0026-895X/01/5904-894–900$3.00
MOLECULAR PHARMACOLOGY

Mol Pharmacol 59:894–900, 2001

Vol. 59, No. 4
556/891083
Printed in U.S.A.

Sensitized Increase of *Period* Gene Expression in the Mouse Caudate/Putamen Caused by Repeated Injection of Methamphetamine

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Received September 8, 2000; accepted December 5, 2000

This paper is available online at http://molpharm.aspetjournals.org

ABSTRACT

Methamphetamine (MAP) causes the sensitization phenomena not only in MAP-induced locomotor activity, dopamine release, and Fos expression, but also in MAP-induced circadian rhythm. Cocaine-induced sensitization is reportedly impaired in *Drosophila melanogaster* mutant for the *Period* (*Per*) gene. Thus, sensitization may be related to induction of the *Per* gene. A rapid induction of *mPer1* and/or *mPer2* in the suprachiasmatic nucleus after light exposure is believed to be necessary for light-induced behavioral phase shifting. Although the caudate/putamen (CPu) expresses *mPer1* and/or *mPer2* mRNA, the function of these genes in this nucleus has not yet been elucidated. Therefore, we examined whether MAP affects the expression of *mPer1* and/or *mPer2* mRNA in the mouse CPu. Injection of MAP augmented the expression of *mPer1* but not *mPer2* or *mPer3* in the CPu, and this MAP-induced increase in *mPer1* expression lasted for 2 h. Also, the MAP-induced increase of *mPer1* mRNA was strongly antagonized by pretreatment with a dopamine D1 receptor and *N*-methyl-D-aspartate (NMDA) receptor antagonist, but not by a D2 receptor antagonist. Interestingly, application of either the D1 or the D2 agonist alone did not cause *mPer1* expression. The present results demonstrate that activation of both NMDA and D1 receptors is necessary to produce MAP-induced *mPer1* expression in the CPu. Repeated injection of MAP caused a sensitization in not only the locomotor activity but also *mPer1* expression in the CPu without affecting the level of *mPer2*, *mPer3*, or *mTim* mRNA. Thus, these results suggest that MAP-induced *mPer1* gene expression may be related to the mechanism for MAP-induced sensitization in the mouse.

A core clock mechanism in the mouse suprachiasmatic nucleus (SCN) seems to involve a transcriptional feedback loop in which CLOCK and BMAL1 function as positive regulators and the three *mPeriod* (*mPer*) genes, *Per1* (Sun et al., 1997; Tei et al., 1997), *Per2* (Albrecht et al., 1997; Shearman et al., 1997; Takumi et al., 1998), and *Per3* (Zylka et al., 1998) are involved in negative feedback (Dunlap, 1999). In addition, it was determined that two mouse cryptochrome genes, *mCry1* and *mCry2*, act in the negative limb of the clock feedback loop (Kume et al., 1999). It is already well known that the SCN contains a master pacemaker that regulates behavioral and physiological circadian rhythms such as locomotor activity, body temperature, and endocrine release (Inouye and Shibata, 1994). Interestingly, expression of the *Per* gene occurs not only in the SCN but also in other brain areas such as the cerebral cortex, caudate/putamen (CPu), and cerebellum (Albrecht et al., 1997; Shearman et al., 1997). However, the function of clock genes outside of the SCN has not been fully elucidated.

Destruction of the SCN abolishes the circadian rhythms of many physiological functions (Inouye and Shibata, 1994). On the other hand, there are at least two oscillators outside the SCN: a food-associated oscillation entrained by daily restricted feeding (Mistlberger, 1994) and methamphetamine (MAP)-induced oscillation produced by its daily injection (Shibata et al., 1994, 1995). In addition, oral administration of MAP through drinking bottle initiates a circadian rhythm with a long free-running period even after SCN ablation (Honma et al., 1987). Based on these facts, it has been suggested that other circadian oscillators such as the MAP-induced rhythm exist in areas other than the SCN. Thus, it is possible that *mPer* mRNA outside of the SCN regulates the SCN-independent circadian rhythm, and rapid induction of *mPer* outside of the SCN by MAP may entrain the SCN-independent oscillation.

This study was partially supported by grants awarded to S.S. from the Japanese Ministry of Education, Science, Sports, and Culture (11170248, 11233207, and 11145240), and by a grant-in-aid for Encouragement of Young Scientists to T.M. from the Japan Society for the Promotion of Science (11771503).

ABBREVIATIONS: SCN, suprachiasmatic nucleus; MAP, methamphetamine; CPu, caudate/putamen; NMDA, *N*-methyl-D-aspartate; *Per*, *Period*; *Tim*, *timeless*; PB, phosphate buffer; PFA, paraformaldehyde.

Recently it was reported that sensitization to repeated cocaine exposure, a phenomenon also seen in humans and animal models and associated with enhanced drug craving, is eliminated in flies mutant for *period*, *clock*, *cycle*, and *double-time*, but not in flies mutant for *timeless* (Andretic et al., 1999) We demonstrated that the MAP-induced free-running oscillation of rat locomotion with drinking application of MAP exhibits a sensitization phenomenon (Nikaido et al., 1999). Therefore, the next progressive step was to examine whether MAP induces *Per* expression in the CPu, and whether sensitization is involved in MAP-induced *Per* expression but not *timeless* expression.

