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Effects of SKF-38393, a dopamine D_1 receptor agonist on expression of amphetamine-induced behavioral sensitization and expression of immediate early gene arc in prefrontal cortex of rats

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Abstract

Repeated administrations of psychostimulants into rodents produce behavioral sensitization. We examined whether a dopamine D_1 agonist can reverse behavioral sensitization once established by repeated amphetamine (AMP) administrations and determined the mRNA expression levels of the D_1 and D_2 receptors, metabotropic glutamate receptor 1 (mGluR1), and activity-regulated cytoskeleton-associated protein (arc) in rats. Rats were pretreated with six intermittent AMP injections. Following a 14-day withdrawal period, the rats were divided into six groups and treated with either SKF-38393 (SKF; dopamine D_1 agonist), SCH-23390 (SCH; selective D_1 antagonist), YM-09151-2 (YM; selective D_2 antagonist), SKF+SCH, SKF+YM or physiological saline once daily for 5 days. Three days or 4 weeks after the reversal treatments, all the rats were rechallenged with AMP. D_1 and D_2 antagonist treatments produced no significant decreases in locomotor activity or stereotyped behavior rate, respectively. In the SKF treatment group, stereotyped behavior rate decreased markedly after the three-day and four-week withdrawal periods. SKF+SCH treatment inhibited the effect of SKF treatment. The rats in the other groups that received AMP with or without SKF were decapitated 1 h after treatment, and the mRNA levels of the D_1 and D_2 receptors, mGluR1, and arc were measured by TaqMan real-time reverse transcriptase-polymerase chain reaction (RT-PCR). AMP administration significantly increased arc level. SKF also increased arc level significantly after the first single injection and after repeated injections of AMP during the pretreatment. There was no significant difference in arc expression level between the saline and SKF treatment groups after the AMP challenge, suggesting that arc expression level is not involved in the reversal effects of SKF in AMP sensitization.

Keywords: Amphetamine; Dopamine D_1 agonist; Psychostimulants; Behavioral sensitization; Neuroplasticity; TaqMan RT-PCR; arc

1. Introduction

In Japan, amphetamine (AMP) and methamphetamine (MAP) are two of the most popular drugs that are abused. Drug addiction is a major social problem. Eighty-five percent of the people who have abused these drugs for over 5 years, have psychological problems (Wada, 1990). In the USA, Rawson suggested that significant MAP problems may persist or even expand (Rawson et al, 2002). MAP users are at much higher risk of infection with HIV than opiate users. Partly because MAP enhances libido, users of the drug typically also have many more sexual partners (Gibson et al., 2002).

Repeated intermittent administrations of psychostimulants, such as AMP, MAP and cocaine, produce behavioral sensitization characterized by either a progressive enhancement in the behavioral activity induced by these drugs or an enduring behavioral hypersensitivity to these drugs after treatment in animals (Utena, 1966; Robinson and Becker, 1986; Tadokoro and Kuribara, 1986). Behavioral sensitization persists for months and is thought to represent a permanent change in the neurobiology of an organism (Kalivas and Stewart, 1991). This phenomenon can be used in developing an animal model for drug-induced psychosis and drug craving in humans (Robinson

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and Becker, 1986; Robinson and Berridge, 1993; Lieberman et al., 1997; Laruelle, 2000).

Behavioral sensitization is closely associated with dopaminergic and glutamatergic systems in the brain (Steketee, 2003; Vanderschuren and Kalivas, 2000) (for review: Steketee, 2003; Vanderschuren and Kalivas, 2000). The mesocorticolimbic dopamine system, which arises from the ventral tegmental area and innervates the nucleus accumbens among other regions, has been implicated in processes associated with drug addiction, including behavioral sensitization. Another important region is the frontal cortex, including the medial prefrontal cortex (mPFC). mPFC, defined as the cortical region that has reciprocal innervation with the mediodorsal nucleus of the thalamus, is also a terminal region of the mesocorticolimbic dopamine system. mPFC contains pyramidal glutamatergic neurons that serve as the primary output of this region. These pyramidal neurons are modulated by numerous neurotransmitter systems, including gamma aminobutyric acidergic interneurons and dopaminergic, noradrenergic, serotonergic, glutamatergic, cholinergic and peptidergic afferents. Indeed, ibotenic acid lesions in mPFC inhibit the induction of behavioral sensitization to cocaine (Li et al., 1999a,b). Damage to the dorsal prefrontal cortex caused by ibotenic acid prevents behavioral sensitization to cocaine (Pierce et al., 2000). These findings provide a rationale for examining the role of PFC in behavioral sensitization, because the changes in the interactions between the aforementioned neurotransmitter systems in this region may lead to alterations in behavioral responses. In PFC, Lu et al. (1999) have reported that metabotropic glutamate receptor 1 (mGluR1) mRNA level increased 3 days after withdrawal from five daily injections of amphetamine (5 mg/kg/day) (Lu and Wolf, 1999). Moreover, repeated exposures to cocaine (20 mg/kg) for 10 days, followed by a 14-h withdrawal period, induced marked effects on D_1 and D_2 dopamine receptor mRNA expression levels in PFCz (Schmidt-Mutter et al., 1999).

