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# Long-lasting change in 5-$HT_{2A}$ receptor-mediated behavior in rats after a single footshock

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**Abstract**

To investigate the long-term functional change in the 5-$HT_{2A}$ receptor after acute stress, we examined the effect of single footshock on head shake behavior induced by the 5-$HT_{2A}$ receptor agent (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI) in rats. Head shakes were evoked in a dose-dependent manner by 0.1–10 mg/kg of DOI, and the maximal response was attenuated by a single footshock given 24 h before. This suggests that there is a decrease in the number of functionally effective 5-$HT_{2A}$ receptors. The single footshock-induced reduction in head shakes evoked by DOI was observed immediately and 24 h after footshock, and lasted until 1 and 2 weeks after footshock. Because there were no changes in the [$^3$H]ketanserin binding of the frontal cortex 1 week after footshock, decreases in head shakes were not due to the down-regulation of 5-$HT_{2A}$ receptors evoked by footshock.


*Keywords:* DOI; 5-$HT_{2A}$ receptor; Head shake; 5-HT (5-hydroxytryptamine, serotonin); Stress

## 1. Introduction

Several studies have indicated that acute single stress changes serotonin (5-hydroxytryptamine, 5-HT) neurotransmission in the brain. To investigate the changes in the presynaptic components of 5-HT neurotransmission evoked by stress, in vivo microdialysis studies of 5-HT release were performed during or immediately after stress. These studies reported that acute single stress, such as electric footshock, restraint, forced swimming, and conditioned fear stress, increased extracellular 5-HT concentrations in the medial prefrontal cortex, amygdala, hippocampus, and other brain regions of rats (Kawahara et al., 1995; Yoshioka et al., 1995; Hashimoto et al., 1999; reviewed at Rueter et al., 1997).

Postsynaptic serotonergic responses to stress are mediated by a number of different 5-HT receptor subtypes. Among the subtypes, 5-$HT_{1A}$ and 5-$HT_{2A}$ receptors have special roles in serotonergic responses to stress, and have been suggested to be involved in affective disorders and anxiety disorders. Single restraint stress did not affect 5-$HT_{1A}$ agent-induced behavioral responses 24 h after stress (Kennett et al., 1985). Yamada et al. (1995) reported that an acute single physical stress, such as tail pinch, restraint by taping, and electric footshock, decreased 5-$HT_{2A}$ receptor-mediated head shake behavior induced by the 5-$HT_{2A/2C}$ agent (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI), but failed to alter the $B_{max}$ and $K_d$ of [$^3$H]ketanserin binding in the rat prefrontal cortex and hippocampus. Chaouloff et al. (1994) reported opposite results, showing that a single restraint stress did not affect DOI-induced head shake behavior 2 h after stress. These previous studies examined the short-lasting effects of a single stress (immediately to 24 h after stress), but a long-lasting effect of the single stress on 5-$HT_{2A}$ receptor function has not been examined.

In recent years, to examine the effects of 5-HT-related drugs on anxiety, we have studied conditioned fear stress in rats as an animal model of anxiety (Hashimoto et al., 1996, 1997; Inoue et al., 1996a,b; Muraki et al., 1999; Li et al., 2001). Rats are individually subjected to scrambled footshocks from the grid floor of the shock chamber. After 1 to 14 days, rats are again placed in the shock chamber without

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shocks. In this situation, rats react with the species-specific defensive response of freezing (Fanslow, 1980). Even 14 days after footshock, rats exhibit freezing behavior when they are again placed in the shock chamber without shocks (Li et al., 2001). This result suggests that an acute single footshock is enough to cause a long-lasting functional change in the brain. Van Dijken et al. (1992a,b,c, 1993) also indicated that acute single stress caused long-lasting behavioral and endocrinological changes in rats even several weeks after footshock.

In the present study, to investigate the long-lasting functional change in the 5-$HT_{2A}$ receptor system after an acute single stress, we examined the effect of a single electric footshock on head shake behavior induced by the 5-$HT_{2A/2C}$ receptor agent DOI in rats immediately after footshock, and at 24 h, 1 and 2 weeks after footshock. We also examined the effect of a single electric footshock on [$^3$H]ketanserin binding in the frontal cortex of the rats at 1 week after footshock.

## 2. Material and methods

The study was approved by the Hokkaido University School of Medicine Animal Care and Use Committee and was in compliance with the Guide for the Care and Use of Laboratory Animals, Hokkaido University School of Medicine.

