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Behavioral sensitization and alteration in monoamine metabolism in mice after single versus repeated methamphetamine administration

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Abstract

To address the functional alterations of monoaminergic neuronal systems in mice after single and repeated administration of methamphetamine, we examined the tissue contents of monoamines and their metabolites in addition to locomotor activity estimated by horizontal locomotion and rearing measurements. In male ICR mice, the repeated treatment regimen (intraperitoneal administration of 1.0 mg/kg methamphetamine once per day for five consecutive days) induced hyperlocomotion with a plateau level on test day 4. The initial behavioral response (within 5 min after injection) to the drug appeared to include context-dependent sensitization. Mice after the initial repeated treatment regimen showed behavioral sensitization to the same dose of methamphetamine 5 days after the final injection (test day 11). On test day 11, the first 150 min, but not the nocturnal behavior (during the dark hours), were significantly enhanced after the drug challenge. A marked reduction of the content of L-dihydroxyphenylalanine and the ratio of 3,4-dihydroxyphenylacetic acid to dopamine was observed in the striatum + accumbens of mice after single and repeated administration of methamphetamine. As for serotonin metabolism, the ratio of 5-hydroxyindolacetic acid to serotonin significantly increased in mice after single administration of methamphetamine, although it decreased in mice after repeated administration of methamphetamine. Norepinephrine metabolism (the ratio of 3-methoxy-4-hydroxyphenylglycol to norepinephrine) was not affected in the striatum + accumbens or thalamus + hypothalamus of the mice after repeated or single methamphetamine treatment. These results suggest that dopaminergic and serotonergic neuronal activities were altered during the development of behavioral sensitization. The ratio of 3-methoxytyramine to dopamine was not affected, suggesting that the methamphetamine treatment selectively inhibited the monoamine oxidase pathway for dopamine inactivation.

Keywords: Methamphetamine; Behavioral sensitization; Nocturnal behavior; Monoamine metabolism; Dopamine; 5-HT (5-hydroxytryptamine; serotonin)

1. Introduction

Repeated administration of psychostimulants such as cocaine and amphetamines enhances locomotor activities in response to treatment of the same or related drugs in rodents. This phenomenon is defined as behavioral sensitization or reverse tolerance. Behavioral sensitization is considered to be a primary stage for drug addiction and craving and certain aspects of drug-induced psychosis (for reviews, see Robinson and Becker, 1986; Robinson and Berridge, 1993; Hyman, 1996). The drugs of abuse that induce behavioral sensitization enhance dopaminergic neurotransmission, which mediates hyperlocomotion and reinforcing effects. One of the major pathways of dopaminergic neurons involved is the mesoaccumbens dopamine system (for reviews, see Uhl et al., 1993; Nestler et al., 1996; Pierce and Kalivas, 1997). The neurochemical aspects of dopaminergic neurotransmission have been extensively studied in sensitized animals (Robinson and Becker, 1986 and references herein). However, the simultaneous measurement of dopamine and other monoamines, such as serotonin (5-hydroxytryptamine; 5-HT) and norepinephrine, and their metabolites in sensitized animals compared with that in animals with single drug administration has not been investigated.

In the present study, we first measured the locomotor activity in mice after acute and chronic methamphetamine treatment and showed the details of developmental profile of behavioral sensitization compared with locomotor activity after an acute drug injection. We next measured three monoamines (dopamine, serotonin, and norepinephrine) and their metabolites simultaneously to know how and whether chronic—in addition to acute—treatment of methamphetamine would affect the monoamine metabolism in

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several mouse brain regions. It was predicted that the changes in the metabolism turnover of brain contents of monoamines involved in central locomotor control might differ between the treatment groups (acute and chronic). However, the present study indicated that the changes had no significant differences.

2. Materials and methods

2.1. Animals

Male ICR mice (5 weeks old upon purchase; Japan SLC, Shizuoka, Japan) were housed in groups of three to six in a temperature-controlled (22 ± 1 °C) and humidity-controlled (50 ± 5%) environment under a 12-h light/dark cycle (lights on at 0700 h) with food and water available ad libitum except during the locomotor activity measurements using the Animex Auto apparatus (see Section 2.2.1). Animal handling and care were conducted according to the National Institutes of Health *Guide for Care and Use of Laboratory Animals* (NIH publication No. 85-23, revised 1985) and all experiments were approved by the Institutional Animal Research Committee. After at least 7 days of habituation in this facility, mice were used in the experiments as follows.

2.2. Behavioral analyses

2.2.1. Locomotor activities during the 30-min measurement period

Mice were weighed (30–34 g on day 1) and divided into three groups: according to the protocol shown in Table 1, they were injected intraperitoneally (i.p.) with 0.1 ml/10 g vol of sterile saline or 1.0 mg/kg methamphetamine hydrochloride dissolved in saline once per day for six consecutive days (test days 1–6) and on test days 9 and 11. All mice were placed into a transparent acrylic test box (30 × 30 × 35 cm) on the Animex Auto apparatus (System MK-110; Muromachi Kikai, Tokyo, Japan) in a quiet room for 30 min prior to injection. The 30-min habituation period was sufficient to allow locomotor activity to stabilize based on our preliminary experiments (data not shown). Then, mice received a drug injection and were immediately returned to the same test box and subjected to horizontal activity counts (sensitivity parameter = 580) for 30 min at 5-min intervals. After the measurements, mice were returned to their home cages. All experiments were performed between 1000 and 1700 h. No mouse reduced body weight, but all maintained or gained body weight during the methamphetamine injection period (data not shown). On day 11, some mice were sacrificed by cervical dislocation and decapitation 30 min after the injection. The brains were immediately removed, and the striata and the regions of thalami and hypothalami were isolated, weighed, and frozen in liquid nitrogen until assay by high-performance liquid chromatography (HPLC; see Section 2.3).