Li et al. (2000) reported that cocaine-induced behavioral sensitization (locomotor activity) can be reversed by a dopamine receptor agonist (Li et al., 2000) without the need for continuous medication. Additionally, there have been a number of reports on the reversal effects of D_1 agonists on other psychostimulant-related behaviors and mental activities in animals and humans. D_1 receptor agonists effectively suppress self-administration and seeking behaviors for cocaine. Self-administration and seeking behaviors are suppressed in rats (Barrett et al., 2004; Alleweireldt, et al., 2003; Haile and Kosten, 2001; Caine et al., 1999), monkeys (Mutschler and Bergman, 2002) and humans (Haney et al., 1999) by the administration of D_1 receptor agonists after subchronic treatment of cocaine abuse. Haney et al. (1999) reported that ABT-431, a selective D_1 dopamine receptor agonist, produces significant dose-dependent decreases in the subjective effects of cocaine, including ratings of “high” and “stimulated”, and suppresses cocaine craving. However, pergolide, a D_1/D_2 dopamine receptor agonist, increased the ratings of “I want cocaine” (Haney et al., 1998). These results suggest that D_1 agonists have potential utility for the treatment of cocaine abuse. To our knowledge, however, no report on the effect of D_1 receptor agonists on AMP-induced behavioral sensitization has been published yet.

The long-lasting behavioral effects of psychostimulants are presumably caused by neuroplastic changes at the circuit, cellular, and molecular levels, mainly in the dopaminergic and glutamatergic systems (Nestler, 2005) (for review: Nestler, 2005). It is therefore reasonable to analyze the expression patterns of neuroplasticity-related genes to gain insight into the molecular mechanism of behavioral sensitization. The activity-regulated cytoskeleton-associated protein (arc) is suitable for this analysis, first because arc has been implicated in neuronal plasticities, such as LTP (Guzowski et al., 2000) and neuritic elongation (Ujike et al., 2002), and second because arc is up-regulated in the prefrontal cortex by the administration of psychostimulant drugs, including amphetamine (Klebaur et al., 2002), methamphetamine (Kodama et al., 1998) and cocaine (Freeman et al., 2002). The strong association of arc with neuronal plasticity is also supported by the fact that newly synthesized arc mRNA is not only transported into dendrites but also accumulates specifically at synaptic sites that have experienced strong activity (Steward et al., 1998).

On the basis of these findings, we evaluated the effects of a D_1 agonist on AMP-induced behavioral sensitization (locomotor and stereotyped activities) and the mRNA expression levels of the D_1 and D_2 receptors, mGluR1 and arc in the prefrontal cortex of rats.

2. Materials and methods

2.1. Behavioral experiments

2.1.1. Animals

Male Sprague–Dawley rats, initially weighing 280 to 300 g (Charles River Laboratories, Japan), were housed individually with free access to food and water under a 12-h light/12-h dark cycle (lights on at 6:00 a.m.) and handled for 1 week before treatment was started.

2.1.2. Drugs

D-Amphetamine sulfate (AMP), SKF-38393 (SKF; Sigma) and SCH-23390 (SCH; Sigma) were dissolved in 0.9% physiological saline. YM-09151-2 (YM) was dissolved in 0.1 N HCl and neutralized with NaOH. All doses were calculated for the salt form of the drugs. Each drug was injected in a volume of 1.0 ml/kg body weight. AMP was administered intraperitoneally (i.p.). SKF, SCH and YM were administered subcutaneously (s.c.). The control rats were injected with saline (1.0 ml/kg body weight).

2.1.3. Pretreatment regimen

The AMP pretreatment regimen carried out in a 13-day period. All the animals received six intermittent AMP injections (1.0 mg/kg i.p.) once a day to produce behavioral sensitization. Pretreatment AMP was administered on Tuesday, Thursday and Saturday. The pretreatment regimen was always started on Thursday. This intermittent regimen has been shown in our laboratory to result in robust behavioral sensitization (Utena

1966; Tadokoro et al., 1986; Hirabayashi et al., 1993; Kuribara, 1995a,b; Ida et al. 1995). The rats were acclimated to the test room in the cages for locomotor activity measurement for 30 min before the injections. The control rats were injected with saline (1.0 ml/kg body weight, i.p.). All the animals were treated exactly the same.