Treatment with MAP is known to increase locomotor activity and Fos expression in the CPu (Graybiel et al., 1990). Pharmacological studies have further revealed that both MAP-induced hyperlocomotion and Fos expression in the CPu are attenuated by pretreatment with dopamine D1, D2, or NMDA receptor antagonists (Ujike et al., 1989; Kuribara and Uchihashi, 1993; Kuribara, 1994, 1995, 1996; Yoshida et al., 1995). Thus, it has been suggested that D1, D2, and NMDA receptors play an important role in the sensitization induced by repeated injection of MAP. This evidence suggests that MAP-induced *Per* expression may be involved in the activation of both dopamine and NMDA receptors. Therefore, in the first part of our present experiment, we examined the pharmacological characteristics of MAP-induced *Per* gene expression in the mouse CPu. Then, in the latter part, we examined the expression pattern of *Per* and *timeless* mRNA using animals sensitized to MAP.

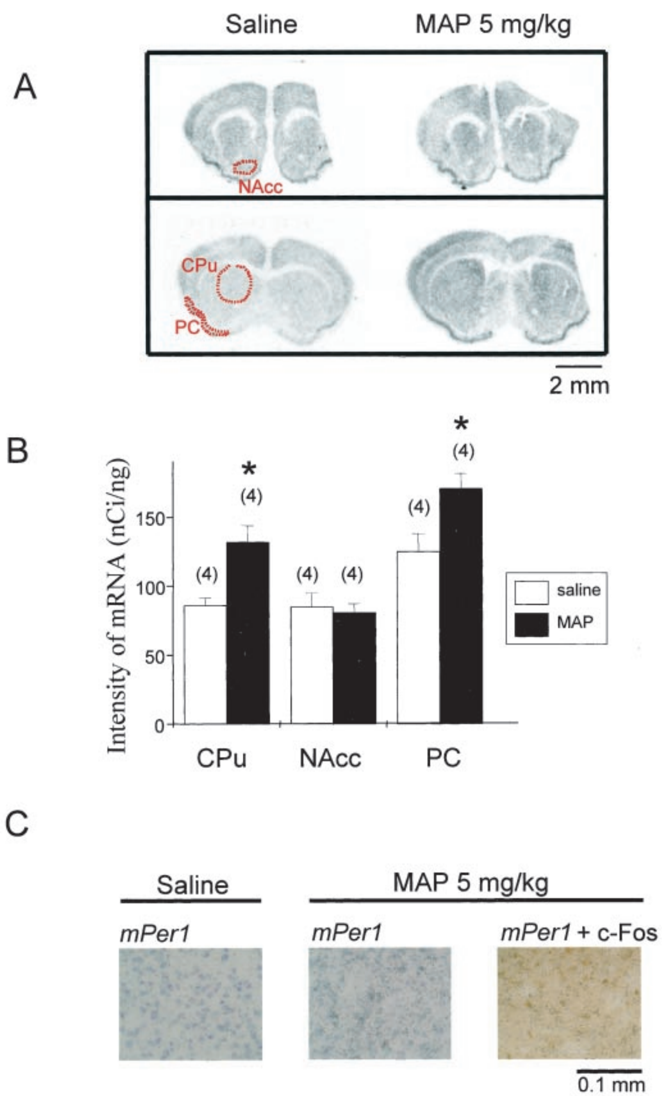

Fig. 1. Effect of 5 mg/kg of MAP on *mPer1* expression in CPu, accumbens (NAcc), and piriform cortex (PC). Mice were decapitated 60 min after MAP injection. A, representative in situ hybridization autoradiograms of *mPer1* on X-ray film. The brain areas surrounded by a broken line exhibit the NAcc, CPu, and PC areas. B, radioisotope in situ hybridization was performed for quantitative analysis purposes. Numbers in parentheses indicate the number of animals (*$P < 0.05$ in comparison with saline by Student's *t* test). C, emulsion autoradiograms of *mPer1* in the dorsal caudate. A 5-mg/kg injection of MAP increased *mPer1* expression (black dots, middle panel), and there were many dots on cells showing the Fos immunoreactivity (right panel). Fos immunoreactive cells were stained by a brown color.

