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Note

Effects of *Psilocybe argentipes* on Marble-Burying Behavior in Mice

Yoshihiro MATSUSHIMA,[1] Osamu SHIROTA,[2] Ruri KIKURA-HANAJIRI,[3] Yukihiro GODA,[3] and Fumio EGUCHI[1,†]

[1]*Department of Health and Nutrition, Takasaki University of Health and Welfare, 37-1 Nakaorui, Takasaki, Gunma 370-0033, Japan*
[2]*Laboratory of Pharmacognosy and Natural Product Chemistry, Faculty of Pharmaceutical Sciences at the Kagawa Campus, Tokushima Bunri University, 1314-1 Shido, Sanuki, Kagawa 769-2193, Japan*
[3]*Division of Pharmacognosy, Phytochemistry, and Narcotics, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan*

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***Psilocybe argentipes* is a hallucinogenic mushroom. The present study examined the effects of *P. argentipes* on marble-burying behavior, which is considered an animal model of obsessive-compulsive disorder. *P. argentipes* significantly inhibited marble-burying behavior without affecting locomotor activity as compared with the same dose of authentic psilocybin. These findings suggest that *P. argentipes* would be efficient in clinical obsessive-compulsive disorder therapy.**

Key words: *Psilocybe argentipes*; hallucinogenic mushrooms; psilocybin; marble-burying behavior; obsessive-compulsive disorder

Obsessive-compulsive disorder (OCD) is a mental illness that is widespread (2–3% of the population worldwide) and chronically disabling.[1)] This psychiatric condition is characterized by recurring obsessions and compulsions that significantly interrupt the daily functioning of the patient. Currently, selective serotonin (5-HT)-reuptake inhibitors (SSRIs) represent first-line pharmacotherapy for OCD. Nevertheless, the onset of action is slow, and 30–50% of patients do not respond at all to these agents.[2,3)]

Rodents use bedding material to bury noxious materials like glass marbles. No habituation to these novel objects occurs on serial testing or by prehousing with marbles.[4)] Moreover, SSRIs that have been used to treat human OCD inhibit marble-burying behavior without affecting locomotor activity. Hence marble-burying behavior has generally been considered as an animal model of OCD.[5)]

Francisco *et al.*[3)] recently found that psilocybin is associated with acute reductions in OCD symptoms in several subjects without significant adverse effects. Psilocybin is the main psychoactive substance in many hallucinogenic mushrooms. The structural similarities of psilocybin and psilocin to serotonin, a neurotransmitter also derived from tryptophane, is believed to be the cause of their action on the serotonin receptor in the brain and of hallucination (Fig. 1). Psilocybin is a potent 5-HT_{1A} and 5-$HT_{2A/2C}$ receptor agonist. The mechanisms of action of OCD are largely unclear, but are probably diverse for various subjects, since OCD presents a broad range of symptoms. Hallucinogenic mushrooms produce a variety of psychoactive agents aside from psilocybin, such as psilocin, baeocystin, norbaeocystin, bufotenin, and aeruginascin. Hence they show therapeutic action in a huge variety of subjects beyond the authentic materials. Hallucinogenic mushrooms have potential value as a novel form of pharmacotherapy, but no previous investigations have investigated the medicinal use of hallucinogenic mushrooms without psychotherapy. In addition, few animal model studies have examined the use of psilocybin in OCD, and the effects on the marble-burying behavior have yet to be determined.

The present study investigated the effects of hallucinogenic mushrooms on marble-burying behavior as compared with the effects of antipsychotic agents such as fluvoxamine. Mycelia of *Psilocybe argentipes*, one of the most popular hallucinogenic mushrooms in Japan, were used as the microbiological material. The amount of psilocybin in *P. argentipes* was determined by high-performance liquid chromatography (HPLC). We also examined the effects of authentic psilocybin on marble-burying behavior in comparison with *P. argentipes*.