2.1.3.1. Experiment 1. The rats were randomly divided into six groups. Each group received six intermittent i.p. injections of 1.0 mg/kg AMP once a day for the pretreatment. Each group received a five-day reversal treatment from day 27 to day 31 (all the subjects were given a 14-day withdrawal period from the end of the 13-day pretreatment period) in their home cages. For the saline treatment group, the rats were subcutaneously injected once daily with physiological saline (1.0 ml/kg) for 5 days. For the SKF treatment group, the rats were subcutaneously injected once daily with SKF (3.0 mg/kg) for 5 days. For the SKF+SCH treatment group, the rats were subcutaneously injected once daily with SCH (1.0 mg/kg) after SKF (3.0 mg/kg, s.c.) injection for 5 days. For the SCH treatment group, the rats were subcutaneously injected once daily with SCH (1.0 mg/kg) for 5 days. For the SKF+YM treatment group, the rats were subcutaneously injected once daily with YM (1.0 mg/kg) after SKF (3.0 mg/kg, s.c.) injection for 5 days. For the YM treatment group, the rats were subcutaneously injected once daily with YM (1.0 mg/kg) for 5 days. Double injections during the reversal treatment were given 30 min apart. These injections were given in the animals' home cages. On day 34 (3 days after the end of the reversal treatment) all the subjects were again intraperitoneally challenged with 1.0 mg/kg AMP in their cages for locomotor activity measurement.

2.1.3.2. Experiment 2. The rats were divided into two groups: the saline and SKF treatment groups. In these groups, the AMP pretreatment regimen and reversal treatment were the same as those in Experiment 1 except for the withdrawal time. These two groups were exposed to a 4-week withdrawal period from the end of the reversal treatment and challenged with 1.0 mg/kg AMP on day 60.

2.1.4. Behavioral sensitization measurement

During the AMP pretreatment regimen and challenge test, the animals received AMP injections in Plexiglas test cages (area: 40×40 cm; height: 20 cm) and monitored with an infrared activity sensor (O'HARA & Co., Ltd., Tokyo, Japan) equipped with infrared beams (400 photocell beams projected on the floor like a cone) positioned 50 cm above the center of the floor for 180 min. The test cages were linked to a computer that recorded photocell beam breaks. Locomotion activity was estimated by determining the number of crossovers. The number of crossovers was continuously recorded and accumulated at 10 min intervals. Moreover, on the first, sixth and challenge injections of AMP, we recorded the behavior on a videotape to assess stereotyped behavior rate for 120 min. Eight minutes after the injection, the animals were rated for 2 min and successively at 10 min intervals for up to 120 min.

An investigator blind to the drug treatments measured how long the animals engaged in focused stereotyped activity (i.e., repetitive head movements, rearing, sniffing, biting and licking). The chronometer was started after the subjects exhibited a stereotyped behavior for 2 to 3 s in the absence of locomotor activity. Data are presented as the percentage of time the subjects displayed a specific stereotyped response during the observation period. Our measurement of stereotyped behavior rate was in accordance with the method of Panayi et al. (2002).

In this study, locomotor activity was measured for 3 h and stereotypy was measured for 2 h. This is because there is almost no stereotypy 2 h after AMP injection (7% or less in first AMP injection) and because AMP is still active on locomotor activity after this time in our studies (Fig. 2).

2.2. Gene expression in prefrontal cortex

2.2.1. Animal preparation

The rats were handled and treated using the same protocol as that of Experiment 1. On days 13 (after six AMP injections), 31 (after SKF or saline treatment) and 34 (after AMP challenge), each rat was decapitated 1 h after injection and the cortex was dissected on an ice-cold plate. Rats of the same age that were drug-free were also decapitated and designated as "naive". Moreover, twelve rats were decapitated 1 h after a single injection of AMP, SKF or saline. All the brain samples were stored at −80 °C until use.

2.2.2. RNA extraction, quantitation, quality check, and cDNA synthesis

Brain tissues were homogenized and total RNA was extracted using the RNeasy lipid minikit in accordance with the manufacturer's instructions with an additional on-column DNase treatment step (Qiagen, Valencia, CA), and quantification was carried out by absorption at 260 nm. RNA integrity was checked by assessing the sharpness of the 18S and 28S units of ribosomal RNA bands by agarose gel electrophoresis.

Approximately 50 ng of each total RNA sample was reverse-transcribed using a SuperScript II RT kit (Qiagen) in a total reaction volume of 21 μl, containing 50 ng of random hexamer, 10 mM dNTP mix and 50 units of SuperScript II RT. The reaction mixture was incubated at 42 °C for 50 min and terminated by heating to 70 °C for 15 min. Negative controls, including those without RNA and reverse transcriptase, were used to confirm the absence of genomic DNA contamination. We detected no genomic DNA contamination in any of the controls.

2.2.3. TaqMan probes and primers

TaqMan primers and probes for the rat D_1 receptor (P/N 4324034), D_2 receptor (assay ID — Rn00561126_m1), mGluR1 (assay ID — Rn00566625_m1) and arc (assay ID — Rn00571208_g1) were synthesized by Applied Biosystems and optimized according to the manufacturer's protocol.