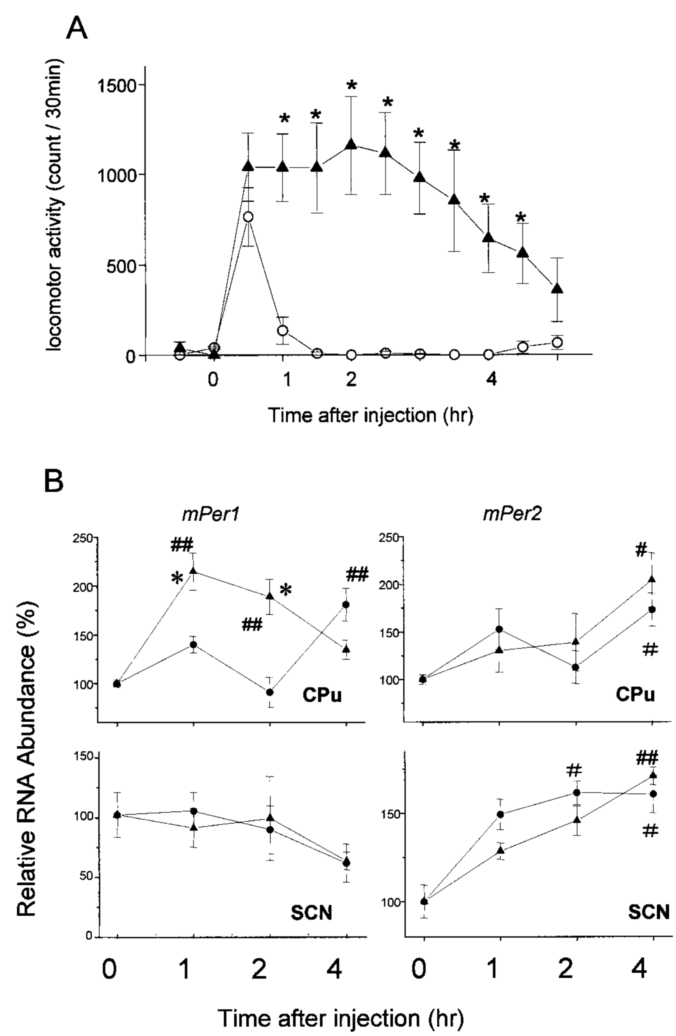

Fig. 2. Time course of MAP-induced locomotor activity (A) and *mPer* expression in the CPu or SCN (B). A, MAP increased mouse locomotion for 4 h. ○, saline injection ($n = 4$); ▲, 5 mg/kg MAP injection ($n = 4$) (*$P <$ 0.05 in comparison with saline by Student's *t* test). B, relative value of *mPer1* and *mPer2* mRNA expression in the CPu. ●, saline injection; ▲, 5 mg/kg MAP injection. *Per* expression observed in the saline group immediately after saline injection was set as 100%. Three to four animals made up each point (*$P < 0.05$ in comparison with saline by Student's *t* test; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ in comparison with 0-min point by Dunnett's test).

Materials and Methods

Animals. In all experiments, we used 4- to 6-week-old male *ddY* mice (Takasugi, Saitama, Japan) maintained under a 12 h:12 h light/dark cycle. All animals were allowed free access to food and water and were treated in accordance with the Law no. 105 and Notification no. 6 of the Japanese Government.

Locomotor Activity Measurement. For assessment of the locomotor activity, mice were housed individually in transparent plastic cages (31 × 20 × 13 cm). Motor activity was measured using an infrared area sensor (F5B; Omron, Tokyo, Japan), and the activity count (number of movements) was recorded by computer and stored on disk at 5-min intervals.

Sample Preparation. Mice were deeply anesthetized with ether and intracardially perfused with 0.1 M phosphate buffer (PB), pH 7.4, containing 4% paraformaldehyde (PFA). Brains were removed, postfixed in 0.1 M PB containing 4% PFA for 24 h at 4°C, and transferred into 20% sucrose in PB for 72 h at 4°C. Brain slices (40 μm thick) including the CPu, accumbens, piriform cortex, and SCN were made using a cryostat (HM505E; Microm, Walldorf, Germany) and placed in 2× standard saline citrate until processing for hybridization.

In Situ Hybridization. The quantity of *mPer1*, *mPer2*, *mPer3*, or *mTim* mRNA expression in the various brain areas was studied by means of in situ hybridization. Slices were treated with 1 μg/ml proteinase K in 10 mM Tris-HCl buffer, pH 7.5, containing 10 mM EDTA for 10 min at 37°C followed by 0.25% acetic anhydride in 0.1 M triethanolamine and 0.9% NaCl for 10 min. The slices were then incubated in the hybridization buffer [60% formamide, 10% dextran sulfate, 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 0.6 M NaCl, 1× Denhardt's solution (0.02% Ficoll, 0.02% polyvinyl pyrrolidone, 0.02% bovine serum albumin), 0.2 mg/ml transfer RNA, 0.25% sodium dodecyl sulfate] containing ^{33}P-labeled cRNA probes for 16 h at 60°C. Radioisotope ([α-^{33}P]UTP)-labeled antisense cRNA probes (PerkinElmer Life Sciences, Boston, MA) were made from restriction enzyme-linearized cDNA templates [nucleotide positions: *mPer1* (538–1752), *mPer2* (1–638), *mPer3* (814–1955), *mTim* (236–909)] kindly provided by Dr. Okamura (Kobe University, Kobe, Japan). After a high-stringency posthybridization wash in 2× standard saline citrate/50% formamide, slices were treated with RNaseA (10 μg/ml) for 30 min at 37°C.

The radioactivity of each slice visualized on BioMax MR film (Eastman Kodak, Rochester, NY) was analyzed using a microcomputer interface to an image analysis system (MCID; Imaging Research Inc., ON, Canada) after conversion into optical density by ^{14}C-autoradiographic microscales (Amersham Pharmacia Biotech, Buckinghamshire, UK). For data analysis, we subtracted the intensities of the optical density in sections from the corpus callosum from those in the SCN, CPu, piriform cortex, and accumbens and regarded this value as the net intensity for these areas. The intensity values of sections from the rostral to the caudal part of the SCN, CPu, piriform cortex, and accumbens (three to five sections per mouse brain) were then summed; the sum was considered to be a measure of the amount of *mPer1*, *mPer2*, *mPer3*, or *mTim* mRNA in this region. To express the relative mRNA abundance (Figs. 2–5 and 7), the intensity values of vehicle treatment were adjusted to 100.