Five-week-old male ICR mice (Charles River Laboratories Japan, Kanagawa, Japan) were used in each experiment. For $\geq$7 d before behavioral tests, all the animals were maintained in an air-conditioned room under controlled temperature ($22 \pm 2\,^{\circ}C$) and relative humidity ($55 \pm 5\%$). The mice were housed in plastic cages with sawdust and kept under a light-dark cycle (lights on 07:00–19:00). They were allowed ad-libitum access to food (MF; Oriental Yeast, Tokyo) and water, except during the experiments. All procedures involving animals were conducted in accordance with the "Guidelines for Animal Experiments" of Takasaki University of Health and Welfare. Marble-burying behavior tests were conducted between 09:00 and 17:00. The mice were placed individually in plastic cages ($22.5 \times 38.5 \times 18.5$ cm) containing 25 glass marbles (diameter, 15 mm) evenly spaced on sawdust (depth, 5 cm) without food or water. At the same time, the locomotor activity of the mice was measured using an MDC system (MDC-W01,

† To whom correspondence should be addressed. Tel: +81-27-352-1290; Fax: +81-27-353-2055; E-mail: eguchi@takasaki-u.ac.jp
Abbreviations: OCD, obsessive compulsive disorder; 5-HT, 5-hydroxytryptamine; SSRIs, selective serotonin reuptake inhibitors

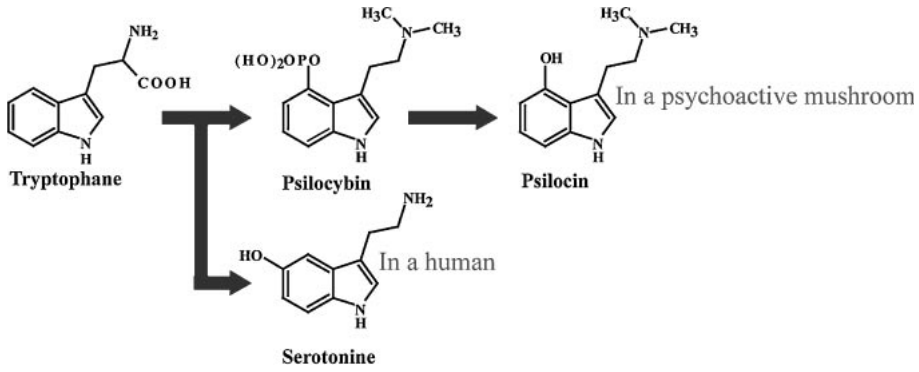

Fig. 1. Chemical Structures of Tryptophane Derivatives in Metabolic Biotransformation.

MDC-WR1; Brain Science Idea, Osaka, Japan) comprising a sensor monitor mounted above the cage to detect changes in heat across multiple zones of the cage. Body heat radiated by the animal was detected with the sensor head of the monitor. Thus, the system allowed monitoring and counting of all spontaneous movements. The results for marble-burying behavior were expressed as the number of marbles buried at least two-thirds deep within 30 min and average total counts of locomotor activity for 30 min.

Fluvoxamine maleate (Tocris Cookson, Ellisville, MO), psilocybin, and psilocin were dissolved in saline for the marble-burying behavior test. Authentic psilocybin and psilocin were prepared as reported previously (psilocybin, psilocin, and hallucinogenic mushrooms such as *P. argentipes* are controlled as narcotic drugs by law in Japan).[6] The fungus *P. argentipes* from our laboratory collection was cultivated in potato-dextrose liquid medium, consisting of 20% potato extract and 2% dextrose, at 28 ± 2 °C for 14 d. Cultivated mycelia were freeze-dried and powdered using an electric blender (WB-1; Osaka Chemical, Osaka, Japan) for 30 s. Powdered mycelia were dissolved in saline for the marble-burying behavior test. Fluvoxamine was administered intraperitoneally, while the other agents were administered orally at a volume of 0.1 ml/10 g body weight 30 min before the marble-burying behavior test.

