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H. 知的財産権の出願・登録状況（予定も含む）

なし。

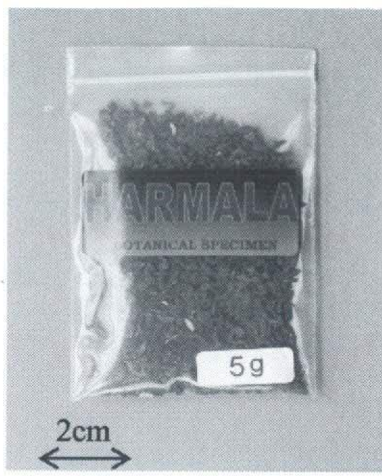

写真1.　都内・渋谷で購入したハルマラ

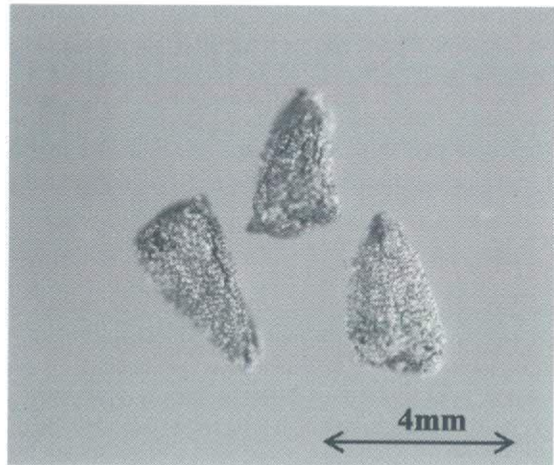

写真2.　購入したハルマラ種子の拡大写真

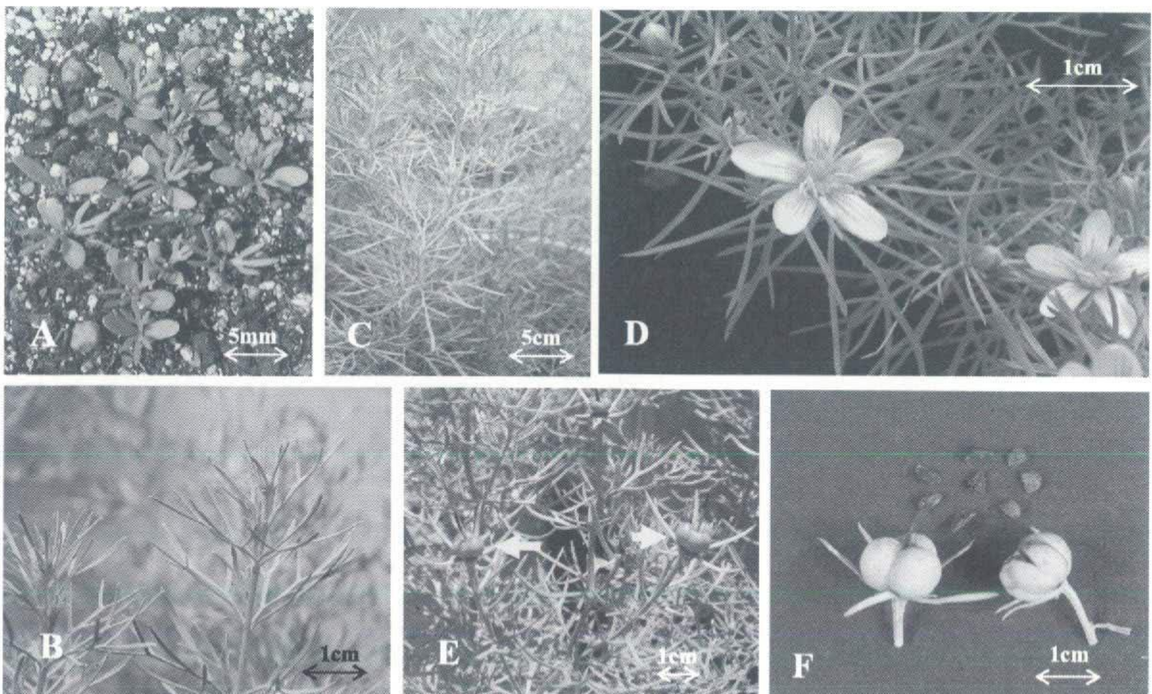

写真3.　*Peganum harmala* の生育状況.