2.2.4. Real-time quantitative PCR

Transcripts were measured by TaqMan real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) using the TaqMan Universal PCR Master Mix kit (Applied

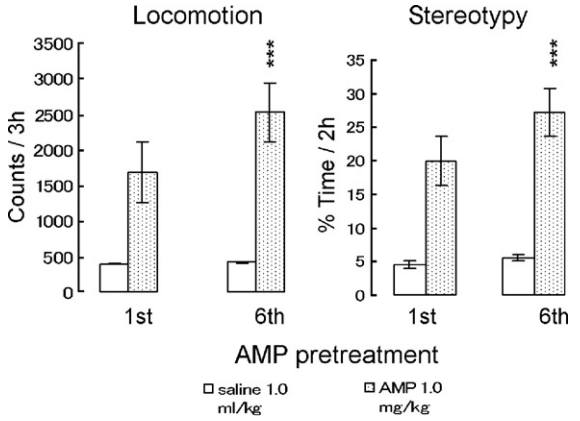

Fig. 1. Changes in mean 3 h overall locomotion activity counts after six intermittent administrations of saline or AMP (1.0 mg/kg) (left side). Values are expressed as means±S.E.M. The asterisks (***) represent significant differences from the activity count at the 1st administration within group ($p<0.001$). $N=24–122$ in each group. Changes in mean 2 h overall rates of stereotypy after six intermittent administrations of saline or AMP (1.0 mg/kg) (right side). Values are expressed as means±S.E.M. The asterisks (***) represent significant differences from the rate of stereotypy at the 1st administration within group ($p<0.001$). $N=12–122$ in each group.

Biosystems, Foster City, CA) and ABI Prism 7900 Sequence Detection System (Applied Biosystems). The conditions for the PCR were 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 1 min.

As for the control, we employed a probe specific for the glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gene, which was used previously as a successful endogenous control (Greisbach et al., 2002; Molteni et al., 2002), and the β-actin gene. Because there was no remarkable difference in the results between both genes, we present this data corrected for *GAPDH* in this study. Unknown samples were run in triplicate.

2.3. Data analysis

The mean overall locomotion activity count for 180 min after the drug administration was calculated for individual groups of rats. These data were first analyzed by one-way or two-way analysis of variance (ANOVA) followed by Tukey's test. Statistical differences in the quantitative analysis of gene expression were estimated by one-way ANOVA followed by Bonferroni's post-hoc test.

3. Results

3.1. Behavioral experiments

3.1.1. Experiment 1

3.1.1.1. Establishment of behavioral sensitization by six intermittent AMP injections. As shown in Fig. 1, repeated administrations of AMP-induced sensitization to locomotor activity [F(drug × administration) = 25.393, $p<0.001$] and stereotyped activity [F(drug × administration) = 7.717, $p<0.01$]. The activity counts and the rates of stereotypy at the sixth administration of AMP were significantly higher than those at the first administration. On the other hand, the repeated administrations of saline elicited no significant change in locomotor [F(drug × administration) = 0.224, NS] or stereotyped [F(drug × administration) = 0.891, NS] activity.

3.1.1.2. Effects of reversal treatment with SKF-38393. As shown in Fig. 2, the SKF treatment group showed no significant change in locomotor activity compared with the saline-treated control group. On the other hand, the rates of stereotyped behavior of the SKF treatment group at the challenge administration were significantly lower than those of the saline-treated control group.

As shown in Fig. 3, the SKF treatment group showed no significant change in locomotor activity compared with the saline-treated control group at any of the ten-min-interval time points [F(drug × time) = 0.466, NS]. The rates of stereotyped behavior of the SKF treatment group at the challenge administration were lower than those of the saline-treated control group at any of the ten-min-interval time points [F(drug × time) = 2.180, $p<0.05$].