Powdered mycelia from *P. argentipes* (0.5 g) were extracted with 50 ml of methanol for 24 h at room temperature. The crude extract was filtered through a 0.45-μm polyvinylidene fluoride (PVDF) filter (Whatman, Florham Park, NJ) before injection into the HPLC apparatus. The liquid chromatograph consisted of a HTEC-500 electrochemical detection system and EPC-500 chromatographic data system (Eicom, Kyoto, Japan). The chromatographic column used contained Partisil-10 octadecyl silane, 10 μm (250 mm × 4.6 mm inside diameter; GL Science, Tokyo). The mobile phase comprised 90% 0.1 mol/l aqueous citrate-phosphate buffer (pH 3.1) and 10% ethanol. The flow-rate was 1 ml/min, and the impressed voltage was 750 mV. All measurements were performed at room temperature.

Values were expressed as means ± standard error of the mean. Data was assessed by multivariate analysis of variance, followed by Dunnett's test. Values of $p < 0.05$ were regarded as statistically significant.

The present study investigated the effects of the hallucinogenic mushroom *P. argentipes* on marble-burying behavior, which is considered an animal model

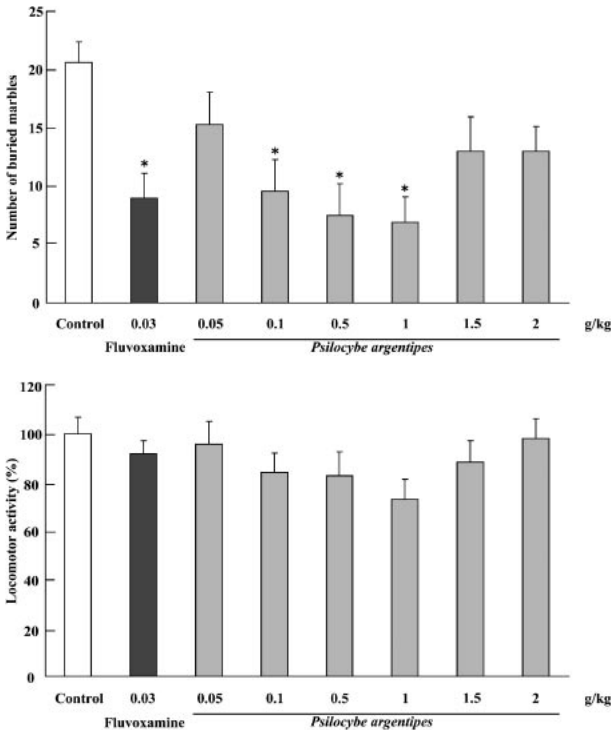

Fig. 2. Effects of *Psilocybe argentipes* on Marble-Burying Behavior and Locomotor Activity in Mice.

Values are expressed as mean ± S.E.M. for 10 mice. $^{*}p < 0.05$, compared to the control group.

of OCD. SSRIs used in clinical treatment inhibit marble-burying behavior without affecting locomotor activity.[7] *P. argentipes* at a dose of 0.05–2 g/kg showed a trend toward inhibited marble-burying behavior, while a dose of 0.1–1 g/kg significantly reduced the number of buried marbles (Fig. 2). Moreover, *P. argentipes* did not significantly affect locomotor activity at any dose (Fig. 2). The relationship between dose and number of buried marbles showed an inverted bell curve. This represents a very unusual result for the marble-burying behavior test, since many chemicals show a proportional relationship, high doses reducing both the number of buried marbles and locomotor activity.[7] Generally, *P. argentipes* shows a dose-proportional hallucinogenic effect.[8] The reduction in marble-burying behavior with *P. argentipes* was hence probably not associated with the hallucinogenic effect. In addition, hallucinogenic effects strongly involve agonistic effects on 5-HT_{2A} receptors.[9] The reduction in marble-burying behavior with *P. argentipes*