Table 1
Schedule of drug administration

Groups	Test day							
	1	2	3	4	5	6	9	11
A	SAL	SAL	SAL	SAL	SAL	SAL	SAL	SAL
B	SAL	SAL	SAL	SAL	SAL	SAL	SAL	METH
C	SAL	METH	METH	METH	METH	METH	SAL	METH

Drug solutions were prepared daily and administered by i.p. injection in a volume of 0.1 ml/10 g of body weight.
SAL = saline; METH = methamphetamine (1.0 mg/kg).

2.2.2. Locomotor activities during the 24-h measurement period

Mice of Groups A and C (Table 1) were used for this experiment as described previously (Kitanaka et al., 2003). The 24-h locomotor experiment was carried out with different groups of mice from that used for the 30-min locomotor activity described in Section 2.2.1. All mice were placed into a transparent acrylic box (37 × 24 × 27 cm) at 0930 h with an infrared sensor that detects thermal radiation from animals (for horizontal locomotion; Supermex; Muromachi Kikai) and a beam sensor (for rearing; 24 cm wide, equipped at 7 cm height; Muromachi Kikai) in a quiet, ventilated chamber (53 × 45 × 45 cm) under a 12-h light/dark schedule (lights on at 0700 h) for 30 min prior to injection. Then, mice received a drug injection (Table 1) at 1000 h and were immediately returned to the same test box and were subjected to measurement of horizontal locomotor activity and rearing for 23 h 30 min at 30-min intervals. During the measurements, mice were fed standard pellets and tap water ad libitum.

2.3. Measurement of contents of monoamines and their metabolites

Each frozen brain sample was homogenized with a Teflon/glass homogenizer in a 20–40 vol (wt/vol) of ice-cold 0.1 N perchloric acid with 30 μM Na_2EDTA containing 3,4-dihydroxybenzylamine hydrobromide and isoproterenol as an internal standard for catechols and indoles, respectively. The homogenates were centrifuged at 10,000 × *g* for 10 min at 4 °C and the supernatants were filtered through a 0.20-μm membrane filter (Millipore, Bedford, MA, USA). The filtrates (10 μl) were injected directly into an HPLC system (system controller, model SCL-10A; autoinjector, model SIL-10A; pump, model LC-10AD; Shimadzu, Kyoto, Japan) equipped with a reversed-phase ODS column (MCM column 150; 4.6 × 150 mm; MC Medical, Osaka, Japan) and an electrochemical detector (Coulochem Model 5100A; ESA, Chelmsford, MA, USA). The column temperature was maintained at 24 °C, and the detector potentials were set at +0.30, +0.05, and −0.30 V on the conditioning cell, detector 1, and detector 2, respectively. The mobile phase was a 1000:35.2:85.8 (vol/vol) mixture of a buffer (50 mM

Na_2HPO_4, 50 mM citric acid, 4.4 mM 1-heptanesulfonic acid, and 0.1 mM Na_2EDTA, pH 3.7), acetonitrile, and methanol, and the flow rate was set at 0.9 ml/min.

2.4. Reagents

Methamphetamine hydrochloride was purchased from Dainippon Pharmaceutical (Osaka, Japan). All standard reagents for HPLC were from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of the highest commercially available purity.

2.5. Statistical analysis

Values are shown as means with bars representing the standard errors of the means (S.E.M.). Statistical analysis was performed using a one-way or two-way repeated-measure analysis of variance (ANOVA) followed by Scheffé's comparisons or *t* test, as indicated. A P value of < 0.05 was considered as a statistically significant difference.

3. Results

3.1. Locomotor activities during the 30-min measurement period

Mice of all three groups (Table 1) received 0.1 ml/10 g saline injection on day 1 and then subjected to their own group schedule. This procedure was needed to reduce the variance of data on locomotor activities on test day 2.

3.1.1. Locomotor activity: Group A

Mice in Group A that received 0.1 ml/10 g saline injection for five consecutive days after test day 1 showed similar locomotor activity when compared with every 5-min interval among each test day. The behavioral locomotor activity on test days 9 and 11 did not differ significantly from those observed in test days 1–6 [test day × 5-min activity interval interaction, $F(35,900) = 1.168$, $P = 0.2348$] (Fig. 1A). Concerning the time course pattern within each test day, locomotor activity of test day 1 alone significantly decreased when compared between periods of 5–10 and 25–30 min by repeated-measure one-way ANOVA followed by Scheffé's test [test day 1, $F(5,144) = 4.209$, $P = 0.0015$; test day 2, $F(5,144) = 1.012$, $P = 0.4135$; test day 3, $F(5,102) = 0.418$, $P = 0.8351$; test day 4, $F(5,102) = 0.446$, $P = 0.8149$; test day 5, $F(5,102) = 0.646$, $P = 0.6652$; test day 6, $F(5,102) = 0.789$, $P = 0.5604$; test day 9, $F(5,102) = 3.244$, $P = 0.0099$, but not significant by Scheffé's test; test day 11, $F(5,102) = 1.084$, $P = 0.3754$].

