Obsessive-compulsive disorder (OCD) is a psychiatric condition with a lifetime prevalence of 1% – 3% that is characterized by recurrent and persistent thoughts, impulses, or images (obsessions) and/or repetitive, seemingly purposeful behaviors (compulsions) (for example, doubting, checking, and washing) (1, 2). Although classified as an anxiety disorder, patients with OCD demonstrate a high incidence of comorbid depression (2). Currently, serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors (SRIs) and especially selective 5-HT reuptake inhibitors (SSRIs) are the first-line agents for the pharmacological treatment of OCD (3). However, up to 50% of patients with OCD failed to respond in an SSRI trial (4). Recently, the noradrenergic and specific serotonergic antidepressant (NaSSA) mirtazapine has been suggested to be effective for OCD symptoms in open trial (5).

Some clinical evidence indicates that glutamatergic abnormalities are associated with OCD symptoms (6, 7). The uncompetitive \(N\)-methyl-D-aspartate (NMDA) receptor antagonist memantine has been reported to exhibit augmentation effects in severe OCD in a single-blinded case-control study (8). \(N\)-Acetyl-L-cysteine (NAC) is a readily available amino acid compound that is thought to attenuate glutamatergic neurotransmission (9, 10). NAC also is an antioxidant and is commonly used for treatment of acute acetaminophen overdose. NAC augmentation has been reported to be effective in treating SRI-refractory OCD in a single case (11).

Marble-burying behavior is considered to be a potential model of OCD on the basis of behavioral similarity (12). Indeed, SSRIs such as fluvoxamine and paroxetine, which are used to treat human OCD symptoms (3), inhibit marble-burying behavior without affecting locomotor activity in mice (12, 13). Moreover, uncompetitive NMDA-receptor antagonists such as memantine and amantadine inhibit marble-burying behavior without affecting locomotor activity (14). However, the effect of NAC on marble-burying behavior has been not reported in this model. Therefore, we investigated the effect of NAC on marble-burying behavior in mice.

Five-week-old male ICR mice (Nihon SLC, Shizuoka) were used in each experiment. The mice were housed in groups of five per cage. For at least 7 days before the behavioral tests, the mice were housed in a room under controlled temperature (23°C ± 2°C), relative humidity (60% ± 10%), and ambient lighting (cycle of 12 h light and 12 h dark, with the period of light starting at 07:00 h) conditions. The animals had free access to food (CE-2; Clea Japan, Tokyo) and water in their home cages. All animal care and use procedures were performed in com-
pliance with the regulations established by the Experimental Animal Care and Use Committee of Fukuoka University followed the Guidelines of the Science Council of Japan.

Fluvoxamine maleate was generous gifts from Solvay Pharmaceutical (Tokyo). Mirtazapine was purchased from LKT Laboratories, Inc. (St. Paul, MN, USA). NAC and α-tocopherol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fluvoxamine was dissolved in distilled water. Mirtazapine was suspended in 0.5% methylcellulose. NAC and α-tocopherol were dissolved in saline.

The marble-burying behavior test was performed as described previously (14). All experiments were conducted between 10:00 and 17:00 h. Mice were placed individually, without food and water, in clear plastic boxes (30 × 30 × 28 cm), each containing 25 glass marbles (1.5 cm in diameter) that were evenly spaced on 5-cm-deep sawdust. Concurrently, the locomotor activity patterns of the mice were measured using an automated activity counter (NS-AS01; Neuroscience, Tokyo) placed 15-cm above the same plastic boxes. Activity was measured under the illumination of a 100-W bulb.

The dependent measure of marble-burying behavior was the number of marbles buried to at least two-thirds of the depth of the sawdust, within 30 min. On the first day, we selected mice that buried more than 20 glass marbles. We carried out the drug evaluations on the next day. Mirtazapine and NAC were administered intraperitoneally (i.p.) 30 min before the test. Fluvoxamine and α-tocopherol were administered orally 60 min before the test. All drugs were administered at a volume of 0.1 mL/10 g of body weight. The observer did not know what agent was being tested.

The results obtained in the marble-burying behavior test were analyzed using one-way or two-way analysis of variance (ANOVA), followed by the Tukey-Kramer post-hoc test to determine whether there were differences between the groups. A probability level of $P < 0.05$ was accepted as statistically significant. Values are expressed as means ± standard error of the mean (S.E.M.).

Fluvoxamine (30 mg/kg) significantly reduced the number of buried marbles [$F(2,28) = 7.757, P < 0.01$ by one-way ANOVA; $P < 0.01$ by the Tukey-Kramer post-hoc test, Fig. 1A]. No significant change in total locomotor activity during the marble-burying behavior was observed at this dose (Fig. 1C). Similarly, mirtazapine (3 mg/kg) significantly reduced the number of buried marbles [$F(2,19) = 5.27, P < 0.05$ by one-way ANOVA; $P < 0.05$ by the Tukey-Kramer post-hoc test, Fig. 1B]. No significant change in total locomotor activity during the marble-burying behavior was observed at this dose.

