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# Repeated administration of Yokukansan inhibits DOI-induced head-twitch response and decreases expression of 5-hydroxytryptamine (5-HT)$_{2A}$ receptors in the prefrontal cortex

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ABSTRACT

Behavioral and psychological symptoms of dementia (BPSD) are commonly seen in patients with Alzheimer's disease (AD) and other forms of senile dementia. BPSD have a serious impact on the quality of life of dementia patients, as well as their caregivers. However, an effective drug therapy for BPSD has not been established. Recently, the traditional Japanese medicine Yokukansan (YKS, Yi-gan san in Chinese) has been reported to improve BPSD in a randomized, single-blind, placebo-controlled study. Moreover, abnormalities of the serotonin (5-HT) system such as 5-HT$_{2A}$ receptors have been reported to be associated with BPSD of AD patients. In the present study, we investigated the effect of YKS on head-twitch response induced by 2,5-dimethoxy-4-iodoamphetamine (DOI, 5 mg/kg, i.p.) in mice, a behavioral response that is mediated, in part, by 5-HT$_{2A}$ receptors. Acute treatment with YKS (100 and 300 mg/kg, p.o.) had no effect on the DOI-induced head-twitch response, whilst 14 days repeated treatment with YKS (300 mg/kg, p.o.) significantly inhibited this response. Moreover, repeated treatment with YKS (300 mg/kg, p.o.) decreased expression of 5-HT$_{2A}$ receptors in the prefrontal cortex, which is part of the circuitry mediating the head-twitch response. These findings suggest that the inhibition of DOI-induced head-twitch response by YKS may be mediated, in part, by altered expression of 5-HT$_{2A}$ receptors in the prefrontal cortex, which suggests the involvement of the 5-HT system in psychopharmacological effects of YKS.



## 1. Introduction

The majority of patients with Alzheimer's disease (AD) and other forms of senile dementia display various psychiatric symptoms, such as hallucinations, depression, delusions and anxiety at some point during the course of their illness. These are collectively known as the behavioral and psychological symptoms of dementia (BPSD). The BPSD are a major cause of distress to family members and caregivers (Finkel, 2003). Furthermore, the presence of BPSD may have a negative impact on the course of the disease (Rubin et al., 1988; Walsh et al., 1990). However, an effective drug therapy for BPSD has not been established. Recently, a traditional Japanese medicine Yokukansan (YKS, Yi-gan san in Chinese) has been demonstrated to improve BPSD, such as hallucinations, agitation and irritability, in a randomized, single-blind, placebo-controlled study (Iwasaki et al., 2005b). Moreover, YKS has been reported to improve psychosis and sleep disturbance in patients with dementia with Lewy bodies (Iwasaki et al., 2005a; Shinno et al., 2007). However, the mechanism of these effects remains unknown.

Head-twitches (mice) and wet-dog shakes (rats), induced by drugs such as the serotonin (5-HT)$_{2A/2C}$ receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) and its structural analogs, are thought to be mediated via central 5-HT$_{2A}$ receptors, and these models have been used as in vivo tests of 5-HT$_{2A}$ receptor pharmacology (Barnes and Sharp, 1999). Indeed, DOI-induced head-twitch response is completely antagonized by the 5-HT$_{2A}$ receptor antagonists ketanserin, MDL 100907 and EMD281014 but is not blocked by pretreatment with the selective 5-HT$_{2C/2B}$ receptor antagonist SDZ SER 082 (Bartoszyk et al., 2003; Darmani et al., 1990; Egashira et al., 2004a; Willins and Meltzer, 1997). Moreover, the bilateral microinjection of DOI elicits a dose-dependent head-twitch response when injected into the prefrontal

*Abbreviations:* AD, Alzheimer's disease; ANOVA, analysis of variance; BPSD, behavioral and psychological symptoms of dementia; DOI, 2,5-dimethoxy-4-iodoamphetamine; EPS, extrapyramidal symptoms; LSD, lysergic acid diethylamide; 5-HT, serotonin; YKS, Yokukansan.

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cortex (Willins and Meltzer, 1997), which suggests that the prefrontal cortex is part of the circuitry mediating the head-twitch response. On the other hand, the selective 5-$HT_{2C}$ receptor agonist (*S*)-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine fumarate (Ro 60-0175) does not induce a significant head shake response in rats (Martin et al., 1998). Therefore, 5-$HT_{2A}$ receptors in the prefrontal cortex may be involved in the DOI-induced head-twitch response. In addition, an agonist action at 5-$HT_2$ receptors is likely to be involved in hallucinogenic mechanisms, since there is a close correlation between the human hallucinogenic potency of 5-$HT_2$ receptor agonists and their affinity for 5-$HT_2$ binding sites (Glennon, 1990). This correlation fits best for the 5-$HT_{2A}$ binding site. Moreover, many hallucinogens including lysergic acid diethylamide (LSD), mescaline and phenethylamine, cause the head-twitch response in mice (Corne and Pickering, 1967; Corne et al., 1963). In addition, only the drugs with hallucinogenic potential in humans activated a significant head-twitch response in htr2A$^{+/+}$ mice, but not in htr2A$^{-/-}$ mice (González-Maeso et al., 2007). However, the effect of YKS on the head-twitch response has not been studied. In the present study, we investigated the effect of YKS on the DOI-induced head-twitch response. We also examined the effect of YKS on expression of 5-$HT_{2A}$ receptor protein in the prefrontal cortex, which is part of the circuitry mediating the head-twitch response, and hypothalamus.

