福岡大医紀（*Med. Bull. Fukuoka Univ.*）：47(1), 53-60, 2020

Alterations in Behavior and Brain Monoamine Levels of Olfactory Bulbectomized Rats Following Saiko-ka-ryukotsu-borei-to Administration

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Abstract

The olfactory bulbectomized (OBX) rat is an animal model that shows depression-like behavior because of frontal lobe neurotransmission system impairment due to surgical removal of the olfactory bulb. Behavioral changes in OBX animals are used as indicators to screen antidepressants. We examined the effect of Saiko-ka-ryukotsu-borei-to (SRBT), an often clinically prescribed oriental medicine, on OBX rats by assessing hyperemotionality and monoamine metabolite levels in the brain. Forty-five male Wistar rats were divided into four groups：Sham, OBX-Vehicle (saline), OBX-Imipramine, and OBX-SRBT. The olfactory bulb was bilaterally removed in OBX rats by aspiration through two holes in the skull. Two weeks after the operation, once-daily oral administration of various agents was performed for 14 days, and hyperemotionality was evaluated on specific days. We also determined monoamine metabolite levels, including homovanillic acid (HVA), 3-methoxy-4-hydroxyphenylglycol, and 5-hydroxyindoleacetic acid (5-HIAA) in specific brain regions of rats using HPLC. As a result, SRBT administration tended to inhibit hyperemotionality. Additionally, HPLC showed that OBX rats had significantly higher 5-HIAA levels in the pituitary gland than Sham rats；however, continued administration of SRBT or imipramine led to decrease 5-HIAA levels. Furthermore, OBX rats had significantly lower HVA levels in the prelimbic cortex than Sham rats, and SRBT or imipramine administration did not affect HVA levels. This study indicates SRBT affects depression-like behavior and may correct neurotransmission abnormalities in OBX rats. However, the monoamines in particular brain areas that are influenced by SRBT administration remain unclear.

Key words： **Depression, Saiko-ka-ryukotsu-borei-to, Olfactory bulbectomy, Hyperemotionality, Monoamine metabolite**

Introduction

Depression is a commonly occurring psychiatric disease worldwide[1)]. The Ministry of Health, Labour and Welfare in Japan has stated that psychiatric disorders comprise a major category of diseases along with cancer, cerebral stroke, heart disease, and diabetes, collectively called the “five major diseases”. The decreased quality of life caused by the characteristics of depression is extremely serious. Therefore, it is important to determine the causes of depression and develop medical treatments.

The mainstream hypotheses for the mechanisms of depression are the “serotonin hypothesis” and “dysfunction of the hypothalamic-pituitary-adrenal axis”[2)]. According to the serotonin hypothesis, it has been hypothesized that serotonergic neurotransmission is impaired in

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the brain of depressed patients because depressed patients have decreased 5-hydroxyindoleacetic acid (5-hydroxyindole acetic acid, 5-HIAA), a metabolite of serotonin in the cerebrospinal fluid, and the antidepressant effect is enhanced when tryptophan, a precursor of serotonin, is combined with monoamine oxidase inhibitors. Antidepressants are considered therapeutic agents that produce such effects for depression patients. However, antidepressants have many problems, such as side effects due to low adherence, serotonin syndrome, and discontinuation syndrome. As a medical therapy for depression, herbal medicine can also be used in clinical practice when patients have issues with treatment with existing antidepressants or prolonged depression. For example, Saiko-ka-ryukotsu-borei-to (SRBT), a type of oriental medicine, is often prescribed in the field of clinical psychiatry. It is often used clinically for insomnia, irritability, neurasthenia, and nocturnal crying in children because of its efficacy[3]. Herbal medicines including SRBT are effective for some depression patients; however, evidence regarding their detailed mechanisms is lacking. It is widely known that depression includes various symptoms, but its pathophysiological mechanism is not fully understood; therefore, studies of depression are actively ongoing. Many tentative theories for the mechanism of depression have been proposed, and the serotonin hypothesis is a major theory. Most antidepressants were developed according to the serotonin hypothesis, which is supported by various animal experiments. As an experimental research animal, the olfactory bulbectomized (OBX) rat is an animal model that shows high similarity to depression in humans as a result of neurotransmission system impairment of the frontal lobe due to surgical removal of the olfactory bulb. Behavioral changes in OBX rats (such as changes in hyperemotionality, locomotor activity, and immobility time in forced swim tests) are used as indicators when screening common antidepressants[4]. We hypothesized that SRBT administration can suppress the hyperemotionality of OBX rats and change monoamine metabolite levels in specific brain regions. Generically it is said that monoamine, not only serotonin but also dopamine, has a close relationship with mental disorders, leading to develop the therapeutic drugs[5].