**Fig. 1.** Effects of fluvoxamine (A and C) and mirtazapine (B and D) on marble-burying behavior in mice. The data are presented as the number of buried marbles (A and B) and locomotor activity (C and D). Fluvoxamine (10 and 30 mg/kg) was administered orally 60 min before the test. Mirtazapine (1 and 3 mg/kg) was administered i.p. 30 min before the test. Values are expressed as the mean ± S.E.M. *$P < 0.05$, **$P < 0.01$, compared with the vehicle-treated group (Tukey-Kramer post-hoc test). The number of mice is shown at the bottom of each column.
NAC dose-dependently reduced the number of buried marbles \( F(3,35) = 3.713, \ P < 0.05 \) by one-way ANOVA, Fig. 2A. NAC (150 mg/kg) significantly reduced the number of buried marbles, without significantly affecting total locomotor activity \( (P < 0.05) \) by the Tukey-Kramer post-hoc test, Fig. 2: A and C. On the other hand, the antioxidant \( \alpha \)-tocopherol (10, 30, and 100 mg/kg) had no effect on the number of buried marbles or total locomotor activity \( (P < 0.05) \) by the Tukey-Kramer post-hoc test, Fig. 2: B and D. In addition, a combination of fluvoxamine (10 mg/kg) and NAC (100 mg/kg) did not exhibit an additive effect on the number of buried marbles, without significantly affecting locomotor activity \( (P < 0.05) \) by two-way ANOVA, Fig. 3A. No significant change in total locomotor activity during the marble-burying behavior was observed at these doses (Fig. 3B).

In the present study, we found that NAC inhibited marble-burying behavior, which is considered to be an animal model of OCD (12), without affecting locomotor activity in mice. Therefore, these effects were not attributable to non-specific sedative effects or a deficit of motor function. These findings suggest that the reduced number of marbles buried by mice treated with NAC might reflect an anti-OCD activity.

We previously reported that uncompetitive NMDA-receptor antagonists inhibited marble-burying behavior without affecting locomotor activity (14). NAC also inhibited marble-burying behavior without affecting locomotor activity. NAC is converted to cystine, a substrate for the glutamate/cystine antiporter located on glial cells. The uptake of cystine by glial cells causes the reverse transport of glutamate into the extracellular space where it appears to stimulate inhibitory metabotropic glutamate receptors on glutamatergic nerve terminals and thereby reduce the synaptic release of glutamate (9). Systemic administration of NAC prevents the cocaine-primed drug seeking in rats by increasing activity of the glutamate-cystine antiporter and restoring extracellular glutamate concentrations in the nucleus accumbens (10). Thus, NAC modulates brain glutamate neurotransmission. On the other hand, the antioxidant \( \alpha \)-tocopherol had no effect on the marble-burying behavior, suggesting that the anti-OCD effect of NAC is not due to antioxidative action. Taken together with these findings, the present results suggest that the glutamatergic system plays an important role in the marble-burying behavior.

In this study, fluvoxamine (as a positive control) inhibited marble-burying behavior without affecting locomotor activity. Similarly, mirtazapine inhibited marble-burying behavior without affecting locomotor activity. These results partly support the effectiveness of mirtazapine for OCD symptoms (5). The pharmacological profile of mirtazapine is characterized by a potent antago-

![Fig. 2. Effects of N-acetyl-L-cysteine (A and C) and \( \alpha \)-tocopherol (B and D) on marble-burying behavior in mice. The data are presented as the number of buried marbles (A and B) and locomotor activity (C and D). N-Acetyl-L-cysteine (50, 100, and 150 mg/kg) was administered i.p. 30 min before the test. \( \alpha \)-Tocopherol (10, 30, and 100 mg/kg) was administered orally 60 min before the test. Values are expressed as the mean ± S.E.M. *\( P < 0.05 \), compared with the vehicle-treated group (Tukey-Kramer post-hoc test). The number of mice is shown at the bottom of each column.](image-url)
nism of presynaptic α2-adrenergic receptors on both norepinephrine and 5-HT neurons and by a potent antagonism of postsynaptic 5-HT2 and 5-HT3 but not 5-HT1A receptors. It has been reported that the effect of fluvoxamine on marble-burying behavior is attenuated by the selective 5-HT1A receptor antagonist WAY100635 at 1 mg/kg, while it is enhanced by WAY100635 at 0.1 mg/kg (13), suggesting that the involvement of 5-HT1A receptors. Therefore, the effect of mirtazapine might be mediated, in part, by 5-HT1A receptors.

NAC has been extensively studied in a variety of medical problems (e.g., acute acetaminophen overdose, contrast nephropathy). A double-blind placebo-controlled crossover Phase I trial was conducted to assess the safety and tolerability of NAC in healthy, cocaine-dependent humans (15). NAC is well tolerated in healthy, cocaine-dependent individuals and reduces cocaine-related withdrawal symptoms and craving. Across placebo and NAC conditions, only mild side effects were noted, and the number of subjects reporting side effects did not differ. Hence, its lack of significant side effects might present an advantage over other pharmacological agents.

In addition, a combination of fluvoxamine (10 mg/kg) and NAC (100 mg/kg) did not exhibit an additive effect on marble-burying behavior. These results suggest the probable presence of interaction between serotonergic and glutamatergic systems and that NAC is useful as an alternative drug rather than combined with fluvoxamine in the treatment of SSRIs-resistant OCD.

In conclusion, the results of the present study show, for the first time, that NAC inhibits marble-burying behavior without affecting locomotor activity in mice. Therefore, these findings support the possibility that NAC is a potential treatment for OCD.

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References