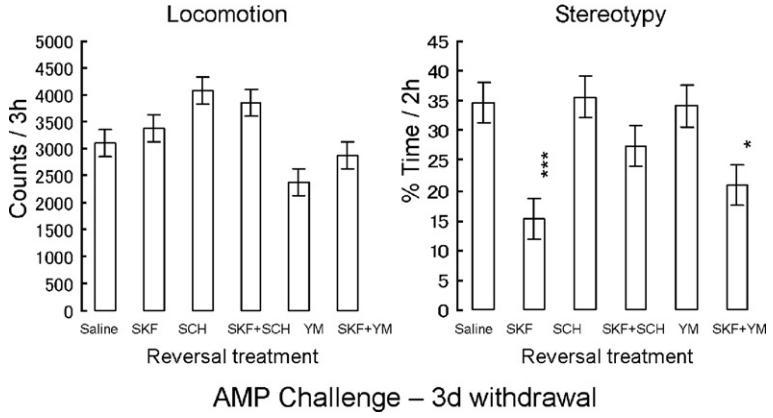

Fig. 2. Overall locomotion activity counts for 3 h after challenge AMP (1.0 mg/kg) administration for rats that were received six AMP (1.0 mg/kg) administrations and five daily reversal treatments (left side). The challenge administration was conducted 3 days after the reversal treatment. Values are expressed as means±S.E.M. $N=10–14$ in each group. Overall rates of stereotypy for 2 h after challenge administration of AMP (1.0 mg/kg) for rats that were received six AMP (1.0 mg/kg) administrations and five daily reversal treatments (right side). The challenge administration was conducted 3 days after the reversal treatment. Values are expressed as means±S.E.M. The asterisks (* and ***) represent significant differences from the rates of stereotypy for the saline treatment group ($p<0.05$ and $p<0.001$, respectively). $N=10–14$ in each group.

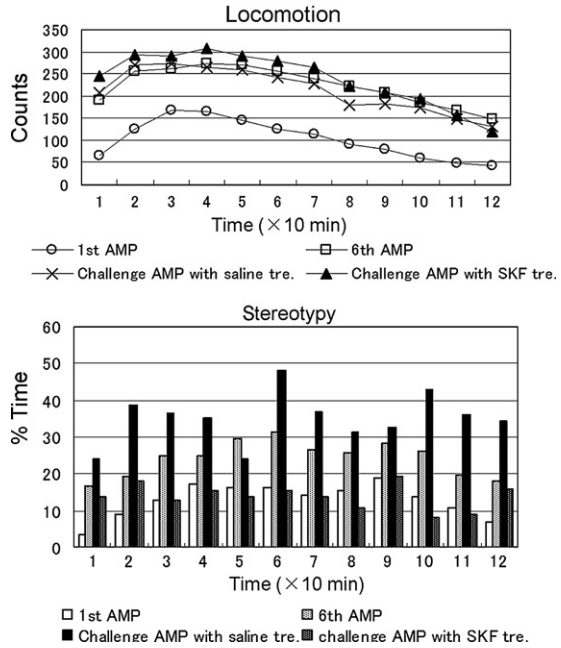

Fig. 3. Time course data of locomotor counts and rates of stereotypy every 10 min for 2 h after first, sixth and challenge administrations of AMP (AMP 1.0 mg/kg). The challenge administration was conducted 3 days after the reversal treatment with saline or SKF. Values are expressed as means ±S.E.M. $N=8$–20 in each group.

3.1.1.3. Effects of reversal treatments with dopamine D_1 and D_2 antagonists. As shown in Fig. 2, D_1 and D_2 antagonist treatments induced no significant changes in either locomotor activity or the rate of stereotyped behavior compared with saline treatment. Moreover, the SKF+SCH treatment group at the challenge administration showed no significant changes in either the locomotor activity or the rate of stereotyped behavior compared with the saline-treated control group. The SKF+YM treatment group showed a significant decrease in the rate of stereotyped behavior, but no significant change in locomotor activity compared with the saline-treated control group.

3.1.2. Experiment 2

As shown in Experiment 1, the SKF treatment group showed no significant change in locomotor activity compared with the saline-treated control group (Fig. 4). On the other hand, the SKF treatment group at the challenge administration had significantly lower rates of stereotyped behavior than the saline-treated control group at the challenge administration (two-tailed Student's *t*-test, $p<0.001$).

3.2. Gene expression

Fig. 5 shows the gene expression levels of the D_1 and D_2 receptors, mGluR1 and arc after the single injections, pretreatment, reversal treatment and AMP challenge.

3.2.1. After single injections

There were significant group differences in the arc mRNA expression level [$F(2,15)=10.979$, $p<0.001$]. There was a significant increase in the arc mRNA expression level above the saline control level in both the AMP and SKF injection groups. There were no significant differences in the D_1 receptor [$F(2,9)=0.720$, NS], D_2 receptor [$F(2,9)=0.672$, NS] and mGluR1 [$F(2,9)=0.164$, NS] mRNA expression levels after the single injections.

3.2.2. After pretreatment

There were significant group differences in the arc mRNA expression level [$F(2,15)=4.690$, $p<0.05$]. There was a significant increase in the arc mRNA expression level above the control level in the AMP pretreatment groups. There were no significant differences in the D_1 receptor [$F(2,9)=0.235$, NS], D_2 receptor [$F(2,9)=0.615$, NS] and mGluR1 [$F(2,9)=0.609$, NS] mRNA expression levels after the pretreatment.

