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Effects of a 5-HT$_7$ receptor antagonist DR4004 on the exploratory behavior in a novel environment and on brain monoamine dynamics in mice

Hiroshi Takeda [a,*], Minoru Tsuji [a], Hideaki Ikoshi [a], Tomoko Yamada [a], Jiro Masuya [b], Makio Iimori [b], Teruhiko Matsumiya [a]

[a] *Department of Pharmacology, Tokyo Medical University, 6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402, Japan*
[b] *Department of Psychiatry, Tokyo Medical University, 6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 160-0023, Japan*

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Abstract

The present study examined whether serotonin (5-hydroxytryptamine; 5-HT)$_7$ receptors play a role in the modulation of emotionality in mice using the selective 5-HT$_7$ receptor antagonist 2*a*-[4-(4-phenyl-1,2,3,6-tetrahydropyridyl)butyl]-2*a*,3,4,5-tetrahydrobenzo (*c*,*d*)indol-2-(1*H*)-one (DR4004). The emotionality of mice was evaluated in terms of exploratory activity in the hole-board test. The mice treated with DR4004 (2.5–10 mg/kg, i.p.) displayed a dose-dependent decrease in locomotor activity by moving less distance in the hole-board, and statistically significant decreases were observed at 5 and 10 mg/kg. On the other hand, DR4004 (10 mg/kg, i.p.) did not affect spontaneous motor activity. In a neurochemical study, decreases in amygdaloid dopamine and 5-HT turnover were observed in mice in which locomotor activity in the hole-board test was attenuated following the administration of DR4004 (10 mg/kg, i.p.). Also, a simple linear regression analysis revealed that locomotor activity on the hole-board was significantly correlated with dopamine and 5-HT turnover in amygdala. Furthermore, co-injection of the selective dopamine reuptake inhibitor 1-(2-[bis(4-fluorophenyl)methoxy]ethyl)-4-(3-phenylpropyl)piperazine (GBR12909; 1.25–5 mg/kg, i.p.) or the selective 5-HT reuptake inhibitor fluvoxamine (20 mg/kg, i.p.) significantly reversed the DR4004 (10 mg/kg, i.p.)-induced decrease in locomotor activity in the hole-board test. These findings constitute the behavioral evidence that 5-HT$_7$ receptors may play a role in the modulation of emotionality. Furthermore, it is also suggested that amygdaloid dopamine and 5-HT neuronal systems may be involved in this modulation.

Keywords: 5-HT$_7$ (5-hydroxytryptamine7) receptors; Exploratory behavior; Hole-board test; Monoamines; Amygdala; (Mouse)

1. Introduction

The brain serotonin (5-hydroxytryptamine; 5-HT) nervous system has been implicated in various brain functions as well as in the pathophysiology and treatment of a wide variety of neuropsychiatric disorders (Bauer et al., 2002; Jones and Blackburn, 2002). A heterogeneous family of at least 14 distinct receptor subtypes has been shown to mediate the effect of 5-HT in the central nervous system (Hoyer and Martin, 1997). Among these, the 5-HT$_1$ and 5-HT$_2$ receptor subtypes have received particular attention as possibly being involved in mediating emotionality and as targets for the treatment of affective disorders such as anxiety and depression (Murphy et al., 1999; Jones and Blackburn, 2002). However, it is still unknown whether other 5-HT receptor subtypes play a role in the modulation of emotionality.

The 5-HT receptor subtype that was most recently identified by molecular cloning is the seven-transmembrane-spanning G-protein-coupled 5-HT$_7$ receptor (Ruat et al., 1993; Shen et al., 1993). Studies using autoradiography, in situ hybridization, radioligand binding and immunohistochemistry techniques have shown that 5-HT$_7$ messenger RNA (mRNA) and receptor protein have a similar abundant

* Corresponding author. Tel.: +81 3 3351 6141x327; fax: +81 3 3352 0316.
E-mail address: ht0417@tokyo-med.ac.jp (H. Takeda).

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distribution in various brain regions, i.e., cerebral cortex, hippocampus, thalamus amygdala and hypothalamus (Ruat et al., 1993; Shen et al., 1993; Gustafson et al., 1996; Neumaier et al., 2001). However, it has been difficult to identify any physiological role for this receptor in the central nervous system, although there is some evidence which suggests that 5-HT_7 receptors may be involved in the regulation of circadian rhythms (Lovenberg et al., 1993; Ehlen et al., 2001).

It has been demonstrated that several receptor agonists and antagonists have affinity for 5-HT_7 receptors, but none of them is selective (Eglen et al., 1997). Thus, the development of selective ligands for 5-HT_7 receptors will be of utmost importance in pharmacologically determining the physiological role of this receptor subtype. Recently, Kikuchi et al. (1999) developed a 2*a*-[4-(4-phenyl-1,2,3,6-tetrahydropyridyl)butyl]-2*a*,3,4,5-tetrahydrobenzo(*c*,*d*)indol-2-(1*H*)-one (DR4004) as a selective 5-HT_7 receptor antagonist, which displaces the binding of [^{3}H]5-carboxamidotryptamine with high affinity and selectivity, and also inhibits the 5-HT-induced stimulation of cyclic AMP accumulation in a mammalian cell line (COS-7 cells) expressing 5-HT_7 receptors. This compound has recently been used as a tool for determining the actual functions of 5-HT_7 receptors (Ehlen et al., 2001; Matsumoto et al., 2002).