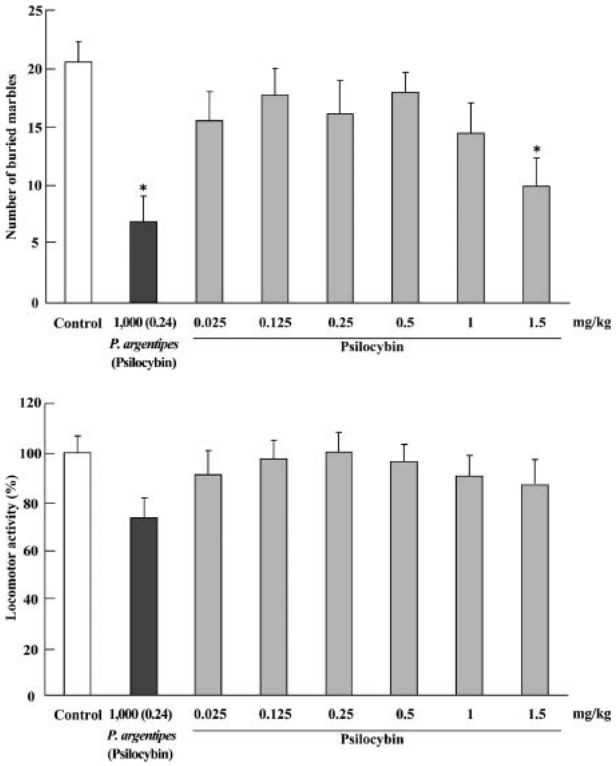

Fig. 3. Effects of Psilocybin on Marble-Burying Behavior and Locomotor Activity in Mice.

Values are expressed as mean ± S.E.M. for 10 mice. $^{*}p < 0.05$, compared to the control group.

thus appears unrelated to agonistic effects on 5-HT_{2A} receptors. The number of buried marbles was comparable between *P. argentipes* and fluvoxamine, which is used in anti-OCD pharmacotherapy. The dose of fluvoxamine was set based on the findings of Ichimaru *et al.*,[10] since fluvoxamine significantly inhibits marble-burying behavior without affecting locomotor activity. In addition, no negative effect was observed, for example, the weight gain or loss or loose stools. Some case reports have described reduced OCD symptoms with ingestion of hallucinogenic mushrooms.[11,12] In one case, hallucinogenic mushroom therapy was maintained as a treatment for OCD symptoms for 4 years.[11] The results show that hallucinogenic mushrooms such as *P. argentipes* can exhibit anti-OCD effects in clinical use.

Quantitative analysis by HPLC showed that mycelia from *P. argentipes* contained 0.024% psilocybin and 0.0008% psilocin. *P. argentipes* at a dose of 0.1–1 g/kg thus contained about 23.8–238 μg/kg psilocybin and 0.8–8 μg/kg, psilocin. This psilocybin content was comparable to other hallucinogenic mushrooms containing high concentrations of psilocybin.[13]

Psilocybin at a dose of 0.025–1.5 mg/kg showed a slight trend toward inhibiting marble-burying behavior, while a dose of 1.5 mg/kg resulted in significantly reduced marble-burying behavior (Fig. 3). In addition, psilocybin did not have any significant effect on locomotor activity during the marble-burying behavior test (Fig. 3). No results of the marble-burying behavior test with psilocybin have previously been described. However, Moreno *et al.* showed that psilocybin markedly decreased human OCD symptoms to varying degrees under controlled conditions.[3] The amount of psilocybin required to exert a significant effect was larger than with *P. argentipes*. These results suggest that the anti-OCD effects of hallucinogenic mushrooms such as *P. argentipes* are probably not the same as those of authentic psilocybin. Hallucinogenic mushrooms produce a wide variety of tryptamine derivatives other than psilocybin.[13] Some of these, such as psilocin, baeocystin, norbaeocystin, bufotenin, and aerginascin, have psychoactive effects, although many hallucinogenic mushrooms produce smaller amounts of them than psilocybin.[13,14] These findings suggest that inhibition of marble-burying behavior by *P. argentipes* is due to the involvement of a variety of psychoactive substances.

In conclusion, *P. argentipes* significantly reduced marble-burying behavior in mice, animal model of OCD, without adversely effecting locomotor activity. Moreover, it inhibited marble-burying behavior more effectively than authentic psilocybin, and at lower doses. These findings suggest that *P. argentipes* exhibits anti-OCD activity in clinical use. In addition, materials other than psilocybin are probably involved in reductions in marble-burying behavior following *P. argentipes* administration.

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