3.1.2. Locomotor activity: Group B

Mice in Group B received 0.1 ml/10 g saline injection for five consecutive days after test days 1 and 9, then they were challenged with 1.0 mg/kg methamphetamine injection on test day 11 (Fig. 1B). Similar to the results of Group A (Fig. 1A), only test day 1 showed a significant decrease of locomotor activities between the periods of the first 0–5

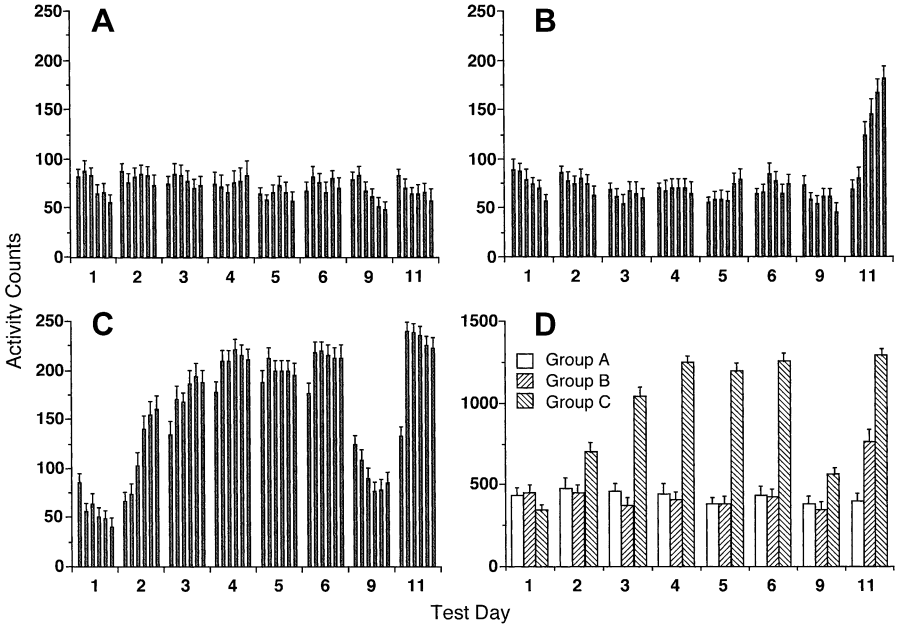

Fig. 1. Horizontal locomotor activity in mice after i.p. injection of 0.1 ml/10 g saline or 1.0 mg/kg methamphetamine. Locomotor activities of the mice in Groups A, B, and C are shown in (A), (B), and (C), respectively. Activity accounts within each 5-min interval during the measuring period (30 min) are shown by six bars of test days 1–11 in (A), (B), and (C). (D) Total locomotor activity for 30 min in each group. The values are shown as mean ± S.E.M. ($n = 18$, 26, and 26 for Groups A, B, and C, respectively).

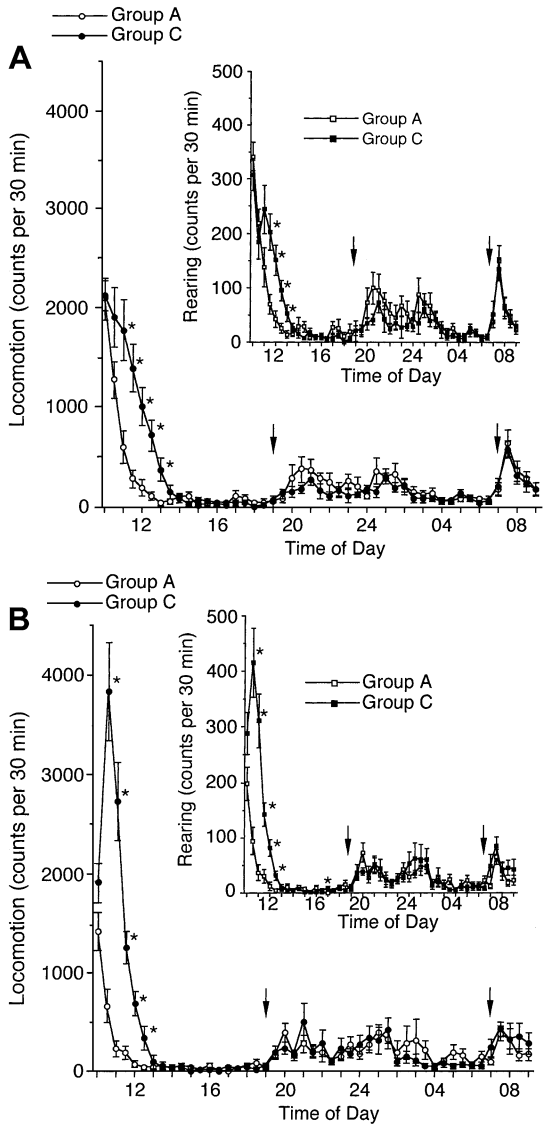

Fig. 2. Horizontal locomotor activity and rearing (inset) in mice after i.p. injection of 0.1 ml/10 g saline or 1.0 mg/kg methamphetamine during 24-h measurement. Each symbol indicates activity accounts within each 30-min interval during the measuring period (the first 30 min of habituation followed by a total of 23 h 30 min measurement). The mice received the injections at 0930 h on test days 2 (A) and 11 (B). Arrows indicate the time when lights were on (0700 h) and off (1900 h). *$P<0.05$, compared with Group A (one-way ANOVA followed by Scheffé's test). The values are shown as mean ± S.E.M. ($n=12$ and 12 for Group A and C, respectively).