Immunohistochemistry of Fos Protein and Emulsion Autoradiography of *mPer1*. The slices were fixed with 4% PFA and processed for immunohistochemistry according to the avidin-biotin-peroxidase complex method. Primary antibody (anti-Fos, 1:5000; Cambridge Research Biochemical, Northwich, UK) was diluted in 0.1 M phosphate buffer containing 1% normal goat serum in 0.3% Triton X-100.

For emulsion autoradiography, slices already processed for immunohistochemistry were dipped into emulsion (NTB2, Eastman Kodak; diluted 1:1 with distilled water) after hybridization with the *mPer1* probe, air dried for 3 h, and stored in light-tight slide boxes at 4°C for 2 weeks. The slides were developed using a D19 developer (Eastman Kodak) and then fixed with Fujifix (Fujifilm, Tokyo, Japan). Subnuclear sliver grain distribution in the CPu was examined using an optical microscope. We did not adopt the quantitative analysis of emulsion autoradiogram because thickness of the coating could not be controlled using the present emulsion-dipping method.

Drugs and Application Schedule. The drugs used in this experiment consisted of methamphetamine HCl (Dainippon Co., Tokyo, Japan); SCH23390 (Funakoshi, Tokyo, Japan); (+)-sulpiride (Sigma, St. Louis, MO); and MK-801, SKF38393, and quinpirole (RBI/Sigma, Natick, MA). All drugs were dissolved into the physiological saline. Drugs were injected during the daytime because spontaneous locomotor activity and *mPer1* expression in the CPu were low at this time (data not shown). To examine the blocking effect of receptor antagonists on MAP-induced *mPer1* expression, these receptor antagonists were injected 15 min before MAP injection.

A single high-dose exposure to MAP or amphetamine sufficiently induces long-term behavioral and neurochemical sensitization (Ohno et al., 1994; Vanderschuren et al., 1999). Therefore, for sensitization experiments, MAP (5 mg/kg) was injected once, and then a small dose of MAP (0.5 mg/kg) was injected again after a 7-day interval.

Statistics. Results are expressed as the mean ± S.E.M. The significance of differences between groups was determined by two-way or one-way analysis of variance followed by Dunnett's test or Student's *t* test.

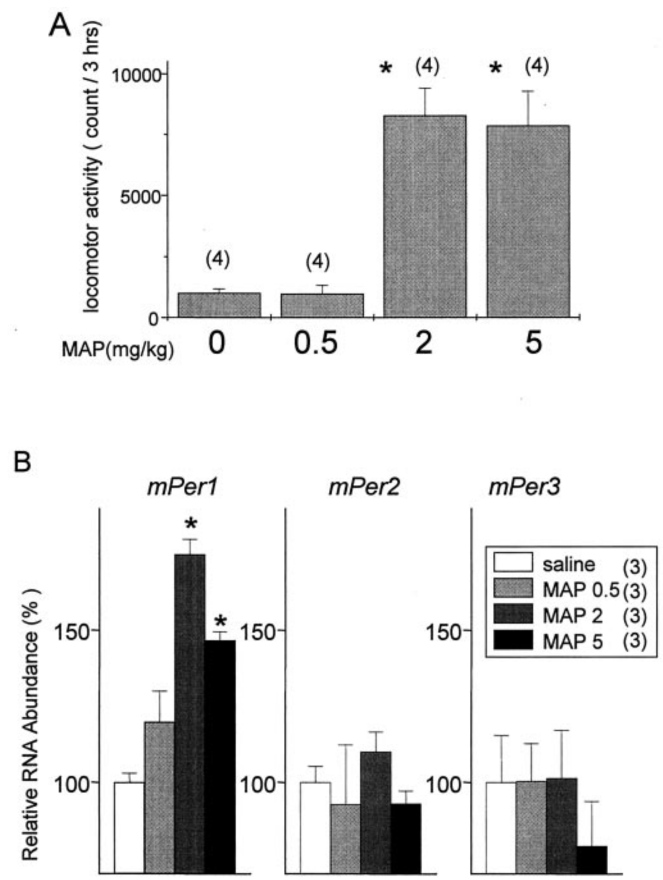

Fig. 3. Dose-response of MAP-induced locomotor activity (A) and *mPer* expression in the CPu (B). A, vertical values exhibit total locomotor counts 3 h after MAP injection. Numbers in parentheses indicate the number of animals (**P* < 0.05 in comparison with saline by Dunnett's test). B, relative value of *mPer1*, *mPer2*, and *mPer3* mRNA expression in the CPu. *Per* expression observed in the saline group was set as 100%. Three animals made up each point (**P* < 0.05 in comparison with saline by Dunnett's test).

Results

Methamphetamine-Induced *mPer1* Expression in the Various Brain Areas. It is well known that dopaminergic neurons innervate the CPu, accumbens, and piriform cortex. Therefore, we examined the amount of *mPer1* expression in these brain areas. Figure 1A shows the representative brain areas responding to MAP and the sampling area for each brain slice. Basal level *mPer1* expression was high in the piriform cortex but low in the CPu and accumbens (Fig. 1, A and B). Unrelated to basal expression, MAP significantly induced *mPer1* expression in the CPu ($P < 0.05$, Student's t test) and piriform cortex ($P < 0.05$, Student's t test) but not in the accumbens (Fig. 1, A and B). Previous papers demonstrated a strong induction of Fos protein in the dorsal CPu (Yoshida et al., 1995); therefore, we examined the Fos expression and *mPer1* induction using CPu slices. Interestingly, *mPer1* mRNA and Fos immunoreactivity were coexpressed in the same striatal cells (Fig. 1C).