A：発芽後子葉から本葉が展開，B, C：成長葉（6月），D：花（7月），
E：果実（7月），F：果実と成熟した種子.

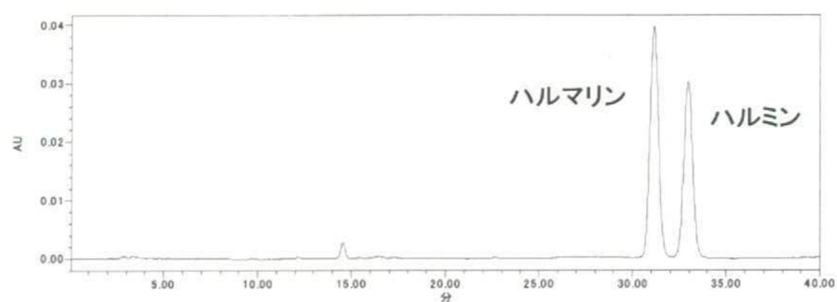

図２．種子の HPLC クロマトグラム
　　測定条件は本文に記載する。

研究成果の刊行に関する一覧表

発表者氏名	論文タイトル	発表誌名	巻	頁	出版年
Funada M, Zhou X, Satoh M, Wada K.,	Profiling of methamphetamine-induced modifications of gene expression patterns in the mouse brain.	Ann N Y Acad Sci	1025	76-83	2004

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Cellular and Molecular Mechanisms of Drugs of Abuse and Neurotoxicity

Profiling of Methamphetamine-Induced Modifications of Gene Expression Patterns in the Mouse Brain

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New York Academy of Sciences

Profiling of Methamphetamine-Induced Modifications of Gene Expression Patterns in the Mouse Brain

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ABSTRACT: Recently described DNA microarray technology allows parallel screening of expression patterns and regulation of hundreds of thousands of genes. In the present study, we used a microarray to examine the gene expressions in the midbrains of mice sacrificed 24 h after completion of a 7-day treatment period consisting of a once-daily treatment with saline (SS), saline followed by a single 2 mg/kg of body weight dose of methamphetamine (METH) (S-METH), or repeated 2 mg/kg METH doses (M-METH) that produced sensitization and place preference (rewarding effect). We used the commercially available cDNA microarray. Approximately 80% of the assessed transcripts in the total brain reached the Affymetrix criteria for "present" and "changed," as well as displaying ≥1.5-fold differences in hybridization intensity difference values in a comparison of SS data to S-METH or M-METH data. S-METH gene expression changes were observed in both up- and down-regulation, with 13 transcripts upregulated and 13 downregulated, whereas the majority of M-METH gene expression changes were observed in down-regulation, with 5 transcripts upregulated and 21 downregulated. We identified several genes that altered expression in both the S-METH and M-METH groups: a transcription factor gene, cellular stress/molecular chaperones, and a cellular regulatory gene.

KEYWORDS: brain; DNA microarray; drug dependence; methamphetamine; mouse; rewarding effect; sensitization

INTRODUCTION

Single or repeated exposure to psychostimulants produces an enduring enhancement of the locomotor-stimulating and reinforcing effects of drugs of abuse.[1,2] This phenomenon, called behavioral sensitization, is currently being evaluated as a potential model for drug addiction and drug-induced psychoses.[1,2] Several studies have documented the altered expression of a number of genes in the brains of animals ex-

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posed to amphetamine or methamphetamine.[3–7] It is important to understand that a response to methamphetamine treatment requires the activity of several pathways, and that different genes can simultaneously receive different inputs. Analysis of many genes in any single experiment provides that different conditions (such as dose of methamphetamine) might result in patterns of expression that differ among genes.

A microarray allows parallel screening of expression patterns and the regulation of thousands of genes.[8,9] Furthermore, it allows for rapid analysis of gene expression. This technology should be very useful for investigation of cellular and molecular mechanisms of drug addiction.

In the present study, we applied recently introduced DNA microarray technology to analyze the molecular mechanism of methamphetamine-induced behavioral sensitization.

MATERIALS AND METHODS

Animals

Male ICR mice (20–25 g) were obtained from Tokyo Animal Laboratories, Inc. (Tokyo, Japan). The mice were maintained on a 12-h light/dark schedule, with laboratory mouse chow and water provided *ad libitum*. All procedures were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, approved by the Japanese Pharmacological Society.