min and the last 25–30 min after saline injection. On test day 11, the locomotor activity significantly increased compared with all other test days [test day × 5-min activity interval interaction, $F(35,1200)=7.514$, $P<0.0001$]. A significant increase in locomotor activity was observed from the first to last 5-min intervals (i.e., interval 0–5 vs. 10–15, 15–20, 20–25, and 25–30; 5–10 vs. 10–15, 15–20, 20–25, and 25–30; 10–15 vs. 20–25 and 25–30; 15–20 vs. 25–30) revealed by repeated-measure one-way ANOVA followed by Scheffé's test [test day 1, $F(5,150)=3.290$, $P=0.0080$; test day 2, $F(5,150)=1.443$, $P=0.2133$; test day 3, $F(5,150)=0.554$, $P=0.7354$; test day 4, $F(5,150)=0.090$, $P=0.9937$; test day 5, $F(5,150)=2.469$, $P=0.0360$, but not significant by Scheffé's test; test day 6 $F(5,150)=1.468$, $P=0.2050$; test day 9, $F(5,150)=1.712$, $P=0.1366$; test day 11, $F(5,150)=44.547$, $P<0.0001$].

3.1.3. Locomotor activity: Group C

Mice in Group C received 1.0 mg/kg methamphetamine once per day for five consecutive days after test day 1 and a saline injection on test day 9, and were challenged with 1.0 mg/kg methamphetamine on test day 11 (Fig. 1C). Statistical evaluation suggested that mice in Group C showed a significant difference in locomotor activity when compared with every 5-min locomotor activity among each test day [test day × 5-min activity interval interaction, $F(35,1200)=13.676$, $P<0.0001$]. By intraday one-way ANOVA followed by Scheffé's test, on test day 1, the locomotor activity significantly decreased (i.e., interval 0–5 vs. 15–20, 20–25, and 25–30), similar to the other two groups [test day 1, $F(5,150)=6.741$, $P<0.0001$]. On test day 2, mice in Group C that received a single injection of 1.0 mg/kg methamphetamine showed an increase in locomotor activ-

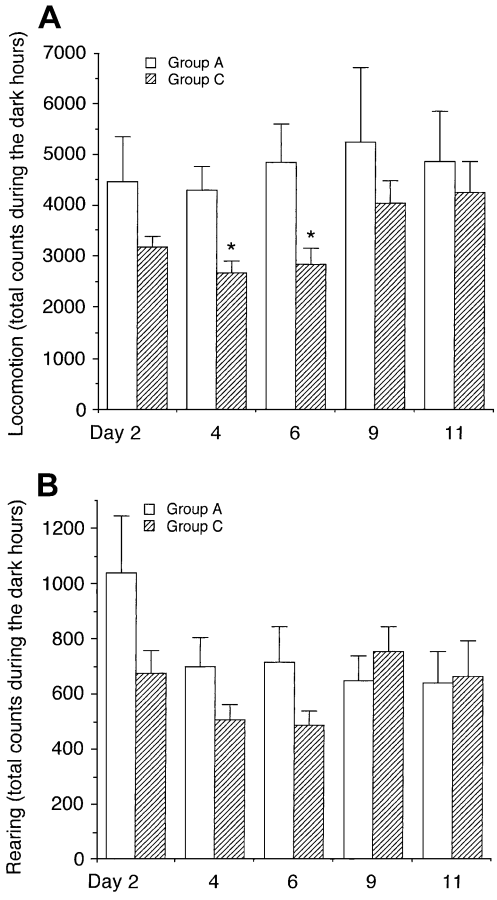

Fig. 3. Total activity counts during the dark period. Total activity counts of horizontal locomotion (A) and rearing (B) during the time between 1900 and 0700 h are shown. *$P<0.05$, compared with Group A (t test). The values are shown as mean ± S.E.M. ($n=12$ and 12 for Groups A and C, respectively).

ities from the first 0–5 min to last 25–30 min, which was a finding similar to that on test day 11 of Group B (Fig. 1B). On test day 9, the locomotor activity significantly decreased when 0–5 min was compared with 10–15, 15–20, 20–25, and 25–30 min [test day 9, $F(5,150)=6.795$, $P<0.0001$].

On all methamphetamine injection days except test day 5, the locomotor activities significantly increased between the period of the first 5 min and of the other intervals [0–5 vs. 5–10 (except test day 3), 10–15, 15–20, 20–25, and 25–30; test day 3, $F(5,150)=8.733$, $P<0.0001$; test day 4, $F(5,150)=7.689$, $P<0.0001$; test day 5, $F(5,150)=1.925$, $P=0.0948$; test day 6, $F(5,150)=5.664$, $P<0.0001$; test day 11, $F(5,150)=51.290$, $P<0.0001$].

3.1.4. Total locomotor activity during the 30-min measurement period

As summarized in Fig. 1D, total locomotor activity for 30 min of the mice in Group C increased while those of mice in Groups A and B remained unchanged [treatment × time interaction, $F(14,536)=34.384$, $P<0.0001$]. Statistical comparison of each test day between groups showed that there were significant differences on all test days except for the saline injection test days [test day 2, $F(2,74)=7.054$, $P=0.0021$; test day 3, $F(2,67)=41.410$, $P<0.0001$; test day 4, $F(2,67)=55.042$, $P<0.0001$; test day 5, $F(2,67)=60.947$, $P<0.0001$; test day 6, $F(2,67)=50.083$, $P<0.0001$; test day 11, $F(2,67)=45.724$, $P<0.0001$; test day 1, $F(2,74)=1.558$, $P=0.2210$; test day 9, $F(2,67)=2.211$, $P=0.125$], and an evaluation by Scheffé's test ($P<0.05$) revealed that locomotor activity of the mice in Group C increased significantly on all test days except on test days 1 and 9 compared with the saline injection groups (Groups B and C in Fig. 1D).