In this study, we examined the effect of continuous administration of SRBT in OBX rats by examining hyperemotionality and monoamine metabolite levels in the brain. This study will enhance the understanding of the relationship between hyperemotionality and monoamine metabolite levels in OBX model rats undergoing continuous SRBT administration.

Materials and Methods

Materials

This study was conducted using male Wistar rats (6 weeks) purchased from CLEA Japan, Inc. The rats were housed three per cage at a controlled temperature of 22±1℃ on a 12 h light/dark cycle (lights on at 8:00 A.M.) with free access to food and water. The rats were kept in this environment for 2 weeks prior to surgery. SRBT was provided by TSUMURA Co., Ltd. in Japan, and imipramine, a type of tricyclic antidepressant, was purchased from Sigma-Aldrich, Japan.

Ethics statement for animal experiments

The study protocol was in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the ethics review committee for animal experiments of Fukuoka University (Approval No. 1704043).

Operation

At 8 weeks of age the rats were anesthetized with a mixture of anesthetic agents (0.4 mg/kg of medetomidine, 2.0 mg/kg of midazolam, and 2.5 mg/kg of butorphanol). The bilateral olfactory bulb was removed by aspiration through two holes in the skull (anterior-posterior : +6 mm, medial-lateral : +1 mm from bregma). After the operation, the rats were housed in single cages (75.5 cm x 21 cm x 17 cm) for development of the OBX model for 14 days (Fig. 1). In total, 34 rats underwent OBX surgery and 11 rats underwent pseudo-operation (not removed olfactory bulbs, just knocked two holes in the skull) were utilized in this study. Success of the surgery was visually confirmed in isolated brain tissue after all behavioral analyses. On day 0 (the day before starting drug administration), we measured hyperemotionality in all rats, and OBX rats that met the criteria (hyperemotionality score >10) were included in the OBX group.

Drug administration

The drugs used in this experiment included SRBT (300 mg/kg) and imipramine (10 mg/kg). The doses

of both drugs were determined based on previous studies[6)7)]. SRBT was administered in a suspension, and imipramine was dissolved in distilled water. The crude drug preparation of SRBT included the following components : Bupleuri Radix 5.0 g, Pinelliae Tuber 4.0 g, Hoelen 3.0 g, Cinnamomi Cortex 3.0 g, Scutellariae Radix 2.5 g, Zizyphi Fructus 2.5 g, Ginseng Radix 2.5 g, Fossilia Ossis Mastodi 2.5 g, Ostreae Testa 2.5 g, and Zingiberis Rhizoma 1.0 g. Forty-five rats were separated into four groups as follows. Sham-Veh : pseudo-operated (not removed olfactory bulbs, just knocked two holes in the skull) and daily administered vehicle (distilled water) for 14 days, n=11 ; OBX-Veh : olfactory bulbectomized, and daily administered vehicle for 14 days, n=11 ; OBX-Imi : olfactory bulbectomized, and daily administered imipramine for 14 days, n=11 ; OBX-SRBT : olfactory bulbectomized, and daily administered SRBT for 14 days, n=12. The average hyperemotionality scores on day 0 were as follows : Sham-vehicle 6.5±2.7, OBX-vehicle 12.8±2.2, OBX-imipramine 12.8±1.5, and OBX-SRBT 13.1±1.1. After a 14-day surgical recovery period, each drug or vehicle was administered at the appropriate dosage via oral administration with a feeding needle once daily for a total of 14 days. The weight of each rat was recorded before daily administration. The hyperemotionality scores were measured 120 minutes after drug administration on days 1 and 14.