The expression and distribution of mRNA and proteins for 5-HT_7 receptors in the midline, thalamus and limbic structures (Ruat et al., 1993; Gustafson et al., 1996; Neumaier et al., 2001) suggest that they may play a role in the regulation of emotion. This hypothesis may be supported by evidence that some compounds that affect emotionality have an affinity for 5-HT_7 receptors; examples include antipsychotics, anxiolytics and antidepressants (Shen et al., 1993; Eglen et al., 1997). Furthermore, it has also been reported that chronic treatment with an antidepressant as well as exposure to stress stimuli affected the density or function of 5-HT_7 receptors (Sleight et al., 1995; Shimizu et al., 1996; Mullins et al., 1999; Yau et al., 2001). However, there is still no conclusive experimental evidence for the regulation of emotion by 5-HT_7 receptors. Therefore, the aim of the present study was to examine whether 5-HT_7 receptors play a role in the modulation of emotionality. Changes in the emotional behavior of mice produced by the selective 5-HT_7 receptor antagonist DR4004 were evaluated using our hole-board apparatus (Takeda et al., 1998, 2003; Tsuji et al., 2000, 2001). Furthermore, to elucidate the mechanisms involved in the expression of these behavioral changes, a neurochemical analysis on monoamine dynamics in mouse brain regions was also performed.

2. Materials and methods

The present studies were conducted in accordance with the Guide for Care and Use of Laboratory Animals adopted by the Committee on Care and Use of Laboratory Animals of Tokyo Medical University and the Japanese Pharmacological Society.

2.1. Animals

Male ICR mice (Charles River, Japan) weighing 25–30 g were housed at a room temperature of 22 ±1 °C with a 12-h light–dark cycle (light on 6:00 a.m. to 6:00 p.m.). Food and water were available ad libitum.

2.2. Apparatus for hole-board test

Exploratory behaviors of mice in a novel environment were measured as previously described using an automatic hole-board apparatus (Muromachi Kikai, Japan) (Takeda et al., 1998, 2003; Tsuji et al., 2000, 2001). The apparatus consisted of a gray box (50 × 50 × 50 cm) with four equidistant holes 30 mm in diameter in the floor. An infrared beam sensor was installed on the wall (65 mm upper and 20 mm lower from floor) to detect the number and duration of rearing and head-dipping behaviors. The horizontal moving distance of mice in the hole-board (locomotor activity) was detected by an overhead color charge-coupled device (CCD) camera (the heads of mice were painted yellow to detect the locus in the hole-board by the color CCD camera and the distance that the painted head moved was used as an indicator of locomotor activity). Head-dipping behaviors were double-checked via an infrared beam sensor and the overhead color CCD camera. Thus, head-dipping behavior was counted only when both the head intercepted the infrared beam and the head was detected at the hole by the CCD camera. Data from the infrared beam sensor and the CCD camera were collected through a custom-designed interface (CAT-10, Muromachi Kikai, Japan) as a reflection signal. All data were analyzed and stored by a personal computer with analytical software (Comp ACT HBS; Muromachi Kikai, Japan).

2.3. Procedure for hole-board test

Groups of mice were injected with DR4004 (2.5–10 mg/kg), 1-(2-[bis(4-fluorophenyl)methoxy]ethyl)-4-(3-phenylpropyl)piperazine (GBR12909; 2.5-10 mg/kg), fluvoxamine (5–20 mg/kg), sulpiride (20 mg/kg) or vehicle (saline or 1% Tween 20 in saline; 10 ml/kg) intraperitoneally (i.p.). Thirty minutes later, each animal was placed in the center of the hole-board and allowed to freely explore the apparatus. Total moving distance, number and duration of rearing and head-dipping behaviors and latency to the first head-dip were automatically recorded for 5 min. In combination studies, GBR12909 (1.25–5 mg/kg, i.p.), fluvoxamine (5–20 mg/kg, i.p.), or sulpiride (20 mg/kg, i.p.) was co-injected with DR4004 (10 mg/kg, i.p.).

2.4. Apparatus and procedure for measurement of spontaneous motor activity

2.4.1. Measurement of the motor activity of mice that had been habituated to a novel environment

The motor activity of mice that had been habituated to a novel environment was measured using an activity-monitoring system (Supermex, Muromachi Kikai, Japan) as previously described (Takeda et al., 2002, 2003). Briefly, mice were placed

in cylinders (19 cm in diameter and 25 cm high) and allowed to explore for 90 min. After this initial acclimation period had expired, motor activity counts in each 10-min segment were automatically recorded by a Supermex sensor (Muromachi Kikai, Japan) for 180 min following the administration of DR4004 (10 mg/kg, i.p.) or vehicle (1% Tween 20 in saline; 10 ml/kg, i.p.). Data from the Supermex sensor were collected through a custom-designed interface (DI-032, Muromachi Kikai, Japan). All data were analyzed and stored by a personal computer with analytical software (CompACT AMS software; Muromachi Kikai, Japan).

2.4.2. Measurement of the motor activity of mice in the home cage

Mice were individually housed in the home cage for 1 week. And then, motor activity counts of mice in each 5-min segment in the home cage were recorded for 120 min following the administration of DR4004 (10 mg/kg, i.p.) or vehicle (1% Tween 20 in saline; 10 ml/kg, i.p.) using an activity-monitoring system (Supermex, Muromachi Kikai, Japan).

2.5. Neurochemical analysis

The concentrations of monoamines and their metabolites were determined as described previously (Takeda et al., 1990, 2003) with minor modifications. Mice were decapitated immediately after the hole-board test, and their brains were rapidly removed and washed slightly with iced saline. Brains were then dissected into five regions (frontal cortex, hypothalamus, amygdala, hippocampus, and midbrain) on an iced plate according to the stereotaxic atlas of mouse brain reported by Paxinos and Franklin (2001). Dissected brain tissue was stored at −80 °C for future analysis. The brain tissue was homogenized with an ultrasonicator in 20 ml/mg wet tissue of ice-cold 0.1 M perchloric acid solution containing 0.1 M of EDTA-2Na, 0.5 M of sodium bisulfate. The homogenized tissue was then centrifuged at 4 °C and 20,000 $\times g$ for 15 min, and the monoamines and their metabolites in the supernatants were directly assayed by high-performance liquid chromatography with coulometric electrochemical detection (Model 500 Coulochem Electrode Array System, ESA, MA, USA).

2.6. Drugs

DR4004 and fluvoxamine were provided by Meiji Seika Kaisha (Kanagawa, Japan). GBR12909 and sulpiride were purchased from Sigma (St. Louis, MO, USA). Fluvoxamine was dissolved in saline. Other drugs were dissolved in Tween 20 until a clear solution was obtained, and then diluted with saline to reach the proper concentrations. The final concentration of Tween 20 in the solution was 1%.

