W. R. Wilson, R. D. Mayfield, *et al*. 1999. G-protein-coupled inwardly rectifying potassium channels are targets of alcohol action. Nat. Neurosci. **2**: 1084-1090.

26. Kobayashi, T., K. Washiyama & K. Ikeda. 2006. Inhibition of G protein-activated inwardly rectifying K^+ channels by ifenprodil. Neuropsychopharmacology **31**: 516-524.

27. Suzuki, T., H. Kato, M. Tsuda, *et al*. 1999. Effects of the non-competitive NMDA receptor antagonist ifenprodil on the morphine-induced place preference in mice. Life. Sci. **64**:

PL151-PL156.

28. Morgan, A. D., M. E. Carroll, A. K. Loth, *et al*. 2003. Decreased cocaine self-administration in Kir3 potassium channel subunit knockout mice. Neuropsychopharacology **28**: 932-938.

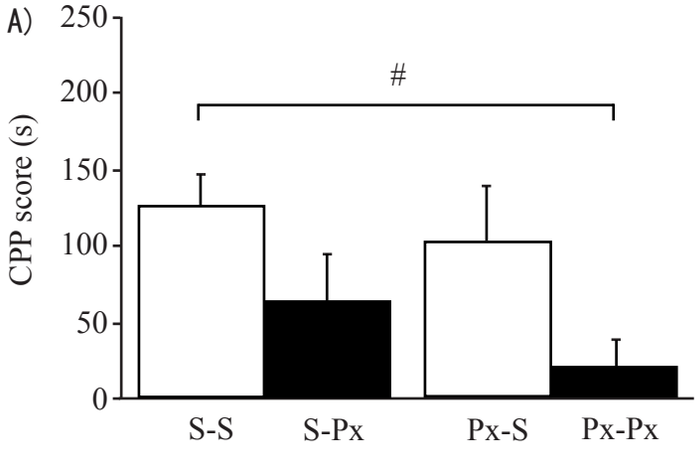
29. Ago, Y., T. Harasawa, S. Itoh, *et al*. 2005. Antidepressant-like effect of coadministration of sulpiride and fluvoxamine in mice. Eur. J. Pharmacol. **520**: 86-90.