Measurement of hyperemotionality

Behavioral changes in OBX rats were measured on day 0, 1, and 14 (Fig. 1) with an OBX-induced hyperemotionality scoring procedure, according to previous studies[8)9)10)]. Responses to the following stimuli were scored : A) attack : a rod was presented 4-5 cm in front of the snout, B) startle : air was blown on the dorsum using a 5-ml syringe, C) struggle : animals were handled with a gloved hand, D) fight : the tail was gently pinched with mosquito forceps, E) vocalization : the degree of squeaking during each of the above procedures was evaluated. The responses, except for item E, were graded as follows ; 0 : no reaction, 1 : slight reaction, 2 : moderate reaction, 3 : marked reaction, 4 : extreme reaction. Item E was graded as follows ; 0 : no vocalization, 1 : occasional vocalization, 2 : marked vocalization. The sum of these scores was recorded as the hyperemotionality score. In each group, rats were observed on the same day, and each test procedure lasted a total of 5 minutes. The observers were blinded with respect to the drug treatment. Only rats that exhibited a hyperemotionality score >10 on postoperative day 14 were selected as OBX group for further study in the drug administration experiments.

Open field test

Locomotor activity was evaluated on days 1 and 14 by placing rats in an open field test chamber (90 cm×90 cm) in a dim room. The luminance was set to 25 lux at the center of the field and 15 lux in the periphery. The animals were tested for 5 minutes in an arena. The total distance that each rat traveled in the arena was recorded for 5 minutes. The data were collected and analyzed using SMART v 3.0 (Panlab Co.).

Determination of monoamine levels

After sacrificing all rats on day 14, each dissected brain was sliced into 2-mm-thick sections. Seven brain regions were rapidly subdissected with a matrix, including the pituitary gland, prelimbic cortex, cingulate cortex, striatum, hypothalamus, dorsal hippocampus, and ventral hippocampus, which were identified by referencing the rat brain atlas[11)] (Fig. 2). Once the tis-

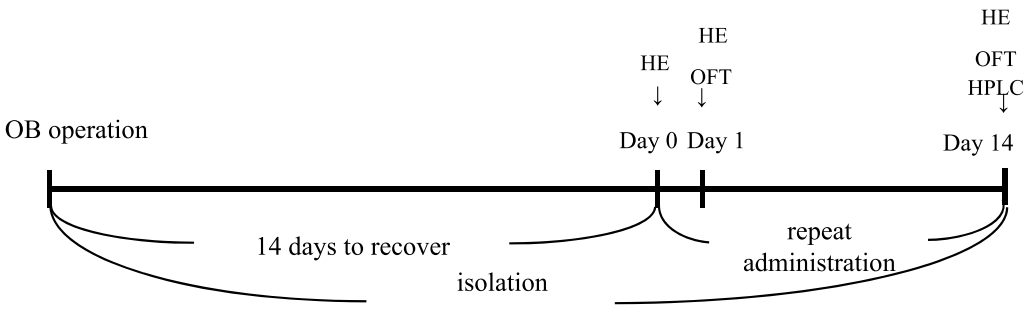

Fig. 1 : The experiment procedure from the operation to the repeat administration.
Abbreviations ; OB for olfactory bulbectomy, HE for hyperemotionality, OFT for open field test, HPLC for high-performance liquid chromatography.

sues were dissected, the samples were immediately placed into pre-weighed tubes and stored at −80℃. On the day of analysis, tissue samples were homogenized and centrifuged at 20,000 g for 15 minutes at 0℃. Sodium acetate was added to adjust the pH of each supernatant prior to chromatographic analysis. Each sample was investigated with high-performance liquid-chromatography (HPLC) to measure levels of monoamine metabolites. We measured homovanillic acid (HVA), 3-methoxy-4-hydroxyphenylglycol, and 5-hydroxyindoleacetic acid (5-HIAA) levels according to methods described in previous studies[5].