2.7. Statistical analysis

The data are presented as the mean ± S.E.M. One-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls multiple comparisons test was used for the statistical evaluation of the data obtained from hole-board test. The spontaneous motor activity in a habituated environment and home cage was statistically analyzed using two-way ANOVA followed by Bonferroni test. In the neurochemical study, norepinephrine, dopamine and 5-HT turnover were determined as the norepinephrine ratio [3-methoxy-4-hydroxyphenyl glycol (ng/g wet tissue)/ norepinephrine (ng/g wet tissue)], dopamine ratio {[3,4-dihydroxyphenylacetic acid (ng/g wet tissue)+homovanillic acid (ng/g wet tissue)]/dopamine (ng/g wet tissue)} and 5-HT ratio [5-

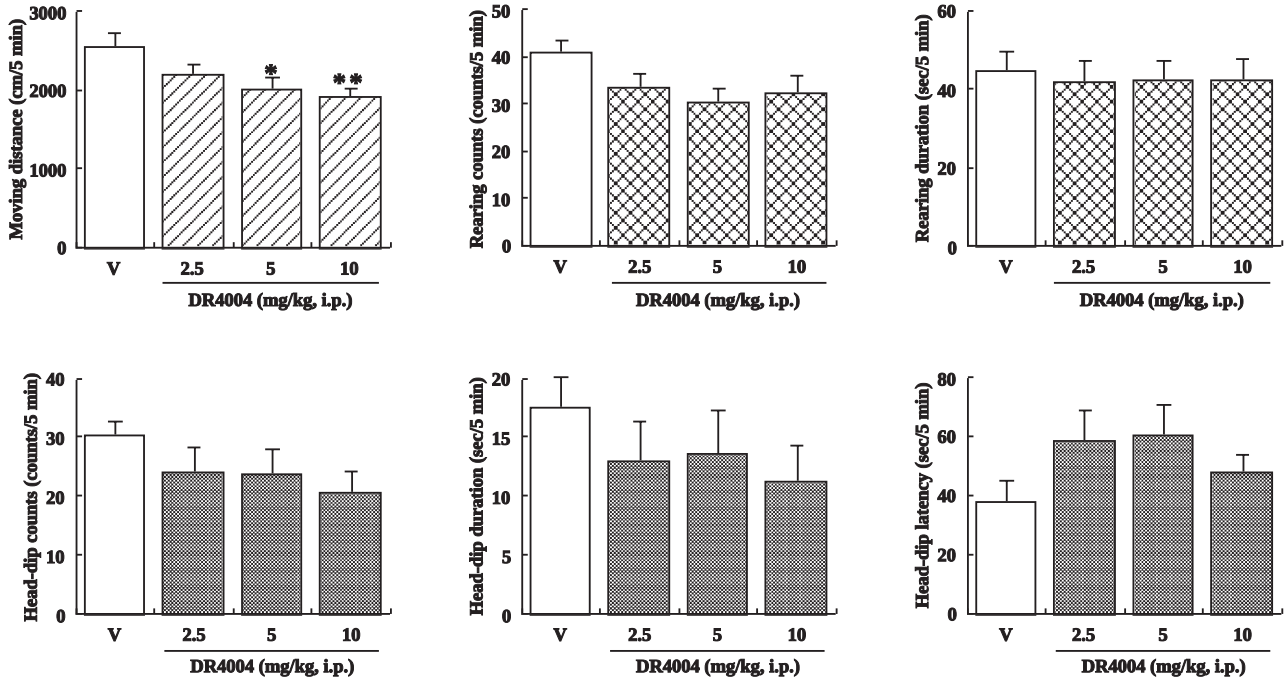

Fig. 1. Effect of DR4004 on exploratory behavior in mice tested on the hole-board. DR4004 (2.5–10 mg/kg, i.p.) or vehicle (V: 10 ml/kg, i.p.) was injected 30 min prior to the measurement of exploratory behavior. Each column represents the mean with S.E.M. of 8 mice. *$P<0.05$, **$P<0.01$ vs. vehicle-treated group.

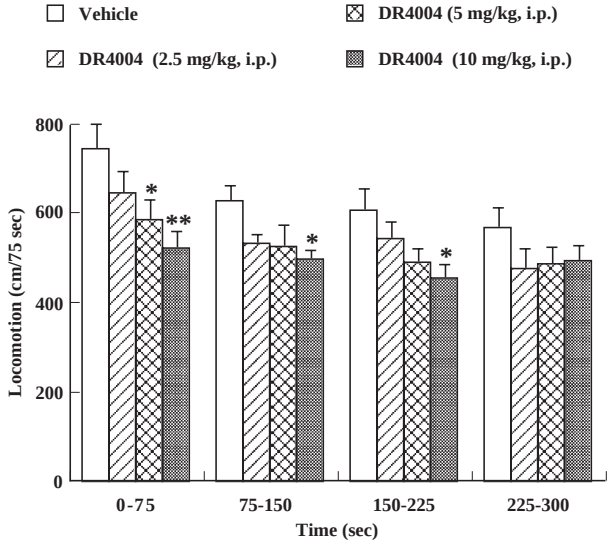

Fig. 2. Time-course of changes in locomotor activity of mice produced by DR4004 in the hole-board test. DR4004 (2.5–10 mg/kg, i.p.) or vehicle (10 ml/kg, i.p.) was injected 30 min prior to the measurement of exploratory behavior. Each column represents the mean with S.E.M. of 8 mice. $*P<0.05$, $**P<0.01$ vs. vehicle-treated group.

hydroxyindole acetic acid (ng/g wet tissue)/5-HT (ng/g wet tissue)], respectively. The neurochemical data were analyzed by Student's *t*-test.