Time Course of MAP-Induced Locomotion and *mPer1* Expression. A 5-mg/kg injection of MAP significantly increased the locomotion, which was maintained for 4 h (Fig. 2A). Two-way analysis of variance revealed an interaction between drug treatment and time course in CPu *mPer1* [$F(3,23) = 13.1$, $P < 0.01$]. Post hoc Dunnett's test demonstrated that the same dose of MAP increased *mPer1* expression in the CPu 1 h ($P < 0.05$) and 2 h ($P < 0.05$) after MAP injection, but not 4 h after injection. On the other hand, MAP did not increase *mPer2* expression in the CPu. In the SCN, MAP did not affect the expression of *mPer1* or *mPer2* at any time point after MAP injection. The increased basal level of *mPer1* and *mPer2* in the CPu and *mPer2* in the SCN may reflect a circadian change in expression of these genes (Fig. 2).

Dose-Response Curve for MAP-Induced Locomotion and *mPer* mRNA. At a dose of 0.5 mg/kg, MAP did not change the locomotion or *mPer* expression in the CPu (Fig. 3). Two milligrams per kilogram was a sufficient dose to produce significant increase in locomotion ($P < 0.05$, Dunnett's test) and also *mPer1* expression in the CPu [$F(3,8) = 29.9$, $P < 0.01$; $P < 0.05$, Dunnett's test]. In this experiment, MAP at any dose did not affect the expression of *mPer2* [$F(3,8) = 0.6$, $P > 0.05$] or *mPer3* [$F(3,8) = 0.5$, $P > 0.05$] in the CPu.

Effect of DA and NMDA Receptor Antagonists on MAP-Induced Locomotion and *mPer1* Expression in the CPu. Next, we examined the pharmacological profile of MAP-induced *mPer1* expression in the CPu. MAP (5 mg/kg)-induced hyperlocomotion was significantly blocked by SCH23390 ($P < 0.01$, Dunnett's test) and sulpiride ($P < 0.01$) but not by MK-801 ($P > 0.05$) (Fig. 4A). Treatment with MAP produced a strong increase in the expression of *mPer1* in the CPu (Fig. 4, B and C) that was completely attenuated by SCH23390 ($P < 0.01$, Dunnett's test), moderately attenuated by MK-801 ($P < 0.01$), and unaffected by sulpiride ($P > 0.05$) (Fig. 4, B and C). The basal level of *mPer1* in the CPu was also significantly reduced by SCH23390 ($P < 0.05$, Dunnett's test) and MK-801 ($P < 0.01$) but increased by sulpiride ($P < 0.05$) (Fig. 4C).

Effect of D1 and D2 Receptor Agonist on *mPer* Expression in the CPu. Because MAP-induced *mPer1* expres-