### 2.1. Animals

Male Sprague–Dawley rats (Shizuoka Laboratory Animal Center, Shizuoka, Japan), weighing 250–300 g, were used. The rats were housed four per cage (38 × 33 × 17 cm), and were maintained in a 12-h light:12-h dark cycle, temperature-controlled environment (22 ± 1 °C), with free access to food and water. Experiments began after a 14-day adaptation period.

### 2.2. Drugs

(±)-2,5-Dimethoxy-4-iodoamphetamine hydrochloride (DOI) was purchased from Sigma (St. Louis, USA). [$^3$H]Ketanserin (60.0 Ci/mmol) was purchased from DuPont New England Nuclear (Boston, USA). Methysergide was a generous gift from Dr. H.Y. Meltzer (Department of Psychiatry, Vanderbilt University School of Medicine, Nashville, USA). DOI was dissolved in saline and was injected subcutaneously (s.c.) at a volume of 1 ml/kg.

### 2.3. Inescapable footshock

Rats were individually subjected to 5 min of inescapable electric footshock [0.2 mA of scrambled shock, on a variable interval schedule, with a mean intershock interval of 60 s (35–85 s) and a shock duration of 30 s] in a plexiglas chamber with a grid floor (19 × 22 × 20 cm, Medical Agent, Kyoto, Japan). Electric shock was provided by a Model SGS-02D Shock Generator (Medical Agent). The same shock intensity duration and parameter, used in previous conditioned fear experiments (Hashimoto et al., 1996, 1997; Inoue et al., 1996a,b; Muraki et al., 1999; Li et al., 2001) was used.

### 2.4. Head shake count

Rats were individually placed in clear plexiglas cages (38 × 33 × 17 cm) for behavioral testing. The frequency of head shakes induced by subcutaneous injections of DOI was counted for 30 min, commencing immediately after injections.

### 2.5. [$^3$H]ketanserin binding

The $K_d$ and $B_{max}$ of [$^3$H]ketanserin at 5-$HT_{2A}$ receptors in the rat frontal cortex were measured as reported previously (Meltzer et al., 1989). According to the method of Heffner et al. (1980), the tissues of the frontal cortex of the rats were dissected and homogenized in 50 mM Tris–HCl buffer containing 5 mM EDTA (pH 7.7 at 25 °C) and centrifuged at 49 000 × $g$ for 10 min twice. The pellets were washed with 50 mM Tris–HCl buffer (pH 7.7 at 25 °C) and centrifuged at 49 000 × $g$ for 10 min. The final pellets were resuspended in 50 mM Tris–HCl buffer and incubated with six different concentrations of [$^3$H]ketanserin for 15 min at 37 °C in 50 mM Tris–HCl buffer. The final tissue concentration was 3.5 mg/ml. The incubations were terminated by rapid filtration over Whatman GF/B filters, which were rinsed twice with ice-cold 50 mM Tris–HCl buffer using an automatic deposit and dispensing system (Harvester) (Brandel, USA). Nonspecific binding was determined in the presence of 1 μM methysergide.

### 2.6. Experimental procedures

#### 2.6.1. Experiment 1 (head shake count/dose–response)

Twenty-four hours after single footshock stress, rats were injected with 0.1, 0.3, 1, 3 and 10 mg/kg of DOI, and head shakes were counted. For each group, there was an unshocked control group.

#### 2.6.2. Experiment 2 (head shake count/time course)

Four experimental groups of rats and four control groups of rats were used. The first experimental group of rats was injected with 3 mg/kg of DOI immediately after a single footshock, and head shakes were counted for 30 min. The second experimental group of rats was injected with 3 mg/kg of DOI 24 h after a single footshock, and head shakes were counted. The third experimental group of rats was injected with 3 mg/kg of DOI 1 week after a single footshock, and head shakes were counted. The fourth experimental group of rats was injected with 3 mg/kg of DOI 2

weeks after a single footshock, and head shakes were counted. For each group, there was an unshocked control group, which was housed, treated and injected with 3 mg/kg of DOI in the same schedule, but without footshock, as the corresponding shock group.