3. Results

3.1. Effects of DR4004 on the exploratory behaviors of mice in the hole-board test

The effects of DR4004 on the exploratory behavior of mice in the hole-board test are shown in Fig. 1. The mice treated with DR4004 (2.5–10 mg/kg, i.p.) displayed a dose-dependent decrease in locomotor activity by moving less distance in the hole-board ($F(3,28)=4.943$, $P<0.01$), and the differences were statistically significant at 5 ($P<0.05$) and 10 mg/kg ($P<0.01$). In contrast, the number and duration of rearing and head-dipping behaviors as well as the latency to the first head-dip were not significantly affected by DR4004.

The effects of DR4004 on the locomotor activity of mice in each 75-sec segment in the hole-board test are shown in Fig. 2. DR4004 (5 and/or 10 mg/kg, i.p.) significantly reduced the locomotor activity within 0–225 s after the start of the hole-board test (0–75 s: $F(3,28)=4.799$, $P<0.01$; 75–150 s: $F(3,28)=3.465$, $P<0.05$; 150–225 s: $F(3,28)=3.653$, $P<0.05$).

3.2. Effects of DR4004 on the spontaneous motor activity of mice

The effects of DR4004 on the spontaneous motor activity of mice are shown in Figs. 3 and 4. DR4004 at a dose that significantly decreased locomotor activity in the hole-board test (10 mg/kg, i.p.) did not statistically affect the motor activity of mice that had been habituated to a novel environment (Fig. 3). Similarly, the motor activity of mice in the home cage was also unaffected by DR4004 (10 mg/kg, i.p.) (Fig. 4).

3.3. Effects of DR4004 on monoamine dynamics in mouse brain regions

The effects of DR4004 on monoamine dynamics in mouse brain regions are shown in Table 1. Repeated behavioral analysis in the hole-board test confirmed that DR4004 (10 mg/kg, i.p.)-treated mice consistently showed a significant decrease in the locomotor activity as shown in Fig. 1 (vehicle-treated group: 2422.8±79.0 cm ($n=8$); 1937.4±101.7 cm ($n=9$); $P<0.01$). Within the brain regions of these mice, significant decreases in the 3,4-dihydroxyphenylacetic acid (dopamine metabolite; $P<0.05$) and 5-hydroxyindole acetic acid (5-HT metabolite; $P<0.01$) concentrations were observed in the amygdala, whereas the concentrations of dopamine and 5-HT were unaffected. Furthermore, decreases in the turnover of both monoamines were also observed, although the decrease in dopamine turnover was not

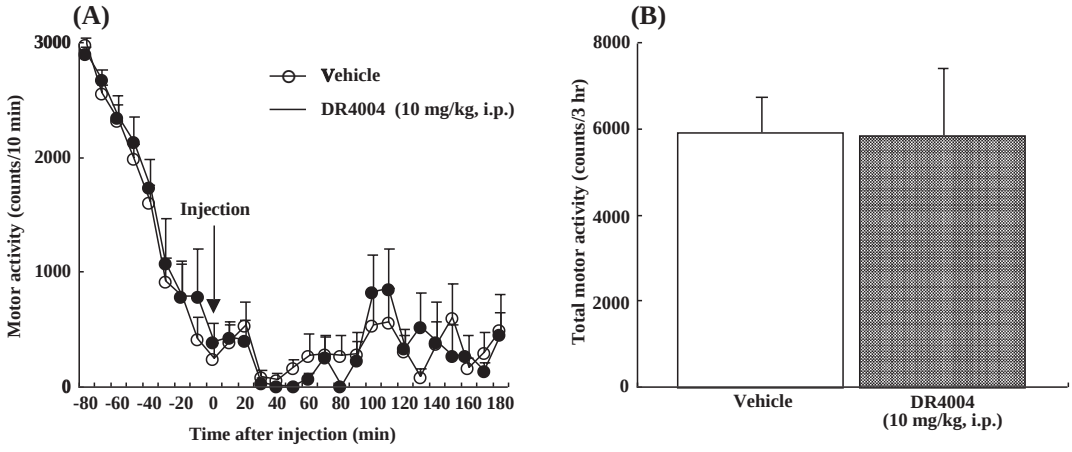

Fig. 3. Effect of DR4004 on spontaneous motor activity of mice that had been habituated to a novel environment. Motor activity counts in each 10-min segment were recorded for 90 min before and for 180 min after the administration of DR4004 (10 mg/kg, i.p.) or vehicle (10 ml/kg, i.p.). Time-course of changes in motor activity and total motor activity for 180 min after drug or vehicle administration are shown in panels A and B, respectively. Each point and column represents the mean with S.E.M. of 8 mice.

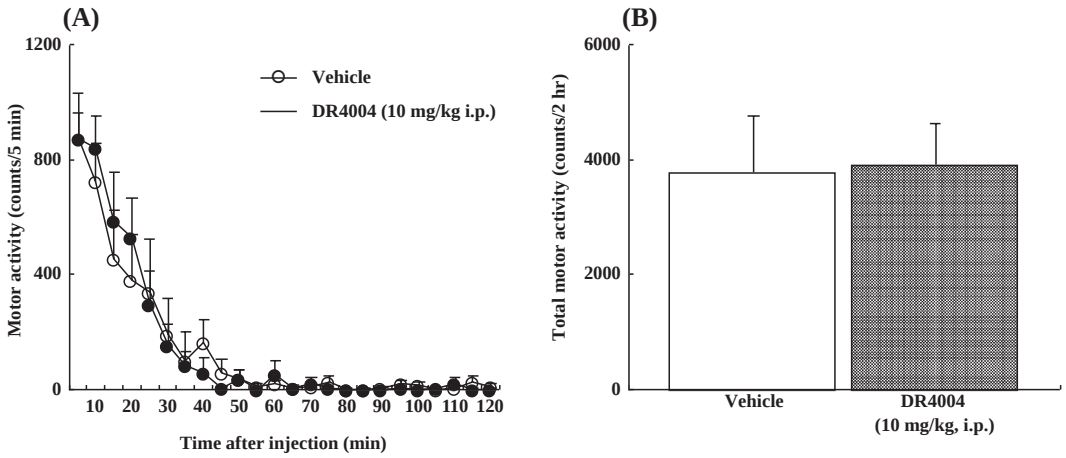

Fig. 4. Effect of DR4004 on spontaneous motor activity of mice in the home cage. Motor activity counts of mice in the home cage in each 5-min segment were recorded for 120 min after the administration of DR4004 (10 mg/kg, i.p.) or vehicle (10 ml/kg, i.p.). Time-course of changes in motor activity and total motor activity for 120 min after drug or vehicle administration are shown in panels A and B, respectively. Each point and column represents the mean with S.E.M. of 7–8 mice.

statistically significant ($P=0.089$). On the other hand, none of the monoamine dynamics in other brain regions were affected by DR 4004 (10 mg/kg, i.p.).