Drugs

Methamphetamine was purchased from Dainippon Seiyaku (Osaka, Japan).

Behavioral Analysis

Methamphetamine-Induced Behavioral Sensitization

The mice were divided into two groups. The acute-group mice received one methamphetamine administration on Day 1 (S-METH). The chronic-group mice received methamphetamine (2 mg/kg, s.c.) once daily for 7 days (M-METH). The effect of repeated treatment with methamphetamine on methamphetamine-induced hyperlocomotion was evaluated by examining the locomotion of these mice. Locomotor activity was monitored automatically by this system (NS-DAS-8, Neuroscience, Japan).

Conditioned Place Preference Procedure

Conditioning sessions (7 for methamphetamine, 7 for saline) were conducted twice daily for 7 days using a shuttlebox (15 × 30 × 15 cm: w × l × h) that was divided into two equal-sized compartments by a sliding wall. One compartment was white with a textured floor, and the other was black with a smooth floor. The shuttlebox treatment compartment selected, and the order of administration of methamphetamine the vehicle were counterbalanced. All conditioning sessions were 50 min in duration, and a minimum of 7 h between administration of methamphetamine and

that of the vehicle. Test sessions were carried out with mice in a drug-free state, at 1 day after the final training session. Mice were placed in the center of the shuttlebox and allowed free access to both compartments. The total time spent in each compartment during a 15-min session was recorded.

Microarray Analysis

Methamphetamine Treatment

Mice in each of the three treatment groups received systemic injections at 9:00 h. Chronic M-METH mice were administered methamphetamine (2 mg/kg) once daily for 7 days. The acute-group mice received once daily administrations of saline for 6 days, followed by a single administration of S-METH on day 7. Control (SS) mice received administrations of saline once daily for 7 days. In the present study, we selected the midbrain as the target region. Twenty-four hours after each of the three groups of mice were administered the last dose of methamphetamine or saline, mice were sacrificed and the midbrain was dissected.

Microarray Hybridization

Poly(A)$^+$ mRNA was prepared from total RNA using an Oligotex-dT30 mRNA purification kit (TaKaRa Shuzo Co., Ltd., Shiga, Japan). The fluorescently labeled cDNA probes were prepared as described. One mg aliquots of mRNA from experimental tissue (S-METH or M-METH) and control tissue (SS) were labeled using a RNA Fluorescence Labeling Core Kit (M-MLV version, TaKaRa Shuzo Co., Ltd.) with Cy5-dUTP and Cy3-dUTP (Amersham, USA), respectively, in each paired case. We used the commercially available cDNA microarray (IntelliGene Mouse Chip Set. 1, TaKaRa Shuzo Co., Ltd.). After hybridization for 16 h, the slides were scanned using the Affymetrix 428 scanner (Affymetrix, Santa Clara, CA).

Data Analysis

The signal intensity of hybridization was evaluated photometrically by the ImaGene computer program (BioDiscovery) and normalized to the averaged signals of housekeeping genes (or global normalization). A cutoff value for each expression level was calculated according to the background fluctuation. The fluctuation can be estimated as the variance of the log ratio of Cy3/Cy5. The display of ≥1.5-fold differences in hybridization intensity difference values between SS data and either S-METH or M-METH data were picked up as an indication of the candidate gene.

RESULTS

Behavioral Analysis

First, in order to fix the schedule of repeated methamphetamine treatment, we confirmed the behavioral property of mice by examining the locomotion of these mice after repeated methamphetamine treatment. The effect of repeated methamphetamine treatment on locomotor activity is shown in FIGURE 1A. In the chronic