3.2.3. After reversal treatment

There were significant group differences in the arc mRNA expression level [$F(2,15)=5.534$, $p<0.05$]. There was a significant increase in the arc mRNA expression level above the naive control level in the SKF treatment group. In the saline treatment group, the arc mRNA expression level was about two and a half times as high as that of the naive control, but there was no statistically significant difference ($p=0.097$). There were no significant differences in the D_1 receptor [$F(2,9)=0.035$, NS], D_2 receptor [$F(2,9)=0.888$, NS] and mGluR1 [$F(2,9)=1.971$, NS] mRNA expression levels after the reversal treatment.

3.2.4. After AMP challenge

There were significant group differences in the arc mRNA expression level [$F(2,15)=38.962$, $p<0.001$]. There was a significant increase in the arc mRNA expression level above the naive control level in both the AMP and SKF treatment groups. There were no significant differences in the D_1 receptor [$F(2,9)=$

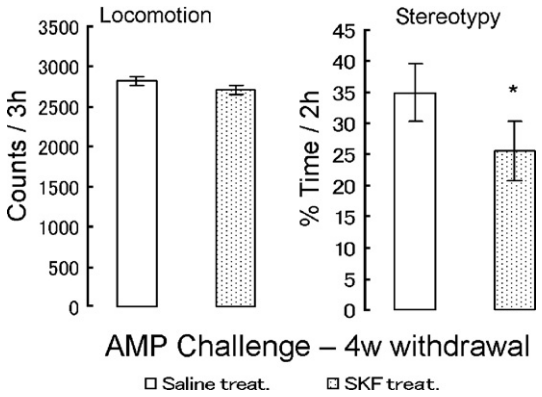

Fig. 4. Overall locomotion activity counts for 3 h after challenge AMP (1.0 mg/kg) administration for rats that were received six AMP (1.0 mg/kg) administrations and five daily reversal treatments (left side). The challenge administration was conducted 4 weeks after the reversal treatment. Values are expressed as means±S.E.M. $N=25$ in each group. Overall rates of stereotypy for 2 h after challenge administration of AMP (1.0 mg/kg) for rats that were received six AMP (1.0 mg/kg) administrations and five daily reversal treatments (right side). The challenge administration was conducted 4 weeks after the reversal treatment. Values are expressed as means±S.E.M. The asterisks (***) represent significant differences from the rates of stereotypy of the saline treatment group ($p<0.001$). $N=25$ in each group.

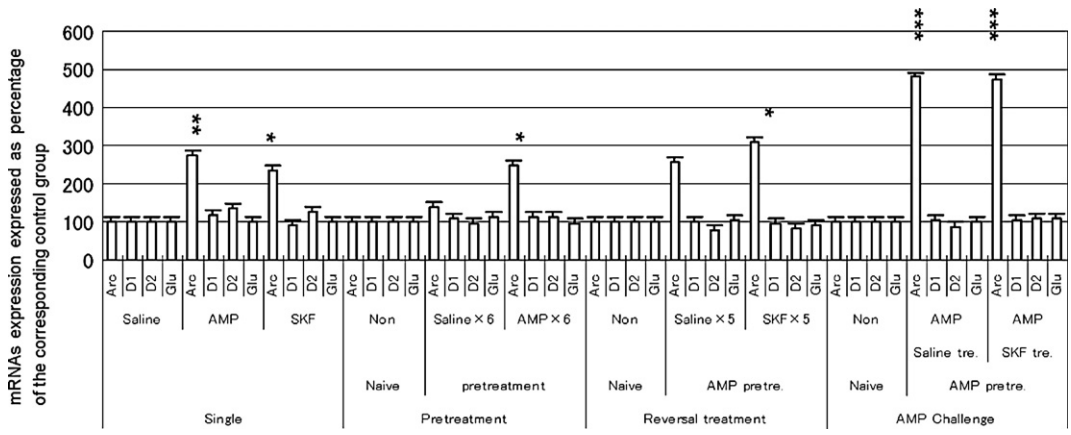

Fig. 5. Mean (±S.E.M.) mRNA expression levels after single injections in prefrontal cortex (left side). The asterisks (* and **) represent a significant difference from the mRNA expression level of the saline-treated control group ($p<0.05$ and $p<0.01$, respectively). $N=4–6$ in each group. Mean (±S.E.M.) mRNA expression levels after six intermittent administrations of AMP or saline pretreatment in prefrontal cortex (left center). The asterisk (*) represents a significant difference from the mRNA expression level of the naive control group ($p<0.05$). $N=4–6$ in each group. Mean (±S.E.M.) mRNA expression levels after reversal treatment in prefrontal cortex (right center). The asterisk (*) represents a significant difference from the mRNA expression level of the naive control group ($p<0.05$). $N=4–6$ in each group. Mean (±S.E.M.) mRNA expression levels after challenge administration of AMP in prefrontal cortex (right side). The asterisks (***) represent significant differences from the mRNA expression level of the naive control group ($p<0.001$). $N=4–6$ in each group.