3.4. Correlations between locomotor activity in the hole-board test and amygdaloid dopamine or 5-HT turnover in mice

Correlations between locomotor activity in the hole-board test and amygdaloid dopamine or 5-HT turnover in mice are shown in Fig. 5. A simple linear regression analysis revealed that individual locomotor activity for all 17 animals was significantly correlated with the dopamine and 5-HT ratios in the amygdala (locomotor activity vs. dopamine ratio: $F(1,15)=14.053$, $P=0.002$, $r=0.696$ (Fig. 5A); locomotor activity vs. 5-HT ratio: $F(1,15)=10.749$, $P=0.005$, $r=0.646$ (Fig. 5B)).

3.5. Effects of dopamine and 5-HT reuptake inhibitors and dopamine D2 receptor antagonist on the exploratory behaviors of mice in the hole-board test

The effects of dopamine and 5-HT reuptake inhibitors and dopamine D2 receptor antagonist on the exploratory behaviors of mice in the hole-board test are shown in Table 2. Neither the selective dopamine reuptake inhibitor GBR12909 (2.5 and 5 mg/kg, i.p.) nor the selective 5-HT reuptake inhibitor fluvoxamine (5–20 mg/kg, i.p.) alone significantly modified exploratory behavior, although a higher dose of GBR12909 (10 mg/kg, i.p.) significantly increased locomotor activity ($F(3,44)=4.037$, $P<0.05$) and decreased head-dip counts ($F(3,44)=3.158$, $P<0.05$). Furthermore, dopamine D2 receptor antagonist sulpiride (20 mg/kg, i.p.) was also ineffective.

Table 1
Effect of DR4004 on the monoamine dynamics in mouse brain regions

	ng/g wet tissue									
	MHPG	NE	DOPAC	HVA	DA	5-HIAA	5-HT	NE ratio	DA ratio	5-HT ratio
Frontal cortex										
Vehicle	117.8±10.0	703.8±20.2	703.8±20.2	126.9±7.0	516.9±96.4	259.1±9.4	566.3±21.9	0.167±0.013	0.979±0.167	0.461±0.021
DR4004	120.3±3.6	671.2±18.7	671.2±18.7	122.4±6.5	437.2±82.7	243.2±5.9	540.2±13.3	0.180±0.007	1.033±0.157	0.451±0.011
Hypothalamus										
Vehicle	378.9±21.0	2509.1±153.7	219.6±8.0	107.5±13.6	468.9±33.6	705.6±49.0	940.8±86.5	0.153±0.008	0.712±0.037	0.761±0.027
DR4004	431.7±34.8	2578.9±114.5	200.1±12.3	123.9±10.0	447.1±25.6	675.6±47.9	855.1±52.8	0.169±0.015	0.727±0.028	0.798±0.049
Amygdala										
Vehicle	157.8±15.0	621.4±31.9	186.7±18.2	91.4±11.6	359.8±61.5	384.7±26.1	663.2±60.0	0.257±0.026	0.874±0.127	0.588±0.021
DR4004	154.5±7.8	617.1±39.7	127.4±12.6[a]	73.5±9.2	331.5±32.5	284.3±12.6[b]	599.8±40.7	0.254±0.012	0.631±0.055	0.484±0.025[b]
Hippocampus									($P=0.089$)	
Vehicle	101.6±6.1	795.1±36.7	68.8±10.3	25.1±2.1	36.1±4.1	313.9±17.3	432.9±23.6	0.129±0.008	2.722±0.256	0.727±0.023
DR4004	105.4±5.6	746.0±10.0	69.6±9.7	25.1±1.3	48.1±5.6	280.4±7.8	412.4±8.9	0.141±0.007	2.169±0.337	0.681±0.017
Midbrain										
Vehicle	194.4±6.2	1017.6±34.9	176.2±6.8	74.2±3.7	269.8±9.6	997.0±22.0	948.9±25.2	0.191±0.008	0.935±0.036	1.052±0.013
DR4004	191.5±14.7	1062.8±44.9	149.5±14.3	75.0±5.3	240.9±20.8	889.6±65.1	820.7±60.0	0.184±0.015	0.944±0.031	1.088±0.021

Mice were decapitated immediately after the measurement of exploratory behavior in the hole-board test. DR4004 (10 mg/kg, i.p.) or vehicle (1% Tween 20; 10 ml/kg, i.p.) were administered to mice 30 min prior to the hole-board test. Each value represents the mean ± S.E.M. of 8–9 mice. [a]$P<0.05$, [b]$P<0.01$ vs. vehicle-treated group. The metabolite ratio of monoamines were calculated as follows: NE ratio= MHPG/NE; DA ratio= (DOPAC+HVA)/DA; 5-HT ratio= 5-HIAA/5-HT. Abbreviations: MHPG, 3-methoxy-4-hydroxyphenyl glycol; NE, norepinephrine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; DA, dopamine; 5-HIAA, 5-hydroxyindole acetic acid; 5-HT, 5-hydroxytryptamine.