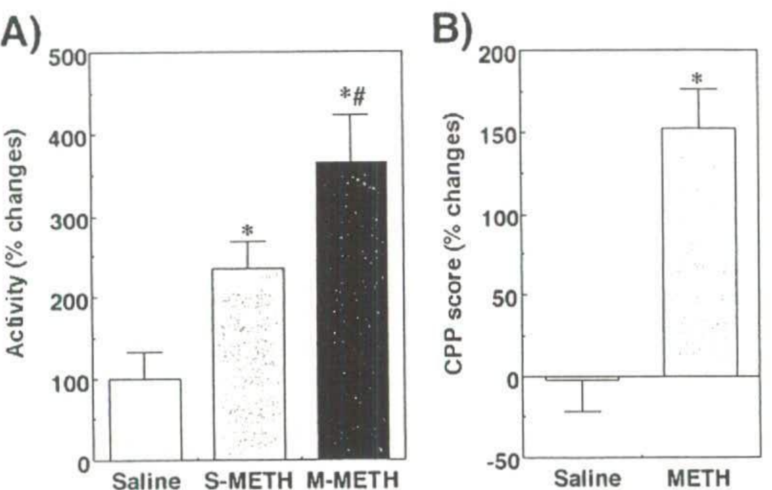

FIGURE 1. (**A**) Effect of repeated administration of methamphetamine on locomotor response to methamphetamine in mice. Methamphetamine (2 mg/kg, s.c.) -induced hyperlocomotion was monitored for 120 min. Chronic methamphetamine group (M-METH) mice received once-daily administrations of methamphetamine (2 mg/kg) for 7 days. The acute-group mice received once-daily administration of saline for 6 days followed by a single administration of methamphetamine on day 7 (S-METH). Each value represents the percentage of baseline (saline-treated group) activity. $^{*}P < .05$ vs. saline-treated group. $^{\#}P < .05$ vs. S-METH group. (**B**) Place preference conditioning produced by methamphetamine administration in mice. Conditioning sessions (7 for methamphetamine; 7 for saline) were conducted twice daily for 7 days. The selected treatment compartment of the shuttlebox and the order of administration of methamphetamine (2 mg/kg, METH) and saline were counterbalanced. Each value represents the percentage of baseline score. $^{*}P < .05$ vs. saline-treated group.

groups, as compared to the acute treatment group, methamphetamine-induced hyperlocomotion was clearly enhanced. These results indicate that this schedule of methamphetamine treatment produces behavioral sensitization to methamphetamine. Next, we examined the rewarding effect of methamphetamine following this methamphetamine treatment schedule. The rewarding effect of methamphetamine was examined using a conditioned place preference procedure.

As shown in FIGURE 1B, methamphetamine produced a significant place preference. These results suggest that the conditioning of methamphetamine treatment for seven days produced a rewarding effect of methamphetamine. Furthermore, the present results indicate that our schedule of methamphetamine treatment produces behavioral sensitization and a rewarding effect. Using this methamphetamine treatment schedule, we examined changes in gene expression in the brain.

Microarray Analysis

Using the ImaGene computer program, we normalized the signal intensity of hybridization to the averaged signals of housekeeping genes. In our study, we detected

TABLE 1. Upregulated gene expression in the mouse midbrain after methamphetamine treatment

Gene	Accession number	S-METH	M-METH
Cytokine-inducible SH2-containing protein	D31943	↑	=
Mouse gene for beta-1-globin	V00722	↑	=
Upstream transcription factor 1	X95316	↑	=
DNA segment, Chr 5	AW413033	↑	=
Murine Glvr-1	M73696	↑	=
Raf-related oncogene	D00024	↑	=
Mus musculus tbc1	U33005	=	↑
ATP-binding cassette, subfamily G (WHITE), member 1	U34920	=	↑
Zinc finger protein	D21850	↑	↑
Glucocorticold-induced leucine zipper	NM010286	↑	↑
Inositol polyphosphate-5-phosphatase, 145 kDa	U52044	=	↑
ESTs	AI326878	↑	=
ESTs	AI528727	↑	=
ESTs	AI116931	↑	=
ESTs	AA764080	↑	=
ESTs	AI324037	↑	=
	Increase	13(11*)	5(3*)

*The asterisk indicates the number of specific genes that changed only in the acute or the chronic methamphetamine treatment group.

genes that changed by 1.5-fold or 1/1.5-fold. TABLE 1 shows the upregulated gene expression results in the midbrain. Focusing on the acute methamphetamine-treated group, we listed the genes in rank order. Upstream transcription factor 1 and SH2-containing protein, which are related to cell-signaling pathways, increased only in the acute states (S-METH). Interestingly, glucocorticoid-induced leucine zipper and zinc finger protein 57, which are related to cellular stress, increased in both acute (S-METH) and chronic states (M-METH). TABLE 2 shows the downregulated gene expression results in the midbrain. Focusing on the acute methamphetamine-treated group, we listed the genes in rank order. Inositol 1,4,5-triphosphate receptor 1 and cyclic nucleotide phosphodiesterase, which are related to cell-signaling pathways, decreased only in the chronic states (M-METH). Zinc finger protein 36, which is related to cellular stress, also decreased in the chronic states (M-METH). Nine of the ESTs decreased in both the acute (S-METH) and the chronic states (M-METH).