0.042, NS], D_2 receptor [$F(2,9)=0.178$, NS] and mGluR1 [$F(2,9)=0.132$, NS] mRNA expression levels after the AMP challenge.

4. Discussion

This study demonstrated (i) that AMP-induced stereotypy is reversed by a D_1 agonist, and (ii) that the reversal effect of this D_1 agonist on stereotypy lasts for 4 weeks.

4.1. Reversal of behavioral sensitization by D_1 agonist

Behavioral sensitization in rodents is characterized by augmented ambulation and stereotypy, and, once established, persists for a long time. It is difficult to reverse behavioral sensitization once established; in our previous studies, chlorpromazine (Hirabayashi and Tadokoro, 1993), haloperidol and ceruletide (cholecystokinin) (Kuribara, 1993a), D_1 and D_2 receptor antagonists, namely, SCH-23390 and YM-09151-2 (Kuribara, 1993b) respectively, and MK-801, which is a noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist (Ida et al., 1995), did not reduce locomotor activity in behaviorally sensitized rats. To our knowledge, there are no other reports on reversal treatment for behavioral sensitization except for a few reports on locomotor activity (King et al., 2000) (King et al., 2000; Li et al., 2000).

In this study, we were able to reverse AMP-induced stereotypy, once established, using a D_1 receptor agonist. Stereotyped behavior is an important indicator in this animal model. We consider an increase in the rate of stereotypy as an important indicator of sensitization, first because the rate of stereotypy increased with repeated AMP administrations, and second because the increase in the rate of stereotypy prevented an increase in locomotor count in our study.

In this study, the direct dopamine receptor agonist SKF produced effects opposite to those of the indirect dopamine receptor agonist AMP. However, from our data, it seems that the effect of reversal treatment requires a selective stimulation of the dopamine D_1 receptor, regardless of whether it is direct or indirect. For example, as mentioned in the Introduction, pergolide, a direct D_1 and D_2 dopamine receptor agonist, increases ambulation count (Li et al., 2000) and enhances cocaine craving (Haney et al., 1998). Moreover, in the cocaine relapse model, D_1 and D_2 class agonists exert opposite effects (Self et al., 1996). D_2 agonists induce cocaine-seeking behavior and enhance the priming effects of cocaine, whereas D_1 receptor agonists inhibit cocaine-seeking behavior triggered by priming injections of cocaine. De Vries et al. (1998) demonstrated that the reinstatement of cocaine-seeking behavior is associated with the expression of behavioral sensitization (De Vries et al., 1998). It is therefore important that AMP-induced stereotypy is also reversed by only D_1 receptor stimulation.

Then how would D_1 stimulation reverse AMP-induced stereotypy? It is difficult to interpret the reversal effect demonstrated here because it is associated with the AMP pharmacology. From their electrophysiological study results, Li et al. (2000) suggested that the reversal of locomotor sensitization occurs as a result of the reversal of an underlying neuroadaptation, namely, the enhanced response of neurons to D_1 receptor stimulation. Subsequently, their group reported that D_1 receptor stimulation enhances mGluR1 phosphorylation (Chao et al., 2002a,b) and mGluR1 surface expression in rat neurons (Chao et al., 2002a,b). These results suggest that reversal of stereotypy induced by D_1 stimulation also requires a reversal "neuroplastic" process as does the development and maintenance of behavioral sensitization (Wolf, 1998).

There was no significant decrease in locomotor activity after SKF treatment in this study. As shown in Fig. 2, repeated SKF treatments did not significantly reduce the sensitized locomotor response, as shown by the time course data. There are three possible reasons the SKF treatment did not reverse the sensitized locomotor response. First, as mentioned above, locomotor activity and stereotyped behavior were viewed as competing behaviors. A decrease in the time of stereotyped activity may lead to the increase in locomotor count. Second, we used a challenge dose that was the

same as the pretreatment dose so as to evaluate the effect for stereotypy. If we had used a very low challenge dose (0.1 mg/kg, for example) to minimize stereotyped behavior, we might have been able to produce a reversal effect for locomotor activity similar to that previously reported by Li et al. (2000). Finally, the rats were observed for 3 h in the test cages. Measuring locomotor activity for a longer period may yield different results.

In this study, we were also able to still reduce the reactivity of stereotypy to AMP 4 weeks after the SKF treatment, suggesting that this is not a temporary phenomenon and that the reversal effect lasts for a long time. This is very important because, if the same condition occurs in clinical situations, it would not be necessary to continue medication to remove the acquired vulnerability to AMP. Wada (2000) suggested that abusers have psychological problems after the cessation of drug abuse and most of them have no chance of receiving continuous medication in Japan. Unfortunately, it is indicated that the reversal effect of SKF becomes weaker with time, because the degree of stereotypy after 4 weeks of withdrawal is the same as that obtained after six AMP pretreatments in our study.