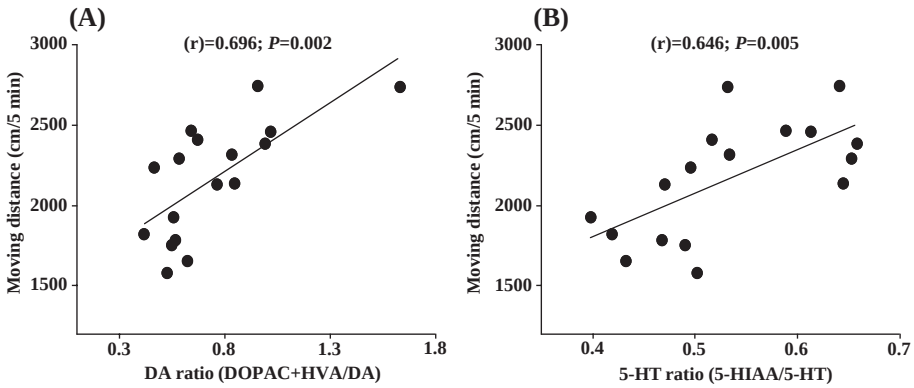

Fig. 5. Correlation between locomotion in the hole-board test and dopamine or 5-HT turnover in the amygdala. The results of a simple linear regression analysis of distance of movement in the hole-board vs. the dopamine (A) or 5-HT (B) ratio are shown for 17 mice.

3.6. Effects of dopamine and 5-HT reuptake inhibitors on the DR4004-induced decrease in locomotor activity of mice in the hole-board test

The effects of dopamine and 5-HT reuptake inhibitors on the DR4004-induced decrease in locomotor activity of mice in the hole-board test are shown in Fig. 6. Co-injection of GBR12909 (1.25–5 mg/kg, i.p.) dose-dependently and significantly reversed the DR4004 (10 mg/kg, i.p.)-induced decrease in locomotor activity ($F(4,55)=7.636$, $P<0.01$) (Fig. 6A). Furthermore, a similar tendency for reversal was also recorded by the co-injection of fluvoxamine (20 mg/kg, i.p.) ($F(4,57)=3.156$, $P<0.05$) (Fig. 6B).

3.7. Effect of dopamine D2 receptor antagonist on the DR4004-induced decrease in locomotor activity of mice in the hole-board test

The effect of sulpiride, a selective dopamine D2 receptor antagonist, on the DR4004-induced decrease in locomotor activity of mice in the hole-board test is shown in Fig. 7. Sulpiride (20 mg/kg, i.p.) alone did not significantly modify the exploratory behavior of mice in the hole-board test (see Table 2). Also, DR4004 (10 mg/kg, i.p.)-induced decrease in locomotor activity of mice in the hole-board test was unaffected by co-injection of sulpiride (20 mg/kg, i.p.).

4. Discussion

The hole-board test, which was first introduced by Boissier and Simon (1962), is a simple method for measuring the response of an animal to an unfamiliar environment. Previously, the hole-board test has been used to assess emotionality, anxiety and/or responses to stress in animals (Rodriguez Echandia et al., 1987). An advantage of this test is that several aspects of behavior can be readily observed and quantified, which makes possible a comprehensive description of the animal's behavior. To make the procedure allow for a more objective quantification of behavioral changes, we recently developed an automatic hole-board apparatus. We previously demonstrated that this system makes it possible to automatically measure changes in various exploratory activities of animals produced by the injection of compounds that affect the emotionality as well as by exposure to stress stimuli (Takeda et al., 1998, 2003; Tsuji et al., 2000, 2001), and therefore, this system may be a useful tool for objectively estimating various emotional states of animals.

In the present behavioral studies using the hole-board test, we found that i.p. administration of DR4004, a selective 5-HT_7 receptor antagonist, partly but also significantly inhibited the exploratory behavior of mice on the

Table 2
Effects of GBR12090, fluvoxamine and sulpiride on exploratory behavior of mice in the hole-board

Drugs (mg/kg, i.p.)	Moving distance (cm)	Rearing		Head-dips		
		Counts	Duration (sec)	Counts	Duration (sec)	Latency (sec)
1% Tween 20	2451.9±77.7	34.1±3.1	36.5±4.2	31.8±3.0	17.7±3.4	48.5±6.1
GBR12909 (2.5)	2557.0±130.6	33.9±3.1	40.8±2.9	33.4±2.9	16.8±2.8	44.3±5.9
(5)	2679.5±149.3	37.8±3.1	39.3±2.9	29.0±3.2	14.5±3.3	48.9±6.2
(10)	3003.8±106.5[a]	43.6±3.3	50.2±4.2	21.4±2.8[a]	7.5±1.1	45.1±8.1
Saline	2489.0±102.1	34.7±6.5	42.5±9.0	30.9±4.8	15.5±2.6	35.5±9.7
Fluvoxamine (5)	2607.0±158.1	30.8±4.7	34.6±7.5	28.1±2.6	20.9±3.0	46.3±8.3
(10)	2797.7±75.3	37.7±4.0	37.4±3.6	24.9±3.2	14.8±2.6	45.4±12.0
(20)	2640.9±141.2	26.7±4.1	31.4±6.1	24.2±2.5	13.0±2.4	45.0±7.2
1% Tween 20	2353.7±92.2	33.4±2.5	35.0±4.3	39.4±5.6	23.9±4.0	32.6±6.7
Slupiride (20)	2607.0±158.1	28.6±3.9	36.1±5.7	34.8±2.8	23.0±2.0	42.7±9.6

Drugs or vehicle (1% Tween 20 or saline) was injected 30 min prior to the measurement of exploratory behavior. Each data represents the mean with S.E.M. of 7–12 mice. [a]$P<0.05$ vs. vehicle-treated group.