Intergroup repeated-measure one-way ANOVA applied to Fig. 1D revealed that there were significant differences in locomotor activity in mice in Group C [$F(7,200)=77.100$, $P<0.0001$]. There was no significant difference among locomotor activities on test days 4, 5, 6, and 11 in Group C. The locomotor activity on test day 2 after drug injection (Group C in Fig. 1D) showed a significant difference compared with that on each 1.0 mg/kg methamphetamine injection day (days 3–6 and 11). The locomotor activity on test day 3 after drug injection also showed a significant difference compared with that on test day 4. Therefore, it is suggested that methamphetamine-induced behavioral sensitization had been developed on test day 4 (see Discussion).

3.2. Locomotor activities during the 24-h measurement period

To address the effect of repeated administration of methamphetamine on nocturnal locomotor activity in mice, we measured horizontal locomotion and rearing for 23 h 30 min after methamphetamine administration. We collected the data on test days 2, 4, 6, 9, and 11 according to Table 1,

Table 2
Tissue contents of monoamines and their metabolites in the striatum + accumbens and thalamus + hypothalamus of the methamphetamine-sensitized mice

	L-DOPA	DA	DOPAC	3-MT	HVA
Striatum + accumbens					
Group A	0.051 ± 0.002	6.03 ± 0.39	0.67 ± 0.08	0.53 ± 0.06	0.76 ± 0.16
Group B	0.018 ± 0.001[a]	7.63 ± 0.36	0.54 ± 0.03	0.55 ± 0.04	0.69 ± 0.08
Group C	0.014 ± 0.002[a]	6.62 ± 0.36	0.40 ± 0.01[a,b]	0.51 ± 0.04	0.67 ± 0.08
Thalamus + hypothalamus					
Group A	0.075 ± 0.004	0.52 ± 0.05	0.24 ± 0.03	N.D.	N.D.
Group B	0.079 ± 0.004	0.51 ± 0.04	0.20 ± 0.00	N.D.	0.14 ± 0.10
Group C	0.072 ± 0.005	0.57 ± 0.04	0.19 ± 0.01[a]	N.D.	N.D.
	NE	MHPG	5-HT	5-HIAA	
Striatum + accumbens					
Group A	1.07 ± 0.02	2.67 ± 0.39	0.94 ± 0.01	0.67 ± 0.05	
Group B	1.12 ± 0.01	2.70 ± 0.13	0.55 ± 0.03[a]	0.53 ± 0.02	
Group C	1.13 ± 0.02	3.11 ± 0.15	0.66 ± 0.04[a]	0.56 ± 0.03	
Thalamus + hypothalamus					
Group A	2.60 ± 0.03	3.08 ± 0.67	1.45 ± 0.07	0.81 ± 0.06	
Group B	2.81 ± 0.03	3.43 ± 0.45	1.32 ± 0.05	0.65 ± 0.04	
Group C	2.70 ± 0.06	3.62 ± 0.57	1.38 ± 0.08	0.56 ± 0.04[a]	

The brain regions of each mouse group were dissected 30 min after the challenge of the drug on day 11. Values are expressed as nanograms per milligram of wet tissues ($n=4$, 10, and 11 for Groups A, B, and C, respectively).

L-DOPA = L-dihydroxyphenylalanine; DA = dopamine; DOPAC = 3,4-dihydroxyphenylacetic acid; 3-MT = 3-methoxytyramine; HVA = homovanillic acid; NE = norepinephrine; MHPG = 3-methoxy-4-hydroxyphenylglycol; 5-HT = 5-hydroxytryptamine (serotonin); 5-HIAA = 5-hydroxyindolacetic acid; N.D. = not detectable.

[a] $P<0.05$, compared with Group A.

[b] $P<0.05$, compared with Group B (one-way ANOVA followed by Scheffé's comparisons).

and the results on test days 2 and 11 are shown in Fig. 2. On test days 2, 4, 6, and 11, mice of Group C showed a significant horizontal hyperlocomotion compared with Group A [test day 2, $F(46,1034)=6.619$, $P<0.0001$ (Fig. 2A); test day 4, $F(46,1034)=12.379$, $P<0.0001$; day 6, $F(46,1034)=21.371$, $P<0.0001$; test day 11, $F(46,1034)=19.141$, $P<0.0001$ (Fig. 2B)]. On test day 9, the hyperlocomotion was detected in mice of Group C during the first 30 min (1000–1030 h) [$F(46,1034)=2.345$, $P<0.0001$], similar to the results shown in Fig. 1C (data not shown). Mice of Group C showed significant rearing compared with Group A [test day 2, $F(46,1034)=2.988$, $P<0.0001$ (Fig. 2A, inset); test day 4, $F(46,1034)=5.264$, $P<0.0001$; day 6, $F(46,1034)=12.985$, $P<0.0001$; test day 11, $F(46,1034)=12.065$, $P<0.0001$ (Fig. 2B, inset)].