Statistical analysis

Analysis of variance and Dunnett's test were performed for each procedure using JMP 12.2 software.

Results

Firstly, we compared OBX group as a whole with Sham group. The OBX group had a significantly lower weight gain rate than the Sham group on day 1 (Fig. 3a). In addition, the OBX group traveled a significantly longer total distance in the open field test (Fig. 3b). Furthermore, the OBX group had a significantly higher hyperemotionality score on day 0 (Fig. 3c).

Next, we compared total hyperemotionality scores

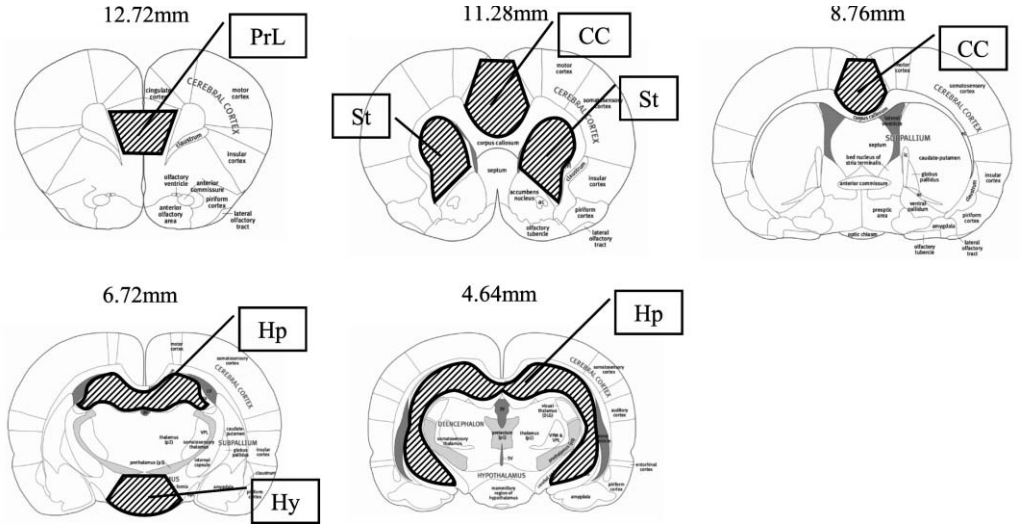

Fig. 2：Each brain region for HPLC examination.
The distance quoted in each case is the distance from rostral to the interaural line. Abbreviations; PrL for prelimbic cortex, CC for cingulate cortex, St for striatum, Hp for hippocampus, Hy for hypothalamus.