### 2.6.3. Experiment 3 ([$^3$H]Ketanserin binding)

One week after a single footshock stress, rats were decapitated and their cerebral cortices were dissected, and the [$^3$H]ketanserin binding assays were performed. Control animals were decapitated without experiencing footshock. The apparent affinity ($K_d$) and the maximal number ($B_{max}$) of [$^3$H]ketanserin binding sites for each rat was determined by nonlinear regression analysis of Scatchard plots.

### 2.7. Data analysis

All data are presented as the means ± S.E.M. of the individual values of the rats from each group. Statistical differences between two groups were analyzed using an unpaired Student's *t*-test (two-tailed). The effects of footshock on the dose–response curve for head shakes induced by DOI were analyzed by a one-way and two-way analysis of variance (ANOVA) followed by a post-hoc Bonferroni/Dunn's test.

## 3. Results

### 3.1. Experiment 1 (head shake count/dose–response)

Head shakes were evoked in a dose-dependent manner by 0.1–10 mg/kg of DOI, and DOI-induced head shakes were attenuated by a single footshock stress given 24 h previously. Two-way ANOVA (footshock × DOI) indicated significant main effects of footshock ($P<0.0001$) and DOI ($P<0.0001$). An interaction between footshock and DOI was not significant. For the effect of DOI in the unshocked control group, one-way ANOVA indicated a significant effect of DOI ($P<0.005$). Post-hoc comparisons indicated that the effect of 0.1 mg/kg of DOI was significantly different from that of 1 mg/kg ($P<0.005$), 3 mg/kg ($P<0.0001$), and 10 mg/kg ($P<0.005$) of DOI. At 3 mg/kg ($P<0.05$) and 10 mg/kg ($P<0.01$) of DOI, there were significantly fewer head shakes in the shocked group than in the respective unshocked control group (Fig. 1).

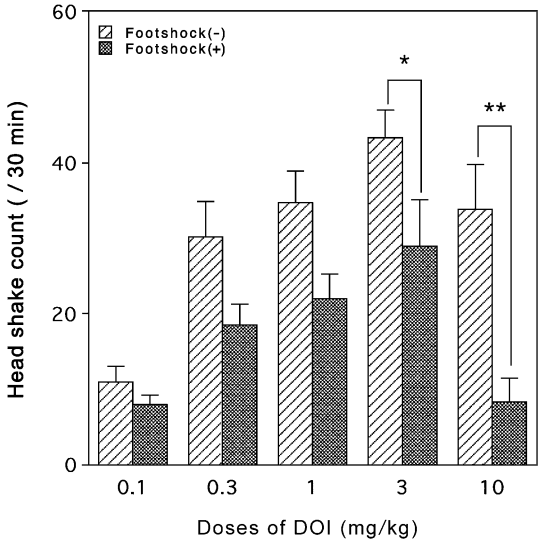


Fig. 1. Effect of a single footshock on DOI-induced head shake behavior in rats 24 h after footshock. Values are the means ± S.E.M. with total counts of head shakes for each group. There were significant main effects of footshock ($P<0.0001$) and DOI ($P<0.0001$) (two-way ANOVA), $n=6$–12, * $P<0.05$, ** $P<0.01$ (Bonferroni/Dunn's test).

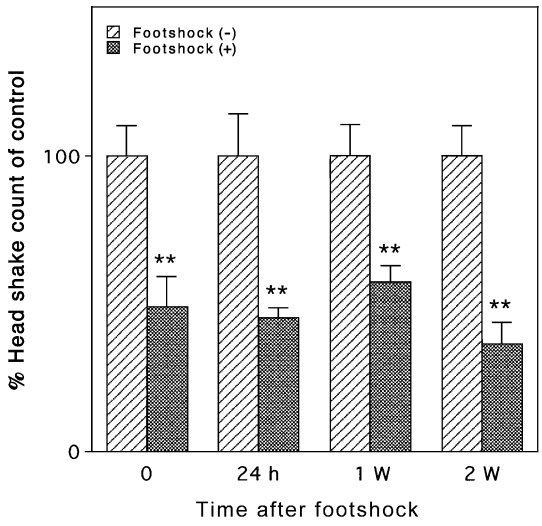


Fig. 2. Long-term effects of a single footshock on DOI-induced head shake behavior in rats. Four experimental groups of rats and four control groups of rats were used. Four experimental groups of rats were shocked and injected with 3 mg/kg of DOI immediately after, and 24 h, 1 and 2 weeks after a single footshock, respectively. Four control groups of rats were housed, treated and injected 3 mg/kg of DOI in the same schedule, but without footshock, as a corresponding shock group. Values are the means ± S.E.M. of the head shake count of expressed as a percentage of the respective control group. The actual numbers of head shakes in the control groups were: 0; 36.9 ± 3.7, 24 h; 26.5 ± 3.6, 1 W; 36.4 ± 3.7, 2 W; 18.4 ± 1.9, $n=8$–16, * * $P<0.01$ (Student's *t*-test), h; hour, W; week.