As described in TABLE 1 and TABLE 2, there are some upregulated and some downregulated genes. These data must be confirmed by RT-PCR or Northern blot analysis.

DISCUSSION

There is considerable evidence that the mesolimbic dopamine system plays an important role in psychostimulants addiction.[1,2,10,11] Microinjection of a psycho-

TABLE 2. Downregulated gene expression in the mouse midbrain after methamphetamine treatment

Gene	Accession number	S-METH	M-METH
Procollagen, type XI, alpha 1	D38162	↓	=
Signal transducer and activator of transcription 1	U06924	↓	=
LIM and cysteine-rich domains 1	AU035886	↓	=
Inositol 1,4,5-triphosphate receptor 1	X15373	=	↓
Protein phosphatase 3, catalytic subunit, gamma isoform	M81475	=	↓
Cyclic nucleotide phosphodiesterase (PDE1A2)	U56649	=	↓
Guanine nucleotide binding protein, beta 1	U29055	=	↓
Transcription factor E2a	X17500	=	↓
TATA box binding protein	U63933	=	↓
Nuclear receptor subfamily 4, group A, member 1	NM010444	=	↓
Acetyl-coenzyme A dehydogenase, medium chain	U07159	=	↓
G protein, gamma transducing activity polypeptide 2	AA028742	=	↓
Aplysia ras-related homolog 9 (RhoC)	X80638	=	↓
Zinc finger protein 36	M58691	=	↓
ESTs	A1605943	↓	↓
ESTs	AA217070	↓	=
ESTs	AI647914	↓	↓
ESTs	AI326244	↓	↓
ESTs	AI256292	↓	↓
ESTs	AW536861	↓	↓
ESTs	AI326325	↓	↓
ESTs	AI314081	↓	↓
ESTs	AW556570	↓	↓
ESTs	AA142505	↓	↓
ESTs	AI853455	=	↓
	Decrease	13(4*)	21(19*)

*The asterisk indicates the number of specific genes that changed only in the acute or the chronic methamphetamine treatment group.

stimulant drug into the ventral tegmental area, the cell body region of the mesolimbic dopaminergic system, produces behavioral sensitization.[12] In the present study, we selected the midbrain as the target region. The acute-group mice were administered a single challenge of methamphetamine, and the midbrain was dissected after 24 h. The chronic-group mice were administered repeated doses of methamphetamine: a dose a day, for seven days. The present behavioral analysis has shown that this schedule of repeated treatment with methamphetamine produces behavioral sensitization and a rewarding effect.

The present study demonstrates that there are some upregulated and some downregulated genes in response to methamphetamine stimulation. Many of these changes in gene expression could be correlated with sensitization to the motor effect of methamphetamine and with its rewarding effect. These data must be confirmed by performing RT-PCR or Northern blot analysis. However, our data may be considered quantitatively; it demonstrates that glucocorticoid-induced leucine zipper and zinc

finger protein 57, which are related to cellular stress, increased in both acute (S-METH) and chronic states (M-METH). Several stressors, such as foot-shock and food-restriction, increase sensitivity to psychostimulants by enhancing their effects on the mesolimbic dopamine system.[2,13] It has been shown that stress-induced sensitization of the motor and addictive effects of psychostimulants may be mediated by stress-induced secretion of glucocorticoids.[13] Our results suggest that modification of the glucocorticoids-mediated system may be involved in the methamphetamine-induced gene expression changes of the midbrain.

We found that DNA microarray analysis reveals some upregulated and some downregulated genes in the midbrain. Our results indicate that single and/or repeated administration of methamphetamine could regulate multiple signaling pathways in the midbrain. However, these data must be confirmed by RT-PCR or Northern blot analysis. Additional studies are needed to delineate that these changes in gene expressions are related to the molecular mechanisms of development of methamphetamine sensitization and psychological dependence.

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植物由来催幻覚成分の薬物依存性
および細胞毒性の評価

課題番号：H16-特別-021

研究報告書

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