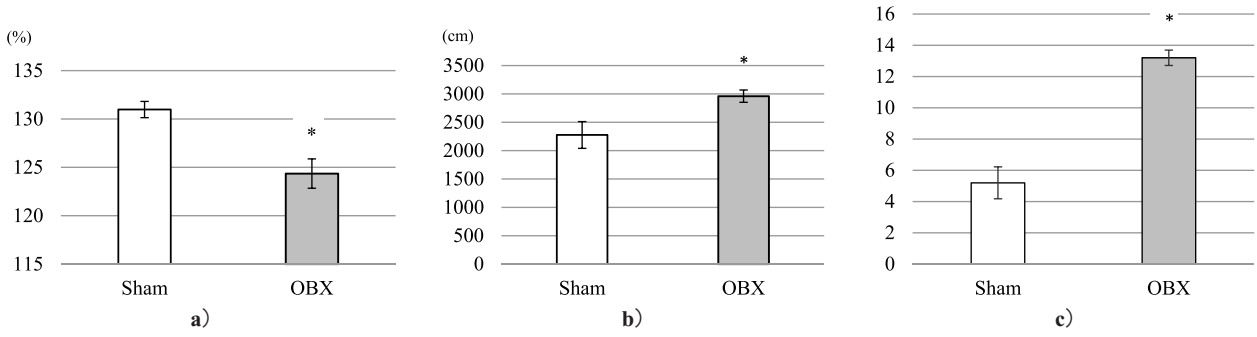

Fig. 3：Measured results of OBX as a whole, compared with Sham.
a) Weight gain rate after finishing recovery period. (p=0.034, vs Sham group)
b) Total distance of OFT after finishing recovery period. (p=0.009, vs Sham group)
c) Each HE score at the time before first administration. (p<.001, vs Sham group)
*：p<0.05, vs Sham
Abbreviations; OBX for olfactory bulbectomy, OFT for open field test, HE for hyperemotionality.

among all OBX groups (vehicle, SRBT, imipramine) and the Sham group. On day 1, all OBX groups showed higher scores than the Sham group, similar to the results of this experiments on day 0 (Table 1a). On day 14, testing for between OBX-SRBT and OBX-Veh approached significance ($p=0.06$, Table 1b). In addition, no significant difference was observed between the OBX-SRBT and Sham, while the OBX-Veh and Sham, and the OBX-Imi and Sham showed significant differences (Fig. 4, Table 1b). These findings suggest that SRBT administration tends to suppress hyperemotionality in rats. As a result of open field test on Day 1, each OBX group had significantly longer distance than sham, but there is no significant difference among 3 OBX groups (Table 2). On day 14, the result showed no significant differences among 4 groups (data not shown).

According to the HPLC results, the amounts of several metabolic products differed in specific brain regions. The OBX-vehicle group had a significantly higher 5-HIAA level in the pituitary gland than the Sham group (Fig. 5a), while the OBX-imipramine and OBX-SRBT groups showed no significant difference from the Sham group (Fig. 5a). The results suggest that continued administration of imipramine or SRBT can reduce 5-HIAA levels in the pituitary gland of OBX model rats. Meanwhile, the HVA level in the prelimbic cortex of OBX rats was significantly lower than that in the Sham group, and administration of imipramine or SRBT did not clearly affect the HVA level (Fig. 5b). No significant difference was found in the levels of other metabolic products of monoamines between the OBX and Sham groups (data not shown).

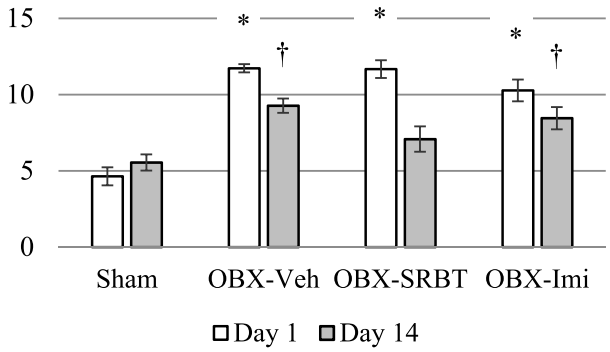

Fig. 4：Average HE total score among the 4 groups, at the time of first and final administration.
(On Day 14：$p_{vehicle}=0.001$, $p_{SRBT}=0.24$, $p_{imipramine}=0.01$, vs Sham group)
*：$p<0.05$, vs Sham Day 1, †：$p<0.05$, vs Sham Day 14

Table. 1：Compared HE total score among 4 groups, vs Sham or vs OBX-Veh on Day 1 (a) and Day 14 (b).