### 3.2. Experiment 2 (head shake count/time course)

Long-term effects of a single footshock session on head shakes were examined with 3 mg/kg of DOI, a dose which evoked maximal effects on head shakes in experiment 1.

Table 1
Effect of a single footshock on [$^3$H]ketanserin binding in the rat frontal cortex 1 week after footshock

| Treatment | $K_d$ (nM) | $B_{max}$ (fmol/mg tissue) |
|---|---|---|
| Footshock (–) | 0.35 ± 0.019 | 11.0 ± 0.3 |
| Footshock (+) | 0.35 ± 0.018 | 10.9 ± 0.8 |

Values are the means ± S.E.M. with the apparent affinity ($K_d$) values and the maximal number ($B_{max}$) of binding sites from regression Scatchard analyses of [$^3$H]ketanserin binding.

Immediately ($P<0.001$), 24 h ($P<0.002$), 1 week ($P<0.001$), and 2 weeks ($P<0.0005$) after footshock, head shake counts of the shock groups were significantly lower than those of the respective unshocked control groups (Fig. 2).

### 3.3. Experiment 3 ([$^3$H]Ketanserin binding)

One week after footshock, there were no significant differences in the $B_{max}$ and $K_d$ of [$^3$H]ketanserin binding in the frontal cortex between the shock group and the unshocked control group (Table 1).

## 4. Discussion

Although DOI has nearly equal affinity for 5-$HT_{2A}$, 5-$HT_{2B}$ and 5-$HT_{2C}$ receptors (p$K_i$ values for 5-$HT_{2A}$, 5-$HT_{2B}$ and 5-$HT_{2C}$ receptors are 7.3, 7.4 and 7.8, respectively, Barnes and Sharp, 1999), DOI-induced head shake behavior is thought to be an index of 5-$HT_{2A}$ receptor function for the following reasons. First, Schreiber et al. (1995) reported that DOI-induced head shake behavior in rats was dose dependently inhibited by several nonselective 5-$HT_2$ receptor antagonists and by the selective 5-$HT_{2A}$ receptor antagonist *R*(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)-4-piperidine-methanol] (MDL 100, 907) (p$K_i$ values for 5-$HT_{2A}$, 5-$HT_{2B}$ and 5-$HT_{2C}$ are 9.4, not detected, and 6.9, Barnes and Sharp, 1999), whereas the selective 5-$HT_{2B/2C}$ receptor antagonist *N*-(1-methyl-5-indolyl)-*N*′-(3-pyridyl) urea hydrochloride (SB 200,646A) (p$K_i$ values for 5-$HT_{2A}$, 5-$HT_{2B}$ and 5-$HT_{2C}$ are 5.2, 7.5, and 6.9, Barnes and Sharp, 1999) failed to inhibit head shake behavior. The order of relative potency correlated with the affinity for 5-$HT_{2A}$ but not 5-$HT_{2C}$ receptors. Second, Willins and Meltzer (1997) reported that the head shake behavior induced by intracortical injection of DOI was inhibited by the systemic administration of the selective 5-$HT_{2A}$ receptor antagonist MDL 100,907, but not by the 5-$HT_{2B/2C}$ receptor antagonist (+)-*cis*-4,5,7*a*,8,9,10,11,11*a*-octahydro-7*H*-10-methylindolo (1,7-*bc*) (2,6) naphthyridine hydrofumarate (SDZ SER 082) (p$K_i$ values for 5-$HT_{2A}$, 5-$HT_{2B}$, 5-$HT_{2C}$ are 6.2, 7.3, and 7.8, Nozulak et al., 1995), and the intracortical injection of the selective 5-$HT_{2C}$ receptor agonist 6-chloro-2-(1-piperazinyl) pyrazine (MK-212) (p$K_i$ values for 5-$HT_{2A}$ and 5-$HT_{2C}$ are 4.76 and 6.47, Roth et al., 1992) failed to induce head shake behavior. Therefore, DOI-induced head shake behavior is attributed to an agonistic effect of DOI at the 5-$HT_{2A}$ receptor.