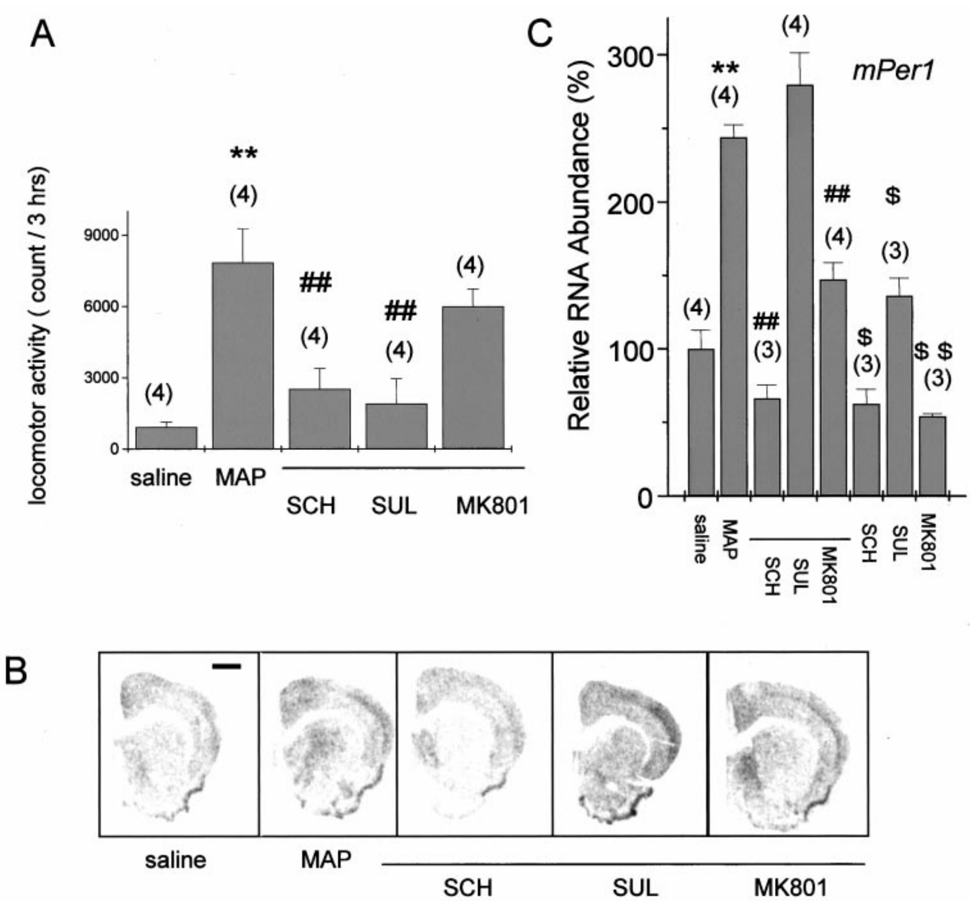

Fig. 4. Effects of D1, D2, and NMDA receptor antagonists on MAP-induced locomotor activity (A) and *mPer1* expression in the CPu (B and C). A, vertical values exhibit total locomotor counts 3 h after saline or MAP injection. Antagonists were injected 15 min before the 5-mg/kg MAP injection. Numbers in parentheses indicate the number of animals ($^{**}P < 0.01$ in comparison with saline by Student's t test; $^{##}P < 0.01$ in comparison with MAP injection only by Dunnett's test). B, representative in situ hybridization autoradiograms of *mPer1* were developed on X-ray film. The D1 receptor antagonist (SCH) and NMDA receptor antagonist (MK-801), but not the D2 receptor antagonist (SUL), antagonized MAP-induced *mPer1* expression. Calibration on the saline panel is 1 mm. C, relative value of *mPer1* mRNA expression in the CPu. *Per* expression observed in the saline group was set as 100%. Antagonists were injected 15 min before the 5-mg/kg MAP injection, and then mice were decapitated 60 min after MAP injection. Numbers in parentheses indicate the number of animals ($^{**}P < 0.01$ in comparison with saline by Student's t test; $^{##}P < 0.01$ in comparison with MAP injection only; $^{\$}P < 0.05$, $^{\$\$}P < 0.01$ in comparison with saline by Dunnett's test). SCH, SCH23390; SUL, sulpiride.

sion in the CPu was attenuated by the D1 receptor antagonist and facilitated by the D2 receptor antagonist, we examined the effect of D1 and D2 receptor agonists on the basal level of *mPer1* expression. Application of D1 receptor agonist SKF38393 did not affect *mPer1* and *mPer2* expression in the CPu (Fig. 5), whereas the D2 receptor agonist, quinpirole, slightly reduced *mPer1* and *mPer2* expression (Fig. 5).

Sensitized Expression of Locomotion and *mPer* mRNA in the CPu with Repeated Injection of MAP. In preparing the four experimental groups, MAP or saline was injected initially into all mice, then half of the MAP- (5 mg/kg) or saline-injected groups received another injection of MAP (0.5 mg/kg) or saline. Figure 6, A and B demonstrates the time course of locomotion (Fig. 6A) after injection of saline or MAP (0.5 mg/kg) and total locomotor counts (Fig. 6B) 60 min after injection, respectively. Small doses of MAP at 0.5 mg/kg did not increase locomotion in the saline-pretreated group but significantly increased locomotion in MAP (5 mg/kg)-pretreated mice [$F(3,12) = 6.9$, $P < 0.01$] (Fig. 6B).

A second injection of MAP (0.5 mg/kg) strongly increased *mPer1* mRNA in the CPu of mice pretreated with MAP at 5 mg/kg [$F(3,12) = 20.1$, $P < 0.01$] (Fig. 7, A and B), but 0.5 mg/kg of MAP did not affect the expression of *mPer2* [$F(3,12) = 1.8$, $P > 0.05$] or *mTim* mRNA [$F(3,12) = 1.1$, $P > 0.05$] (Fig. 7B).

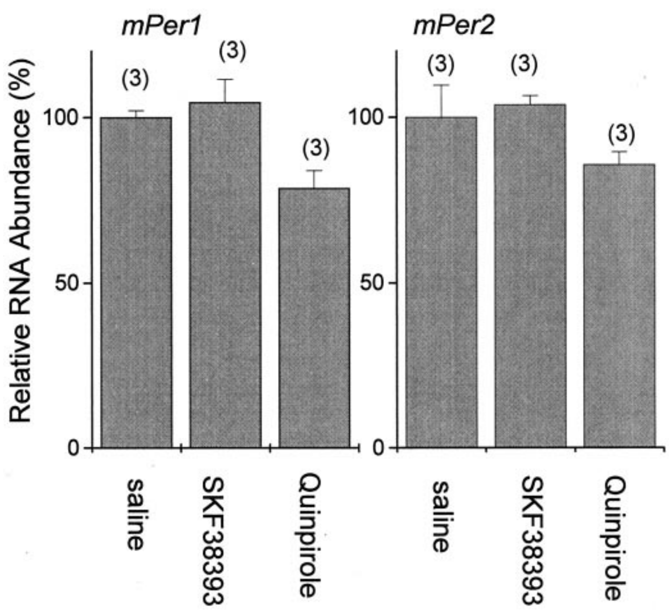

Fig. 5. Effect of the D1 (SKF38393) and D2 (quinpirole) receptor agonists on *mPer1* and *mPer2* expression in the CPu. Mice were decapitated 60 min after each agonist injection. Columns represent the relative value of m*Per1* mRNA expression in the CPu. *Per* expression observed in the saline group was set as 100%. Numbers in parentheses indicate the number of animals.