The total activity counts during the dark period (1900–0700 h) revealed that the total horizontal locomotion during the 12-h dark period was significantly reduced in Group C mice compared with Group A mice (Fig. 3A) on test days 4 and 6, but not on test days 2, 9, and 11. As for the total rearing during the dark periods, there was no significant difference between the two groups on all test days (Fig. 3B).

3.3. Contents of monoamines and their metabolites

To investigate whether single or repeated treatment of methamphetamine would alter the function of monoaminergic neuronal systems, the tissue contents of monoamines and their metabolites in the striatum + accumbens and thalamus + hypothalamus of the mice were measured. As shown in Table 2, the content of L-dihydroxyphenylalanine (L-DOPA), a precursor of dopamine, was significantly reduced in the striatum + accumbens but not in the thalamus + hypothalamus of the mice of Groups B and C compared with Group A on test day 11 (30 min after the final injection). The tissue content of 3,4-dihydroxyphenylacetic acid (DOPAC) was significantly reduced in the striatum + accumbens and thalamus + hypothalamus of Group C mice. The content of 5-HT was significantly reduced in the striatum + accumbens but not in the thalamus + hypothalamus of Groups B and C mice. The content of 5-hydroxyindolacetic acid (5-HIAA) was significantly reduced in the thalamus + hypothalamus but not in the striatum + accumbens of Group C mice. As for norepinephrine metabolism, the tissue contents of norepinephrine and its major metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) did not change by the methamphetamine treatment (Table 1). In our system, 5-hydroxytryptophan, a precursor of 5-HT, could not be detected in any sample (less than 1 pg/mg wet tissue).

As shown in Fig. 4, monoamine metabolism was evaluated by calculating the ratio of the tissue contents of monoamines and their metabolites. A marked reduction of the ratio of DOPAC to dopamine was detected in the striatum + accumbens of mice after single (Group B) and repeated (Group C) administration of methamphetamine in the striatum + accumbens (Fig. 4A). As for serotonin metabolism, the ratio of 5-HIAA to 5-HT significantly increased in the striatum + accumbens of Group B mice (Fig. 4A), although it decreased in the thalamus + hypothalamus of Group C mice after repeated administration of methamphetamine (Group C; Fig. 4B). Norepinephrine metabolism (the ratio of MHPG to norepinephrine) was not affected after repeated or single methamphetamine treatment (Fig. 4).

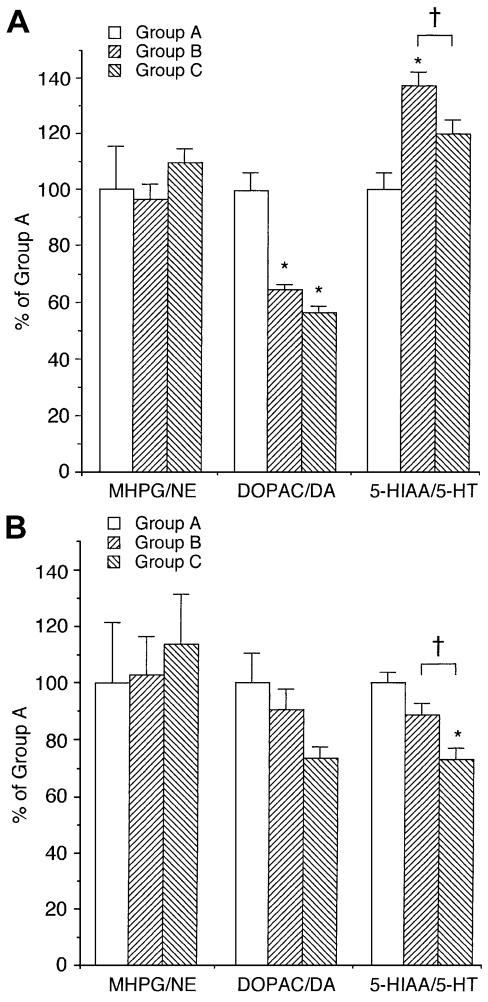

Fig. 4. Monoamine metabolism in the striatum + accumbens (A) and thalamus + hypothalamus (B) of the mice 30 min after the final injection on test day 11. Each column represents the mean ± S.E.M. ($n=4$, 10, and 11 for Groups A, B, and C, respectively). MHPG = 3-methoxy-4-hydroxyphenylglycol; NE = norepinephrine; DOPAC = 3,4-dihydroxyphenylacetic acid; DA = dopamine; 5-HIAA = 5-hydroxyindolacetic acid; 5-HT = serotonin. *$P<0.05$, compared with Group A; †$P<0.05$, compared with Group B (one-way ANOVA followed by Scheffé's test).

4. Discussion

We conducted the treatment protocol (Table 1) to investigate the developmental profile of methamphetamine-induced behavioral sensitization in mice. It is of interest to

analyze the details on how the locomotor response would increase to the maximal level after the drug administration. Immediately after the first methamphetamine treatment, the locomotor activity increased at every 5-min interval up to 30 min to reach the maximal locomotor response (Fig. 1B, test day 11; Fig. 1C, test day 2). Therefore, we decided to measure locomotor activities for up to 30 min after drug injection.