In the present study, the maximal head shake response to DOI was attenuated by footshock, consistent with the study by Yamada et al. (1995) (Fig. 1). This suggested a decrease in the number of functionally effective 5-$HT_{2A}$ receptors as a result of the footshock. Because there was no change in [$^3$H]ketanserin binding (Table 1), the decrease in head shakes was not due to the down-regulation or the post-translational regulation of 5-$HT_{2A}$ receptors in response to footshock. In other studies, immediately after or several hours after single stress, [$^3$H]ketanserin binding in the frontal cortex or hippocampus of rats did not change (Chaouloff et al., 1994; Takao et al., 1995; Yamada et al., 1995), although contradictory results have been reported showing that immediately after restraint stress, the $B_{max}$ of [$^3$H]ketanserin binding in the frontal cortex of rats is increased (Torda et al., 1990).

Other mechanisms may be related to the decrease in DOI-induced head shakes by acute stress, e.g. decreased post-receptor intracellular signal transduction or decreased neural transduction of other neurons which exist downstream of the 5-HT neurons. Several authors have reported that other neurotransmitter systems, such as glutaminergic, dopaminergic, and noradrenergic, etc. modulate DOI-induced head shake behavior (Darmani et al., 1991; Eison et al., 1995; Schreiber et al., 1995; Kim et al., 1998; Gewirtz and Marek, 2000; Vicker et al., 2001). It is possible that the decrease in DOI-induced head shakes produced by acute stress was mediated by stress-induced changes in other neurotransmitter systems. With regard to this point, further investigations will be needed.

Furthermore, the present study showed that a single footshock induced a long-lasting decrease in DOI-induced head shake behavior (Fig. 2). The single footshock-induced reduction in head shakes evoked by DOI was observed immediately and 24 h after footshock, and lasted 1 and 2 weeks after footshock. Although Yamada et al. (1995) demonstrated that tail pinch stress, restraint stress by taping and footshock stress decreased DOI-induced head shake behavior in rats immediately after stress, the long-term effect of an acute single stress on DOI-induced head shake behavior has not been examined. To our knowledge, the present study is the first study showing that a single acute stress evokes a long-lasting decrease in a 5-$HT_{2A}$ receptor-mediated behavioral response.

Yamada et al. (1995) reported that restraint stress in a cylinder and forced swim stress did not change DOI-induced head shake behavior. Chaouloff et al. (1994) indicated that restraint stress did not affect DOI-induced head shake behavior in rats 2 h after stress. From these studies, it is conceivable that acute stress provoking physical pain decreases 5-$HT_{2A}$ receptor-mediated behavioral responses, whereas acute stress without pain does not. However, it is not clear whether the discrepancy between these stress procedures is due to the severity of stress or the type of sensory input.

The functional significance of the change in 5-$HT_{2A}$ receptor-mediated behavioral response induced by stress is not clear. 5-$HT_{2A}$ receptors are widely distributed in the central nervous system, and are strongly expressed in the neocortex. Willins and Meltzer (1997) reported that the microinjection of DOI into the rat medial prefrontal cortex (cingulate cortex area 3) produced head shake behavior. According to this study, 5-$HT_{2A}$ receptors in the medial

prefrontal cortex may be involved in the change in DOI-induced head shake behavior induced by stress. Vaidya et al. (1999) suggested that 5-$HT_{2A}$ receptors mediate the stress-induced down-regulation of brain-derived neurotrophic factor (BDNF) expression, and that this effect could be contribute to the atrophy of hippocampal neurons. In consideration of this study, stress-induced decreases in 5-$HT_{2A}$ receptor function may be related to adaptation to stress. Although 5-$HT_{2A}$ receptor antagonists have been used in the treatment of anxiety disorders clinically, the role of 5-$HT_{2A}$ receptors in fear or anxiety in animal models remains to be elucidated (Inoue et al., 1996b).

In conclusion, we showed that a single footshock session produced long-lasting behavioral changes related to 5-$HT_{2A}$ receptor function. The long-lasting decrease in 5-$HT_{2A}$ receptor function may be related to long-lasting behavioral and emotional changes following a single stress experience.

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