(a)

Day 1	HE score	p (vs Sham)	p (vs OBX-Veh)
Sham	4.64±0.59	—	<.0001*
OBX-Veh	11.73±0.27	<.0001*	—
OBX-SRBT	11.67±0.58	<.0001*	0.99
OBX-Imi	10.27±0.71	<.0001*	0.18

(b)

Day 14	HE score	p (vs Sham)	p (vs OBX-Veh)
Sham	5.55±0.53	—	0.001*
OBX-Veh	9.27±0.47	0.001*	—
OBX-SRBT	7.08±0.83	0.24	0.06
OBX-Imi	8.45±0.73	0.01*	0.72

*：$p<0.05$

Table. 2：Compared average distance of open field test on Day 1 among 4 groups, vs Sham or vs OBX-Veh.

Day 1	Average distance (cm)	p (vs Sham)	p (vs OBX-Veh)
Sham	2276.31±235.98	—	0.002*
OBX-Veh	3154.05±140.61	0.002*	—
OBX-SRBT	3110.41±113.24	0.003*	0.99
OBX-Imi	2930.11±160.24	0.027*	0.68

*：$p<0.05$

Discussion

Previous studies of SRBT have produced several findings in the field of neurotransmission. Chronic stress decreases glucocorticoid receptors in the prefrontal region of rats[12]. Additionally, when dexamethasone is injected into the prefrontal region, the feedback mechanism for the blood corticosterone level in chronically stressed rats fails to function[12]. In one study, repeated administration of SRBT suppressed the reduction of glucocorticoid receptors and improved the feedback system[13], suggesting that SRBT can improve hypothalamic−pituitary−adrenal axis dysfunction. Another study showed that SRBT increased the extracellular concentrations of dopamine and serotonin in the prefrontal region in chronically stressed rats, which indicates that SRBT may promote enhancement of neurotransmitter levels via functional improvement of glucocorticoid receptors[14]. Moreover, a study showed that SRBT ameliorated the impairment of rotarod performance in chronically stressed rats[14].

In the present study, continuous SRBT administration tended to suppress hyperemotionality in OBX rats and led to lower 5−HIAA levels in the pituitary gland compared with the OBX−vehicle group. These results suggest that hyperemotionality in OBX rats may be associated with 5−HIAA levels in the pituitary gland, and SRBT may affect this phenomenon. Previous studies regarded the hyperemotionality of OBX rats as a behavioral change caused by the depressive state[8)15)]. We first determined whether the OBX model was established during the procedure according to weight gain, total distance traveled in the open field test, and the hyperemotionality score on day 0. These results showed that we succeeded in the development of the OBX model. Initially, the OBX model was extremely useful for detecting the effects of existing antidepressants, and it has long been utilized as a preclinical evaluation method for antidepressants. However, its validity as an animal model for depression has been questioned because the theoretical necessity of extracting the olfactory bulb cannot be explained. In 1997, Kelly et al. reviewed the OBX model as a model of depression[16]. By extracting the olfactory bulbs of rodents, behavioral changes (such as hyperactivity, memory impairment, and anxiety reduction), neurochemical changes (such as decreased intracerebral noradrenaline, decreased 5−hydroxytryptamine, and increased 5−HIAA), neuroendocrine changes (increased secretion of corticosterone in the daytime), and neuroimmunological changes (decreased phagocytosis in neutrophils and monocytes) were observed, and these alterations are highly similar to the clinical symptoms of depression[16]. In the present study, the OBX group exhibited behavioral and neurochemical changes similar to those observed in previous studies, and chronic administration of SRBT could ameliorate these changes. However, imipramine administration did not