The expression and development of behavioral sensitization depended on the nature of the pretreatment regimen: doses of drugs, interval of injections, and the period of drug administration (Post, 1980; Robinson and Berridge, 1993). In the present study, we used 1.0 mg/kg methamphetamine to sensitize mice. The dose used induced hyperlocomotion by a single treatment (Fig. 1B) or even by a multiple injection for development of sensitization (Fig. 1C) but not any stereotypic behavior such as head bobbing, as reported by treatment of higher doses of the drug (Nishikawa et al., 1983). Under the protocol of Group C (chronic administration, Table 1), the locomotor response to methamphetamine reached a maximal level on test day 4 and the response plateaued thereafter (Fig. 1), suggesting that methamphetamine-induced behavioral sensitization had already been developed on test day 4. However, the long-lasting manifestation of the behavioral response to the drugs of abuse was expressed even by a single exposure to the drug (test day 3 in Fig. 1C; Vanderschuren et al., 1999).

A previous study reported that conditioned locomotion was induced by saline injection after repeated administration of methamphetamine (Elmer et al., 1996; Itzhak, 1997). The conditioned locomotion, or context-dependent sensitization, is induced by a stimulus of the drug injection under the particular environment of drug injection. The conditioned locomotion reflects different pharmacological properties of psychostimulants (Elmer et al., 1996; Itzhak, 1997). Therefore, it is important to evaluate the contribution of the conditioned locomotion to the development of behavioral sensitization. On test day 9, the locomotor activities of mice in Group C after saline injection were greater than those of mice in Groups A and B, although there was no significant difference by two-way ANOVA (Fig. 1D). The activity counts of Group C for the first and second 5-min intervals on test day 9 were greater than those of Groups A and B (Fig. 1C; 0–5 min: Group A, 78 ± 8; Group B, 72 ± 10; Group C, 124 ± 9; 5–10 min: Group A, 82 ± 10; Group B, 58 ± 7; Group C, 108 ± 11); the conditioned locomotion phenomenon was detected strongly during the first 10-min period immediately after saline injection and gradually settled down to the basal level. The effect might explain the reason why the initial locomotor response to methamphetamine on test day 11 in Group C was significantly reduced compared with those on test days 4–6 by means of the context of intervals of injection (Fig. 1C); the locomotor activity of the first 5-min interval on test day 11 (133 ± 9) was similar to that of test day 9 (i.e., conditioned locomotion), suggesting that the initial (i.e., within first 5 min) locomotor response to the injection of the drug of abuse did not depend on the presence of the drug itself. Therefore, the initial hyperlocomotion observed on test days 4–6 (test day 4, 178 ± 10; test day 5, 188 ± 11; test day 6, 176 ± 10 in Fig. 1C), which was greater than that on test day 11, might be a consequence of conditioned locomotion or context-dependent sensitization immediately after a daily injection rather than by the effect of sensitization.

Relative high dose of methamphetamine (20 mg/kg, i.p. $\times 4$ at 2-h intervals within 1 day) reduces the forced running time (treadmill) accompanied by a significant decrease in the striatal dopamine content in CD-1 mice, suggesting the involvement of the dopaminergic neuronal system in the exhaustive exercise towards fatigue (Kalinski et al., 2001). Based on the present observations, care should be observed when choosing the dose of the drug and the treatment protocol to investigate the effect of the drug on the behavioral sensitization itself. In the present study, we designed the experiments concerning the effects of single and repeated administration of a relatively low dose of methamphetamine (based on the results shown in Fig. 1 and related references, see above) on the spontaneous locomotor activity for 24 h in mice. As shown in Fig. 2, a marked hyperlocomotion for exploratory activity was observed during the period of adaptation in a novel environment on test days 2 and 11. However, after the adaptation, no significant difference in the activity counts for horizontal locomotion and rearing was observed between Groups A and C at each point of 30-min measurement intervals. At the time of 0700 and 1900 h, the status of illumination in the measurement chamber changed as described in Section 2.2.2. Each mouse responded to this change (lights on/off) as shown in Fig. 2, but no significant difference in the activity was observed between Groups A and C. A marked reduction of the total activity counts of horizontal locomotion but not rearing during the dark period was observed on test days 4 and 6 (Fig. 3A). The behavioral sensitization in mice appeared to be developed on test day 4 in our protocol (Table 1 and Fig. 1). Therefore, the results shown in Fig. 3 suggest that methamphetamine administration produces a reduction in subsequent spontaneous horizontal locomotor activity during the initial repeated methamphetamine treatment regimen. On test day 11, mice showed behavioral sensitization against a single methamphetamine injection (Figs. 1 and 2; Kitanaka et al., 2003), but the total activity counts of horizontal locomotion were not affected (Fig. 3A). This suggests that Group C mice on test day 11 contribute a suitable animal model for investigation of the function of monoaminergic neuronal systems on behavioral sensitization without the effect of fatigue induced by methamphetamine (Kalinski et al., 2001).

In contrast to the findings of Kalinski et al. (2001), the tissue content of dopamine was not affected in mice of any

group on test day 11 (Table 2), suggesting that the dopaminergic neuronal system in the striatum + accumbens and in the thalamus + hypothalamus of the mice in the present study could function normally. Evaluation using the value of the ratio of DOPAC to dopamine (i.e., dopamine metabolism) revealed a significant reduction in dopamine metabolism observed in the striatum + accumbens of mice of Groups B and C (Fig. 4A). It might be a result from the selective inhibition of monoamine oxidase pathway for dopamine metabolism in the striatum + accumbens after single and repeated administration of methamphetamine, since the tissue content of 3-MT, an intermediate metabolite of dopamine formed by catechol-*O*-methyltransferase, was not affected in the striatum + accumbens (Table 2). Thus, the possible inhibition of the monoamine oxidase pathway is one of the common molecular aspects of hyperlocomotion and the development of behavioral sensitization induced by single and repeated administration of methamphetamine, respectively. It should be noted that the striatal content of L-DOPA decreased significantly (Table 2), although the mechanism is still unknown. The enzymatic activity of aromatic L-amino acid decarboxylase, an enzyme that forms dopamine from L-DOPA, appears to be still high even under the status of drug treatment in mice, since the tissue content of dopamine is constant (Table 2).