Discussion

In the present experiment, we demonstrated that MAP dose dependently induces the expression of *mPer1* in the CPu and piriform cortex but not in the accumbens or SCN, whereas MAP did not affect the levels of *mPer2* and *mPer3* in these brain areas. Coadministration of D1 receptor antagonist SCH23390 or NMDA receptor antagonist MK-801 significantly attenuated MAP-induced expression of *mPer1*, but D2 receptor antagonist sulpiride did not block this expression. Interestingly, Fos induction in the CPu by MAP injection was reportedly attenuated by either SCH23390 or MK-801 (Konradi et al., 1994, 1996; Ohno et al., 1994; Yoshida et al., 1995). Treatment with MK-801 attenuated MAP-induced *mPer1* expression in the CPu. Previous reports demonstrated that MK-801 inhibited amphetamine-induced glutamate release in the ventral tegmental area (Wolf and Xue, 1999), MAP-induced striatal dopamine release (Finnegan and Taraska, 1996), and striatal Fos expression (Ohno et al., 1994). Thus, NMDA receptor mechanisms are also involved in MAP-induced biochemical responses such as *mPer1* and Fos expression. From a pharmacological point of view, above-mentioned articles have suggested that there may be common neural mechanisms between the expression of Fos and of *mPer1* induced by MAP. The present double-staining experiment demonstrated a dense expression of both Fos and *mPer1* mRNA in the dorsomedial regions of the CPu, which also support the above possibility.

In the present experiment, MAP increased the level of *mPer1* but did not affect the levels of *mPer2* and *mPer3* in the CPu. Application of forskolin induced *Per1* but not *Per2* expression in Rat-1 cells with the induction of cyclic AMP-responsive element binding protein phosphorylation; then it initiated the oscillation of *Per2* expression (Yagita and Oka-

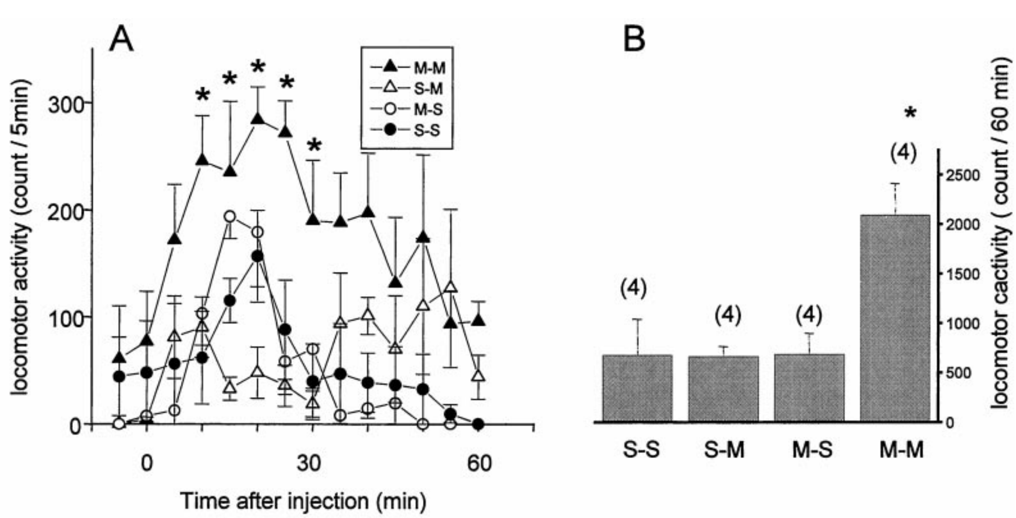

Fig. 6. Sensitization of locomotor activity induced by repeated application of MAP. A, time course of locomotor activity. M-M, MAP (5 mg/kg) injection followed by MAP (0.5 mg/kg) injection with 7-day withdrawal; S-M, saline injection followed by MAP (0.5 mg/kg) injection with 7-day interval; M-S, MAP (5 mg/kg) injection followed by saline injection with 7-day withdrawal; S-S, saline injection followed by saline injection with 7-day interval (*$P < 0.05$ in comparison with S-M group by Student's *t* test). B, total activity counts over a duration of 60 min (*$P < 0.05$ in comparison with S-M group by Dunnett's test). Numbers in parentheses indicate the number of animals.

mura, 2000). Interestingly, we found that the promoter region of *mPer1* contains a total of four cyclic AMP-responsive elements (Yamaguchi et al., 2000). This cyclic AMP-responsive element site may be responsible for the *mPer1* induction that occurs with MAP application. In fact, not only D1 and NMDA receptor activation (Das et al., 1997) but also MAP application (Muratake et al., 1998) reportedly cause cyclic AMP-responsive element binding protein phosphorylation.

Light exposure strongly increases *Per1* and *Per2* but not *Per3* expression in the SCN of mice, rats, and hamsters (Shigeyoshi et al., 1997; Yan et al., 1999; Horikawa et al., 2000); however, in our study, MAP failed to change *mPer* gene expression in the SCN. We found a significant circadian oscillation of *Per1* and *Per2* in the hamster SCN with a peak at subjective day (Horikawa et al., 2000). Local injection of NMDA into the SCN at subjective night causes the induction of *Per1* in the hamster (Moriya et al., 2000). On the other hand, stimulation of the D1 receptor in the SCN produces Fos induction in early developmental rodents but not in adults (Viswanathan et al., 1994; Weaver and Reppert, 1995; Grosse and Davis, 1999). Thus, the reason why MAP failed to produce *mPer1* expression in the SCN may be related to weak contribution of D1 receptors in the adult SCN. Changes in *mPer1* mRNA levels of CPu detected by in situ hybridization exhibited a circadian rhythm with a peak at early subjective night (data not shown). Therefore, it is interesting to determine whether transiently induced *mPer1* in the CPu at subjective day may cause a phase shift of circadian rhythm of *mPer1* expression in the CPu. The answer requires an examination of circadian time course of *mPer1* expression in the CPu subsequent to MAP treatment, and this important question should be the follow-up to this article.