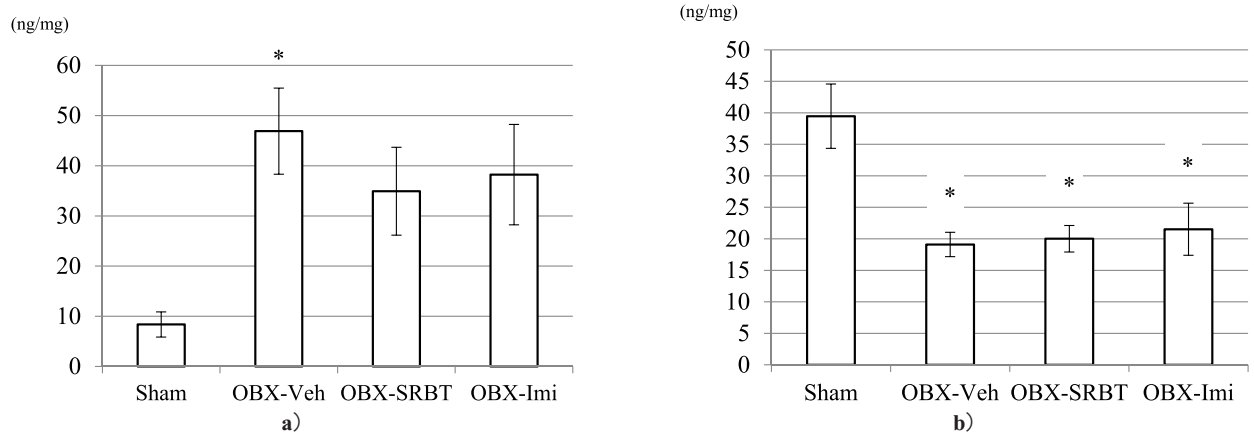

Fig. 5：Compared levels of monoamine metabolites among 4 groups.

a) 5−HIAA level in pituitary gland among the 4 groups after repeat administrations for 14 days. ($p_{vehicle}=0.031$, $p_{imipramine}=0.11$, $p_{SRBT}=0.16$, vs Sham group)

b) HVA level in prelimbic cortex among the 4 groups after repeat administrations for 14 days. ($p_{vehicle}=0.002$, $p_{imipramine}=0.006$, $p_{SRBT}=0.002$, vs Sham group)

a)b)*：$p<0.05$, vs Sham

Abbreviations；HE for hyperemotionality, OBX for olfactory bulbectomy, SRBT for Saiko−ka−ryukotsu−borei−to, Veh for vehicle, Imi for imipramine.

produce a significant difference in the total hyperemotionality score. We might get different result if the term of surveillance was longer. There is also possibility that SRBT may affect the hyperemotionality of OBX rats via a distinct mechanism, which is unverifiable from the present study. Alternatively, we should consider the possibility that the imipramine usage referred to in this experiment was enough to improve several behaviors as shown in previous studies but not enough to change the hyperemotionality score[7]. Regarding the HPLC results, we found that 5-HIAA in the pituitary gland and HVA in the prelimbic cortex were significantly different between the Sham and OBX-Veh. Both SRBT and imipramine administration significantly decreased 5-HIAA levels in the pituitary gland compared with the OBX-Veh. Further studies are needed to understand the mechanisms of neurotransmission. Although the present study measures monoamine metabolites in seven brain areas, it may not be possible to sufficiently measure changes caused by drug administration. In addition, the continuous administration period of 2 weeks may not be sufficient, and further long-term administration may yield different results. This study also has limitations because we used HPLC samples obtained from postmortem brains. To observe the transition of monoamine metabolites in vivo, additional experiments should be conducted using microdialysis. Furthermore, it is preferable to investigate hyperactivity reactions and changes in monoamine metabolite levels when SRBT and conventional antidepressants are simultaneously administered in series because many opportunities exist to combine antidepressants with traditional Chinese medicine in clinical practice.

Conclusions

In conclusion, this study indicates that SRBT affects the depression-like behavior of OBX rats. In addition, SRBT also may have the potential to correct abnormalities in neurotransmission in OBX rats.

Funding sources

This research received no specific grant from any funding agency in the public, commercial, or not-for profit sectors. The authors declare no conflict of interest.

Acknowledgements

We would like to express great appreciation to TSUMURA Co., Ltd. for providing the powdered extract of SRBT. In addition, we thank Lisa Kreiner, PhD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

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「The authors declare no conflict of interest.」