Serotonin neuronal systems may closely interact or modulate the dopamine system (Rocha et al., 1998; Przegalinski et al., 2001; Carey et al., 2001). As for serotonin metabolism (the ratio of 5-HIAA to 5-HT), the opposite results were obtained from the striatum + accumbens and the thalamus + hypothalamus, respectively. In the striatum + accumbens, the serotonin metabolism was significantly up-regulated after a single administration of methamphetamine (Fig. 4A). The up-regulation appeared to be transient; after repeated treatment of methamphetamine, the metabolic change returned towards the basal level. In contrast, the serotonin metabolism decreased significantly in the thalamus + hypothalamus after repeated treatment of methamphetamine (Fig. 4B). These observations suggest that the serotonergic neuronal system is regulated in the opposite manner in the striatum + accumbens and thalamus + hypothalamus to develop behavioral sensitization. The central norepinephrine metabolism (the ratio of MHPG to norepinephrine) is not affected by methamphetamine administration (Table 2, Fig. 4), which is supported by the findings of the relatively high dose of methamphetamine protocol (Kalinski et al., 2001).

Taken together, the findings of the present study suggest that dopaminergic and serotonergic neuronal activities were altered during the development of behavioral sensitization. As discussed above, the degree and direction of modification of the monoaminergic metabolism depend on the treatment protocol (single or repeated) and on the brain regions. In addition to the use of the currently developed monoamine transporter knockout mice (for a review, see Gainetdinov et al., 2002), our method of approach is still useful in finding out the effects of drugs of abuse in naïve animals, which closely resemble human problems.

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References

Carey, R.J., DePalma, G., Damianopoulos, E., 2001. Cocaine and serotonin: a role for the 5-HT_{1A} receptor site in the mediation of cocaine stimulant effects. Behav. Brain Res. 126, 127–133.

Elmer, G.I., Gorelick, D.A., Goldberg, S.R., Rothman, R.B., 1996. Acute sensitivity vs. context-specific sensitization to cocaine as a function of genotype. Pharmacol. Biochem. Behav. 53, 623–628.

Gainetdinov, R.R., Sotnikova, T.D., Caron, M.G., 2002. Monoamine transporter pharmacology and mutant mice. Trends Pharmacol. Sci. 23, 367–373.

Hyman, S.E., 1996. Addiction to cocaine and amphetamine. Neuron 16, 901–904.

Itzhak, Y., 1997. Modulation of cocaine- and methamphetamine-induced behavioral sensitization by inhibition of brain nitric oxide synthase. J. Pharmacol. Exp. Ther. 282, 521–527.

Kalinski, M.I., Dluzen, D.E., Stadulis, R., 2001. Methamphetamine produces subsequent reductions in running time to exhaustion in mice. Brain Res. 921, 160–164.

Kitanaka, J., Kitanaka, N., Takemura, M., 2003. Chronic methamphetamine administration reduces histamine-stimulated phosphoinositide hydrolysis in mouse frontal cortex. Biochem. Biophys. Res. Commun. 300, 932–937.

Nestler, E.J., Berhow, M.T., Brodkin, E.S., 1996. Molecular mechanisms of drug addiction: adaptations in signal transduction pathways. Mol. Psychiatry 1, 190–199.

Nishikawa, T., Mataga, N., Takashima, M., Toru, M., 1983. Behavioral sensitization and relative hyperresponsiveness of striatal and limbic dopaminergic neurons after repeated methamphetamine treatment. Eur. J. Pharmacol. 88, 195–203.

Pierce, R.C., Kalivas, P.W., 1997. A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. Brain Res. Rev. 25, 192–216.

Post, R.M., 1980. Intermittent versus continuous stimulation: effect of time interval on the development of sensitization. Life Sci. 26, 1275–1282.

Przegalinski, E., Siwanowicz, J., Nowak, E., Papla, I., Filip, M., 2001. Role of 5-HT_{1B} receptors in the sensitization to amphetamine in mice. Eur. J. Pharmacol. 422, 91–99.

Robinson, T.E., Becker, J.B., 1986. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res. Rev. 11, 157–198.

Robinson, T.E., Berridge, K.C., 1993. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res. Rev. 18, 247–291.

Rocha, B.A., Scearce-Levie, K., Lucas, J.J., Hiroi, N., Castanon, N., Crabbe, J.C., Nestler, E.J., Hene, R., 1998. Increased vulnerability to cocaine in mice lacking the serotonin-1B receptor. Nature 393, 175–178.

Uhl, G., Blum, K., Noble, E., Smith, S., 1993. Substance abuse vulnerability and D_2 receptors. Trends Neurosci. 16, 83–88.

Vanderschuren, L.J.M.J., Schmidt, E.D., De Vries, T.J., Van Moorsel, C.A.P., Tilders, F.J.H., Schoffelmeer, A.N.M., 1999. A single exposure to amphetamine is sufficient to induce long-term behavioral, neuroendocrine, and neurochemical sensitization in rats. J. Neurosci. 9, 9579–9586.