## 2. Materials and methods

### 2.1. Animals

Male ddY mice (Kyudo, Saga, Japan), aged 4 weeks and weighing 20–25 g, were housed in groups of five in a temperature-controlled room (23±2 °C) on a 12-h light–dark cycle (lights on 07:00–19:00 h), with food and water available ad libitum. The total number of animals used was 141. All procedures regarding animal care and use were carried out based on the regulations established by the Experimental Animal Care and Use Committee at Fukuoka University, Japan.

### 2.2. Drugs

YKS was a generous gift from TSUMURA & CO. (Tokyo, Japan) and was dissolved in distilled water. (±)-2,5-Dimethoxy-4-iodoamphetamine hydrochloride (DOI) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and was dissolved in saline. YKS was administered orally and DOI was administered intraperitoneally (i.p.). We performed oral injection using plastic syringe with sonde for oral injection. Control animals received injections with the drug vehicle (distilled water) via the same route. The doses of YKS were chosen based on a previous report (Koshikawa et al., 1998).

### 2.3. Head-twitch response measurement

Head-twitch response is a distinctive twitching behavior of the head. The procedure followed that previously described by Egashira et al. (2004a). It is easily monitored and usually cannot be mistaken for other head movements such as head-shakes or head-jerks. DOI-induced head-twitch response was observed in a plastic container (10×30×30 cm). Five minutes after i.p. injection of DOI (5 mg/kg), the number of head-twitches was counted for a 5-min period. YKS (100 and 300 mg/kg, p.o.) was injected 60 min before the number of head-twitches was counted. On the other hand, YKS at the same doses was injected p.o. 60 min before the head-twitch response measurement at 24 h after 13 days repeated treatment with once daily YKS. The number of head-twitches was scored using a tally counter by an observer who did not know which agent was being tested. The number of animals in each drug group was as follows: 11 received DOI 5 mg/kg+distilled water (single dose); eight received DOI 5 mg/kg+YKS 100 mg/kg (single dose); nine received DOI 5 mg/kg+YKS 300 mg/kg (single dose); 11 received DOI 5 mg/kg+distilled water (repeated dose); 10 received DOI 5 mg/kg+YKS 100 mg/kg (repeated dose); and 10 received DOI 5 mg/kg+YKS 300 mg/kg (repeated dose).

### 2.4. Open-field test

In this study, we examined the effect of YKS on motor functions such as motor activity and motor coordination to investigate the involvement of motor functions in the effect of YKS on the head-twitch response. In addition, we have already confirmed the execution of several experiments (open-field, catalepsy and rota-rod tests) is not interfering in the results of every one.

Locomotor activity in the open-field test was measured for 3 min as described previously (Egashira et al., 2004a). The activity was measured using an apparatus consisting of a circular floor (diameter, 60 cm) divided by thin red lines into 19 equal blocks. The floor was enclosed by a parapet (height, 50 cm) with an upper opening (diameter, 90 cm). The apparatus was illuminated by a 100 W bulb placed 80 cm above the center of the floor of the apparatus. The activity was measured in a sound-proof dark room under the above-described standard housing conditions. The activity of mice used in this study was measured once before drug treatment. YKS (100 and 300 mg/kg, p.o.) was injected 60 min before the open-field test. On the other hand, YKS at the same doses was injected p.o. 60 min before the open-field test at 24 h after 13 days repeated treatment with once daily YKS. The number of animals in each drug group was as follows: six received vehicle (single dose); six received YKS 100 mg/kg (single dose); six received YKS 300 mg/kg (single dose); 10 received vehicle

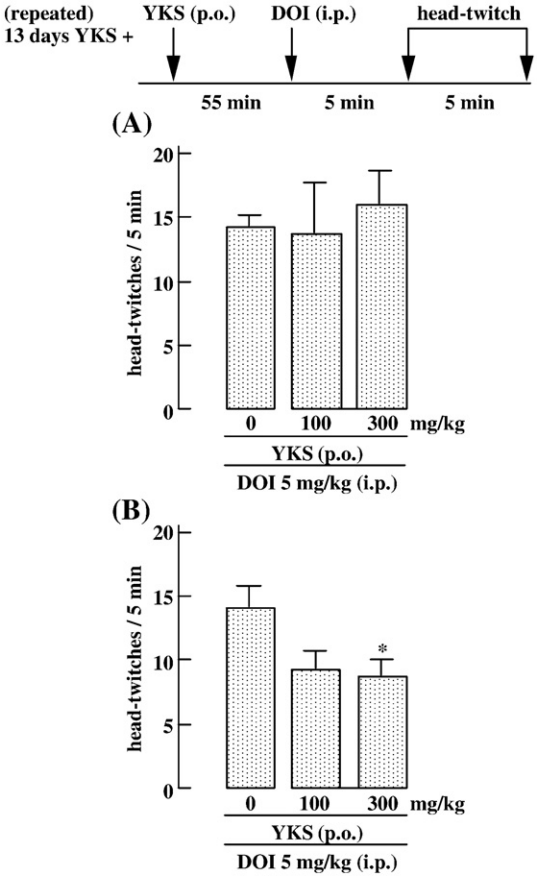


**Fig. 1.** Effect of (A) acute or (B) repeated treatment with YKS on DOI-induced head-twitch response in mice. The number of head-twitches was counted for 5 min, beginning 5 min after treatment with DOI (5 mg/kg, i.p.). YKS was injected p.o. 60 min before the number of head-twitches was counted. On the other hand, YKS was injected p.o. 60 min before the head-twitch response measurement at 24 h after 13 days repeated treatment with once daily YKS. Values are expressed as the mean±SEM ($n$=8–11). *$P$<0.05 compared with DOI+distilled water.