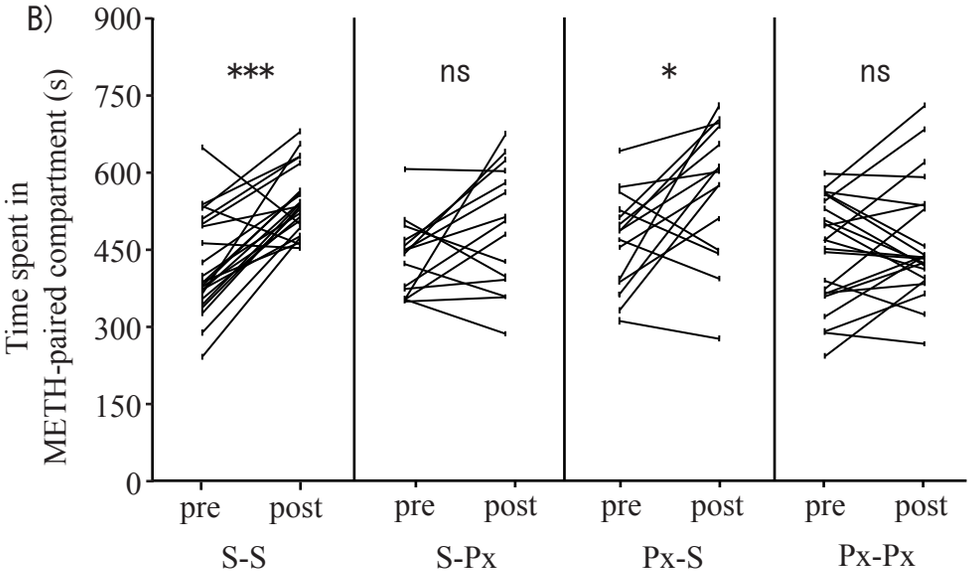
Figure legends

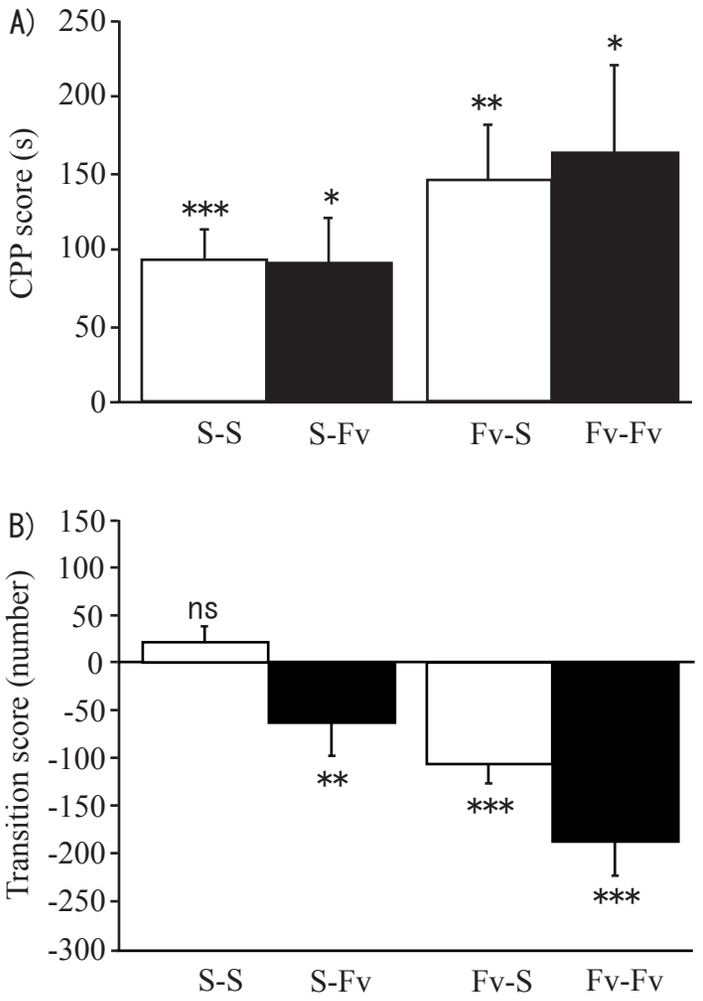
FIGURE 1. Effects of paroxetine on CPP for METH in mice. (A) Reduction of METH CPP by paroxetine (Px) pretreatment. Mice were pretreated with saline (S) in both the conditioning and CPP test phases (S-S), paroxetine only in the CPP test phase (S-Px), paroxetine only in the conditioning phase (Px-S), and paroxetine in both the conditioning and the CPP test phases (Px-Px). The CPP score was defined as the time spent in the drug-paired compartment during the CPP test phase (Day 9) minus the time spent in the same compartment during the preconditioning phase (Day 2). The CPP score of the Px-Px group was significantly lower than that of the S-S group ($^{\#}P < 0.05$). (B) Comparison of time spent in the conditioned compartment before and after conditioning in the four groups. There was a significant CPP in the S-S and Px-S groups, but not in the S-Px and Px-Px groups (when paroxetine was administered in the CPP test phase). $^{***}P < 0.001$, $^{*}P < 0.05$, ns: not significant ($P > 0.05$).

FIGURE 2. Effects of fluvoxamine on CPP for METH and on transitions between compartments. (A) Lack of a significant effect of fluvoxamine (Fv) on METH CPP. Mice were pretreated with saline in both the conditioning and the CPP test phases (S-S), fluvoxamine only in the CPP test phase (S-Fv), fluvoxamine only in the conditioning phase (Fv-S), and fluvoxamine in both the conditioning and the CPP test phases (Fv-Fv). There was a significant CPP in all groups. Fluvoxamine pretreatment in the conditioning phase and/or the CPP test phase failed to inhibit METH CPP (pre- and post-conditioning preference test results were analyzed with paired *t*-tests, $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$). (B) Decreases in transitions between the compartments by fluvoxamine pretreatment. There were significant decreases in transitions in the S-Fv, Fv-S,

and Fv-Fv groups, but not in the S-S group [number of transitions in the pre- and post-conditioning phases was analyzed with paired *t*-tests, ***$P < 0.001$, **$P < 0.01$, ns: not significant ($P > 0.05$)]. The transition score was defined as the number of transitions during the CPP test phase (Day 9) minus the number of transitions during the preconditioning phase (Day 2).


A)
250
200
150
100
50
0
CPP score (s)
#
S-S
S-Px
Px-S
Px-Px


B)
900
750
600
450
300
150
0
Time spent in
METH-paired compartment (s)
***
ns
*
ns
pre
post
S-S
S-Px
Px-S
Px-Px


A)
CPP score (s)
250
200
150
100
50
0
***
*
**
*
S-S
S-Fv
Fv-S
Fv-Fv
B)
Transition score (number)
150
100
50
0
-50
-100
-150
-200
-250
-300
ns
**
***
***
S-S
S-Fv
Fv-S
Fv-Fv