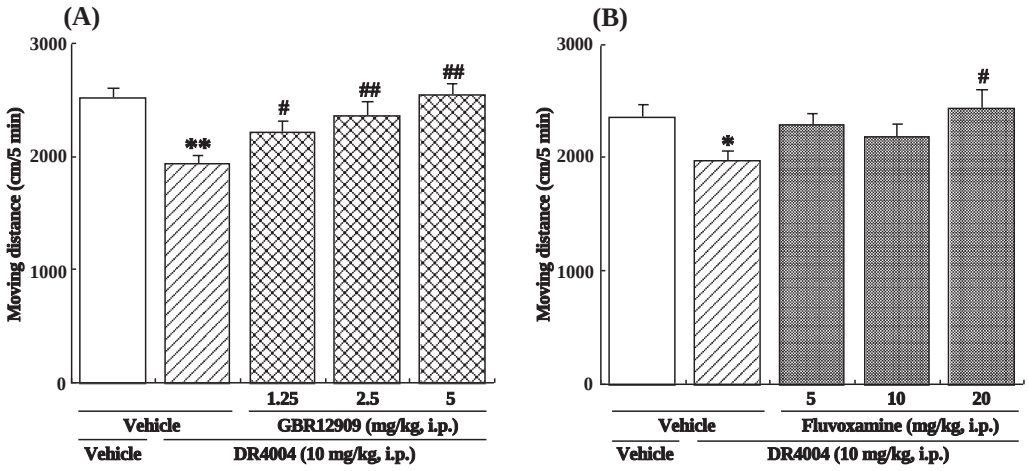

Fig. 6. Effects of GBR12909 (A) or fluvoxamine (B) on the DR4004-induced decrease in locomotion in the hole-board test. GBR12909 (1.25–5 mg/kg, i.p.) or fluvoxamine (5–20 mg/kg, i.p.) was co-injected with DR4004 (10 mg/kg, i.p). Thirty min later, exploratory behaviors of mice on the hole-board were measured for 5 min. Each column represents the mean with S.E.M. of 12–14 mice. *$P<0.05$, **$P<0.01$ vs. vehicle-treated group. #$P<0.05$, ##$P<0.01$ vs. DR4004-treated group.

hole-board with regard to only locomotor activity, i.e., the total distance of movement. Although compounds that affect general motor activity may also produce changes in exploratory behavior in the hole-board test, we confirmed in the present study that DR4004, at a dose that produces a significant effect in the hole-board test, did not affect spontaneous motor activity in mice that had been habituated to a novel environment. Furthermore, the spontaneous locomotor activity of mice in the home cage was also unaffected by injection of DR4004. Therefore, it is unlikely that the behavioral changes observed in the hole-board test are simply related to a decrease in general motor activity. Thus, the present finding suggests that changes in some component of emotionality in a novel environment may be produced by blockade of 5-HT_7 receptors. This suggestion may also be supported by further findings in the present study that although the gradually decreased locomotor activity of mice depends on the passage of time after exposure to the hole-board apparatus; in which these phenomena may have resulted from the habituation to a novel environment, DR4004 showed significant effects only at an earlier stage (0–225 s after exposure to the hole-board apparatus).

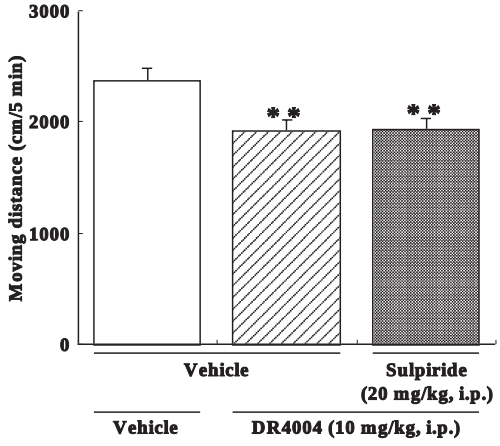

Fig. 7. Effects of sulpiride on the DR4004-induced decrease in locomotion in the hole-board test. Sulpiride (20 mg/kg, i.p.) was co-injected with DR4004 (10 mg/kg, i.p). Thirty min later, exploratory behaviors of mice on the hole-board were measured for 5 min. Each column represents the mean with S.E.M. of 12 mice. **$P<0.01$ vs. vehicle-treated group.

The type of emotional changes produced by DR4004 is still unclear. However, it should be noted that, in addition to the decrease in locomotor activity, DR4004 tended to decrease the number and duration of head-dips, as well as increase the latency to head-dipping in the hole-board test, although these behavioral changes were not statistically significant. Recently, Kanari et al. (2005) reported that the data obtained in the hole-board test show a higher intra-group variability than those in other test of anxiety, i.e., elevated plus-maze and open-field test, which is in agreement with previous report that the hole-board test has a higher sensitivity for measuring anxiety-related behaviors than the elevated plus-maze (Adamec, 1990). Thus, behavioral changes observed in the hole-board test may possibly imply some functional significance, even if they are not statistically significant. Previously, we have found that benzodiazepine anxiolytics increase the number and duration of head-dips in the hole-board test. In contrast, benzodiazepine anxiogenics as well as acute restraint stress decrease the number and duration of head-dips, and increased the latency to head-dipping, suggesting that impairment of head-dipping behavior in the hole-board test may reflect the anxiogenic state of mice (Takeda et al., 1998). Considering these previous findings, the present results in the hole-board test suggest that the blockade of 5-HT_7 receptors by DR4004 might possibly induce the anxiety. To confirm this hypothesis, it would be necessary to further characterize the behavioral effects of DR4004 using multiple tests of emotionality.

It is widely accepted that the brain monoaminergic system plays a critical role in the regulation of emotion and also in the pathogenesis of mood disorders (Maes and Meltzer, 1995; Schatzberg and Schildkraut, 1995; Willner, 1995). Therefore, in the present study, we examined the changes in monoamine dynamics in mouse brain regions after the hole-board test. In these neurochemical studies, a selective decrease in amygdaloid dopamine and 5-HT turnover was observed in mice in which locomotor activity on the hole-board was attenuated by the administration of DR4004. Also, an extended analysis of data obtained from behavioral and neurochemical studies revealed that some emotional changes, as reflected by an attenuation of locomotor activity on the hole-board, were significantly correlated with a decrease in dopamine and/or 5-HT turnover in the amygdala, which suggests that dopamine and 5-HT neuronal activity in the amygdala may be involved in the regulation of locomotor activity on the hole-board. Furthermore, the DR4004-induced decrease in locomotor activity in the hole-board test was suppressed by pharmacological manipulation to enhance dopamine and 5-HT neuronal transmission; i.e., co-injection of GBR12909, a selective dopamine reuptake inhibitor, or fluvoxamine, a selective 5-HT reuptake inhibitor, at doses that alone did not affect exploratory behaviors in the hole-board test. These findings suggest that the decrease in dopamine and 5-HT neuronal activity in the amygdala may be related, at least in part, to the attenuation of locomotor activity in the hole-board test produced by the injection of DR4004. Therefore, it is possible that 5-HT_7 receptors may play a role in the modulation of emotionality by interacting with amygdaloid dopamine and 5-HT neuronal systems.