4.2. Reversal of behavioral sensitization by D_1 and D_2 antagonists

In this study, D_1 and D_2 antagonists did not exert a reversal effect when administered alone. The results of our study are in agreement with those of previous studies showing that sensitization, once established, is not changed by treatment with D_1 and D_2 antagonists (Kuribara, 1995b). A coadministration of the D_1 agonist SKF and the D_1 antagonist SCH cancelled the reversal effect induced by SKF, while that of SKF and the D_2 antagonist YM maintained it. Therefore, it is suggested that the reversal of AMP-induced stereotypy requires D_1 receptor stimulation.

4.3. D_1 receptor, D_2 receptor, mGluR1 and arc mRNA expression levels in PFC

The expression levels of dopamine D_1 and D_2 receptor mRNAs were not changed by our pretreatment schedule of six intermittent AMP injections (1.0 mg/kg, i.p.). As mentioned in the Introduction, Schmidt-Mutter et al. (1999) reported that repeated exposures to cocaine (20 mg/kg) for 10 days followed by a 14-h withdrawal period, induced increasing effects on D_1 and D_2 dopamine receptor mRNA expression levels in PFC. Similarly, Lu et al. (1999) reported that the mGluR1 mRNA level increased on the 3rd day of withdrawal from five daily injections of AMP (5 mg/kg/day). Nevertheless, the mGluR1 mRNA expression level showed no change in our study. Experiments using various AMP doses and time periods for withdrawal and decapitation may help explain this discrepancy.

As mentioned in the Introduction, we analyzed the expression pattern of the neuroplasticity-related gene *arc* to gain insight into the molecular mechanism of behavioral sensitization. *Arc* expression level reportedly increases as a result of subchronic administrations of AMP (Klebaur et al., 2002; Gonzalez-Nicolini et al., 2002), MAP (Fujiyama et al., 2003; Yamagata et al., 2000; Kodama et al., 1998) and cocaine (Samaha et al., 2004; Yuferov et al., 2003; Freeman et al., 2002; Fosnaugh et al., 1995). Similarly in this study, repeated administrations of AMP enhanced *arc* expression in the cerebral cortex.

Interestingly, both single and repeated administrations of SKF significantly increased the *arc* expression level. How does D_1 stimulation enhance *arc* expression? It is suggested that D_1 receptor stimulation activates adenylyl cyclase (Cristina et al., 1998) by stimulating Gs proteins coupled to the D_1 receptor, and adenylyl cyclase activates the cAMP/protein kinase A (PKA)/cAMP-responsive element binding protein (CREB) signal transduction pathway, and CREB phosphorylation induces *arc* in dentate granule cells (Ying et al., 2002). This hypothesis is in agreement with the set of molecular mechanisms involved in learning: the stimulation of dopamine D_1 receptors, the activation of the cAMP/PKA/CREB signal transduction pathway, a transient burst of altered gene expression, and synaptic rearrangement (Berke and Hyman, 2000; Di Chiara, 2000; Dani et al., 2001). This raises the possibility that *arc* plays a role in multiple forms of synaptic plasticity, i.e., not only in AMP-induced behavioral sensitization, but also in neurobehavioral adaptations associated with the reversal effect induced by D_1 receptor stimulation.

Arc levels were elevated after AMP challenges in sensitized rats, compared with those after single AMP injection, although there was no significant difference in *arc* expression level between the saline and SKF treatment groups after the AMP challenge. Therefore *arc* expression level in the homogenate of mPFC does not correlate with the reversal effects of SKF in AMP sensitization. There is nevertheless the possibility that SKF treatment has formed novel neural circuits which are associated with the reversal effects and which also express *arc* after AMP challenge. Therefore topographical information on *arc* induction in the brain and the quantification of other cytoskeleton and synapse-associated genes will provide further insight into this finding. In addition, various time periods for decapitation will provide further information.

Noteworthily, repeated treatments with saline after AMP pretreatment showed an increase in *arc* expression level, suggesting a cross sensitization between AMP and stressful stimulants.

In summary, we have evaluated the effects of a D_1 agonist on AMP-induced behavioral sensitization (locomotor activity and stereotyped behavior) and the mRNA expression levels of the D_1 and D_2 receptors, mGluR1 and arc in the prefrontal cortex of rats. In the SKF treatment group, stereotyped behavior rate significantly decreased after both 3-day and 4-week withdrawal periods. SKF+SCH treatment inhibited the decreasing effect of SKF treatment. AMP administration significantly increased *arc* expression level. The SKF treatment group showed a marked increase in *arc* expression level after both the single SKF injection and the repeated treatments with AMP during the pretreatment period compared with the control groups. *Arc* expression level was further augmented by the treatment with saline after the AMP pretreatment.

There was no significant difference in *arc* expression level, between the saline treatment group and the SKF treatment group after the AMP challenge suggesting that *arc* was a non-specific marker in this investigation.

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