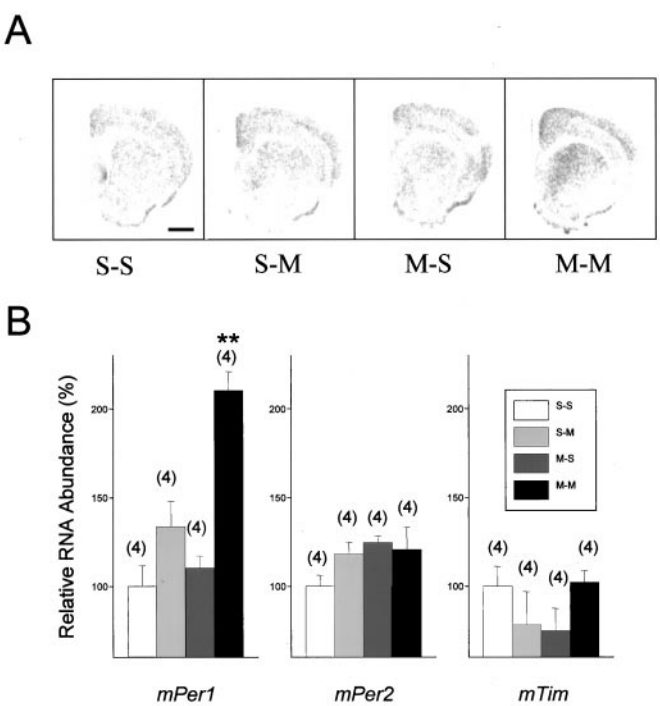

Fig. 7. Sensitization of *mPer1*, but not of *mPer2* or *mTim*, induced by repeated application of MAP. A, representative in situ hybridization autoradiograms of *mPer1* developed on X-ray film. M-M, MAP (5 mg/kg) injection followed by MAP (0.5 mg/kg) injection with 7-day withdrawal; S-M, saline injection followed by MAP (0.5 mg/kg) injection with 7-day interval; M-S, MAP (5 mg/kg) injection followed by saline injection with 7-day withdrawal; S-S, saline injection followed by saline injection with 7-day interval. As shown in panel M-M, a small doses of MAP (0.5 mg/kg) augmented *mPer1* expression in the dorsal caudate of the MAP (5 mg/kg)-pretreated mouse. Calibration on the S-S panel is 1 mm. B, relative value of *mPer1*, *mPer2*, or *mTim* mRNA expression in the CPu. *Per* expression observed in the S-S group was set as 100%. Mice were decapitated 60 min after the last MAP or saline injection. Numbers in parentheses indicate the number of animals (**$P < 0.01$ in comparison with S-S by Dunnett's test).

In this experiment, SCH23390 and MK-801 administration alone lowered the expression of *mPer1* in the CPu, suggesting a tonic activation of the *mPer1* gene through D1 and/or NMDA receptors in the CPu. Actually, MK-801 decreased the MAP-induced *mPer1* expression but slightly augmented the MAP-induced locomotion. On the contrary, sulpiride strongly attenuated the MAP-induced locomotion without affecting *mPer1* expression. These results seemingly indicate that MAP-induced locomotor stimulation is not sufficient for induction of *mPer1* by MAP. One of our previous articles supports this idea by showing that the D1 and NMDA receptor blockade abolished MAP-induced anticipatory behavior without attenuating its induction of hyperlocomotion (Shibata et al., 1995).

In the present experiments, repeated administration of MAP caused sensitization in *mPer1* expression but not in *mPer2*, *mPer3*, or *mTim* expression in the CPu. Therefore, behavioral sensitization is associated with *mPer1* expression in the CPu but not that in the SCN, suggesting the important role of *mPer1* gene expression in MAP-induced behavioral sensitization. Furthermore, we demonstrated that the MAP-induced free-running oscillation of rat locomotion with drinking application of MAP exhibited a sensitization phenomenon (Nikaido et al., 1999). Interestingly, Andretic et al. (1999) reported that flies mutant for *period*, *clock*, *cycle*, and *double-time* lack sensitization to repeated cocaine administration, but flies mutant for *timeless* do not. On the other hand, in contrast to *Drosophila melanogaster,* mutation of the *Clock* gene that regulates circadian rhythm in mice does not affect acute or sensitized responses to cocaine (Sidiropoulou et al., 2000). Thus, in mammals, the *Clock* gene is not required for the induction of behavioral sensitization to cocaine. Therefore, we should investigate whether the sensitized increase in *mPer1* expression reflects the result or cause using *mPer1* gene mutant mice. Taken together, the results seem to indicate that MAP-induced sensitized expression of *mPer1* may be related, at least, to the sensitized phenomenon.

In conclusion, the present results demonstrate that the activation of both D1 and NMDA receptors plays an important role in causing MAP-induced expression of *mPer1* in the CPu. Furthermore, behavioral sensitization induced by repeated MAP injection is associated with sensitization of MAP-induced *mPer1* expression.

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