At present, the mechanisms for the decrease in dopamine and 5-HT neuronal activity in the amygdala produced by DR4004 are still unclear. However, it has been previously demonstrated that 5-HT_7 receptors are positively coupled to adenylate cyclase via the stimulation of Gs-protein (Lovenberg et al., 1993; Ruat et al., 1993; Hoyer and Martin, 1997). Therefore, this receptor subtype may have excitatory effects on localized neuronal cells. Indeed, a recent electrophysiological study using DR4004 revealed that endogenous 5-HT-mediated synaptic transmission in the hippocampus is positively modulated by 5-HT_7 receptors (Matsumoto et al., 2002). Although the location of 5-HT_7 receptors in the amygdala has not been determined in detail, the results of the present neurochemical studies indicate that the blockade of endogenous 5-HT-induced stimulation of 5-HT_7 receptors results in a decrease in both dopamine and 5-HT neuronal activities. Therefore, it is possible that 5-HT_7 receptors might be located presynaptically at dopamine and 5-HT nerve terminals in the amygdala, and may have an excitatory effect on the activities of these neurons. Furthermore, it was recently reported that 5-HT_7 receptor agonist SB-269970-A inhibits 5-HT efflux from the slices of dorsal raphe nucleus (Roberts et al., 2004). Thus, such modulatory role of 5-HT_7 receptors at cell body sites of 5-HT neurons might also be involved in the present findings. Further detailed neuroanatomical studies on the localization and function of 5-HT_7 receptors are needed to confirm these speculations.

The previous finding by Kogan et al. (2002) suggested that dopamine D2 receptor activity might contribute to some of the in vivo effects of DR4004. Namely, DR4004 produced hyperglycemia, and this effect was reduced by raclopride, a dopamine D2 receptor antagonist. It is therefore doubtful whether the behavioral effects of DR4004 observed in the present study are also mediated by dopamine D2 receptor activity. However, we confirmed in the present study that co-injection of sulpiride, a selective dopamine D2 receptor antagonist, did not modify the DR4004-induced decrease in locomotor activity of mice in the hole-board test. This evidence seems to exclude the possibility that dopamine D2 receptor activity might be involved in the present behavioral effect of DR4004.

In addition to dopamine D2 receptors, previous receptor binding studies indicate that DR4004 has affinity also for 5-HT_{1A} and 5-HT_{2A} receptors (Kikuchi et al., 1999; Meneses, 2004). However, we have obtained data in the previous studies that neither 5-HT_{1A} receptor antagonist WAY100635 (Tsuji et al., 2001) nor 5-HT_2 receptor antagonist ketanserin (unpublished observation) showed a significant effect in the hole-board test. Therefore, we consider that the affinity for 5-HT_{1A} and 5-HT_{2A} receptors of DR4004 may not be related to the behavioral changes observed in the hole-board test.

The present observation of specific changes in amygdaloid dopamine and 5-HT turnover in mice in which locomotor activity on the hole-board was attenuated by the injection of DR4004 may be of great significance, since both the brain region and monoamines are thought to be an important neurobiological basis for the mechanisms of the regulation of emotion. In animals, it has been reported that bilateral lesions of the amygdala result in significant changes in social behaviors usually associated with the emotional state (Zola-Morgan et al., 1991), decreased autonomic responses to emotionally salient stimuli (Bagshaw et al., 1965) and impaired fear conditioning (Gallagher et al., 1990). Similar abnormal emotionality resulting from amygdala lesions has also been observed in human studies, e.g., emotional blunting (Aggleton and Brown, 1999), impaired enhanced perception of emotionally salient events (Anderson and Phelps, 2001) and reduced fear conditioning (Bechara et al., 1995). Also, functional magnetic resonance imaging (fMRI) studies have demonstrated activation of the amygdala in response to changes in the emotional state during a fear-conditioning paradigm (LaBar et al., 1998; Buchel et al., 1999). These findings suggest that the amygdala may be a key brain region that controls the emotional state and behavior.

Furthermore, previous animal studies have provided evidence that both the 5-HT and dopamine neuronal systems in the amygdala play a significant role in the regulation of

emotion. Specifically, pharmacological manipulation of dopamine and 5-HT neuronal transmission in the amygdala affected the behaviors of animals in various emotional paradigms (Graeff et al., 1997; Nader and LeDoux, 1999; Guarraci et al., 2000). Additionally, recent fMRI studies in humans suggested that both dopamine and 5-HT are important as specific neuronal substrates involved in the responses of the amygdala to emotional stimuli (Hariri et al., 2002a, 2002b). Based on this evidence, including our present findings, further detailed studies focusing on 5-HT_7 receptors, which may functionally interact with the dopamine and 5-HT neuronal systems in the amygdala, may be useful for understanding the mechanisms that underlie the regulation of normal emotionality, and also the pathophysiology of some neuropsychiatric disorders that result from the dysfunctional regulation of emotion.

In conclusion, the present study demonstrated that DR4004, a selective 5-HT_7 receptor antagonist, decreased both locomotor activity in a novel environment and dopamine and 5-HT turnover in the amygdala in mice. Also, a simple linear regression analysis revealed a significant correlation between locomotor activity on the hole-board and dopamine or 5-HT turnover in the amygdala. Furthermore, the DR4004-induced decrease in locomotor activity was antagonized by pharmacological activation of dopamine and 5-HT neuronal transmission resulting from blockade of their monoamine transporters. These findings therefore constitute the behavioral evidence that 5-HT_7 receptors may play a role in the modulation of emotionality. Furthermore, it is also suggested that the amygdaloid dopamine and 5-HT neuronal systems may be involved in this modulation.

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