The analysis of the effects of drugs on locomotor activity in rodents is an important tool in behavioral pharmacology, and changes in this parameter can have important consequences for more specific processes such as in a psychotic state (e.g., schizophrenia). In fact, activation of the mesolimbic dopaminergic system induces several behavioral changes including an increase in locomotor activity in rodents (1, 2). Furthermore, psychostimulant-induced hyperlocomotion, which is mediated by activation of the mesolimbic dopaminergic system, has been used as an animal model of schizophrenia (3). Psychotic episodes of schizophrenia have been traditionally treated by antipsychotics. Therefore, it is believed that activation of dopamine D2 receptors, particularly in the nucleus accumbens (a terminal region of the mesolimbic dopaminergic system), is linked to episodes of schizophrenia (4). However, the pathophysiological mechanism that underlies schizophrenia, especially the etiological mechanism, is not fully understood.

Genetic and environmental factors play a role in the development of schizophrenia. In animals, genetic and environmental factors can affect schizophrenia-like pathogenesis and pathophysiological changes (5). The C57BL/6J-bgJ/bgJ (beige-J) strain of mouse originated as a spontaneous mutation from C57BL/6J lines and is of particular interest as a homologue of Chediak-Higashi syndrome in humans (6). Even though limited information is available on which molecules have been changed in the pathophysiological changes in beige-J mice, these mice show defects in lysosome-containing cells, neutral protease activity in polymorphonuclear leukocytes, and other immunological functions, especially natural killer (NK) cell functions (7 – 9). A large and growing body of evidence supports the notion that immune dysfunction may play a role in schizophrenia, whereas the involvement of NK cell activities in schizophrenic patients is still controversial (10). Our preliminary study showed that beige-J mice exhibited increased locomotor activity. Therefore, we hypothesized here that dopaminergic functions in the beige-J mouse strain would be different from those in other strains. Thus, the aim of the present study was to investigate locomotor activity accompanied by activation of the dopaminergic system in beige-J mice. Previous studies have indicated that morphine-induced antinociception in beige-J (11) or diabetic (12) mice was enhanced by splenectomy. Furthermore, the hyperactivity of diabetic mice was normalized by splenectomy (13). Therefore, the effects of splenectomy on increased locomotor activity in beige-J mice were also investigated.

Male beige-J mice (National Institute on Genetics,
Mishima), weighing 25 – 35 g (6 – 9 weeks), at the beginning of the experiments, were used. C-57BL/6J and male ddY mice were obtained from Tokyo Animal Laboratories, Inc. (Tokyo). The mice were housed at a room temperature of 22°C ± 1°C with a 12-h light-dark cycle (lights on 8:00 A.M. to 8:00 P.M.). Food and water were available ad libitum. Splenectomies were carried out under sterile and isoflurane-anesthetized conditions. In the present study, beige-J mice and control mice were obtained from different vendors. Thus, these mice were reared in different environments and were maintained as separate colonies across generations. Therefore, we used these animals for experiments after at least 2 weeks habituation periods in our facility. Sham-operated animals were subjected to the same handling, anesthesia, surgical exposure of the spleen, and wound closure as splenectomized animals, as described previously (12). Seven days after surgery (sham or splenectomy), all experiments were initiated.

The spontaneous locomotor activity of mice was measured for 3 h by an ambulometer (ANB-M20; O’Hara Co., Ltd., Tokyo) as described previously (14). Mice were placed in tilting cages for a habituation period of 30 min. Apomorphine-induced hyperlocomotion was measured for 3 h after the injection of apomorphine (1.0 mg/kg, s.c.) in a volume of 10 ml/kg.

The concentrations of dopamine (DA) and its metabolites 3,4-dihydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA) were determined as described previously (14). Sham-operated and splenectomized mice were sacrificed, and the brain was quickly removed and dissected into the limbic forebrain (containing the nucleus accumbens and olfactory tubercles) on an ice-cold glass plate. The tissue samples were homogenized in 2 ml of 0.2 M perchloric acid containing 100 μM EDTA (2Na) and 100 ng isoproterenol, as an internal standard. To remove the proteins completely, the homogenates were placed in cold water for 60 min. The homogenates were then centrifuged at 20,000 × g for 20 min at 0°C, and the supernatants were maintained at pH 3.0 using 1 M sodium acetate. A solution sample of 50 μl was injected by a refrigerated GILSON automatic sampler (Model 231) and analyzed by high performance liquid chromatography (HPLC) with electrochemical detection (ECD). The HPLC system consisted of a delivery pump (880-PU; Jasco, Tokyo), an analytical column (Eicom, MA-5ODS 4.6 × 150 mm; Eicom Co., Kyoto) and a guard column (Eicom). The electrochemical detector (EC-100, Eicom) included a graphite electrode (WE-3G) and was used at a voltage setting of 0.7 V vs. an Ag/AgCl reference electrode. The mobile phase consisted of a 0.1 M sodium acetate / 0.1 M citric acid buffer, pH3.5, containing 13% – 15% methanol, sodium 1-octanesulfonate, and EDTA (2Na). The flow rate was set to 1.0 ml/min with a column temperature of 25°C.

Apomorphine hydrochloride (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in saline. DA, DOPAC, and HVA were purchased from Research Biochemicals, Inc. (Wayland, MA, USA) and dissolved in 0.02 N acetic acid for HPLC. Behavioral data (total activity counts) were statistically evaluated with a Newman-Keuls test. Neurochemical data were statistically evaluated with a one-way ANOVA followed by Dunnett’s test. A value of $P < 0.05$ was considered statistically significant.

Previous research has demonstrated that beige-J mice have shorter life span and lower body weight when compared to a control group of C57BL/6J mice (15). Beige-J mice used in the present study were a bit smaller than those of the same-aged control animals. Our preliminary data showed that beige-J mice exhibited greater spontaneous locomotor activity than ddY mice even in their home cages. In the present study, spontaneous locomotor activity in sham-operated beige-J mice was significantly greater than those in C-57BL/6J and ddY mice. Splenectomized beige-J mice showed a significant decrease in locomotor activity, whereas a decrease in locomotor activity was not observed in splenectomized C-57BL/6J and ddY mice (Fig. 1A). To specify whether the presynaptic or postsynaptic dopaminergic system was involved, we examined the effects of apomorphine on locomotor activity in sham-operated and splenectomized beige-J mice. Apomorphine-induced hyperlocomotion in sham-operated beige-J mice was significantly greater than that in ddY mice (Fig. 1B). On the other hand, apomorphine (1.0 mg/kg) produced behavioral disruption (struggling-like behavior) accompanied by hypolocomotion in C-57BL/6J mice (data not shown). However, splenectomy did not affect the behavioral changes induced by apomorphine in ddY, C-57BL/6J or beige-J mice.

We next examined the effects of splenectomy on the levels of dopamine as well as its metabolites in the limbic forebrain of beige-J mice. Splenectomy significantly reduced DOPAC and HVA levels (DOPAC: $F_{1,8} = 10.3$, $P < 0.05$; HVA: $F_{1,8} = 9.01$, $P < 0.05$) (Fig. 2: A and B). In contrast, DA levels in the limbic forebrain were significantly increased by splenectomy compared to those in the sham-operated group ($F_{1,8} = 24.2$, $P < 0.01$) (Fig. 2C). The high DA ratios in the limbic forebrain of beige-J mice were consistently decreased by splenectomy (Fig. 2D) to the levels seen in ddY mice (0.235 ± 0.01, n = 7).

The present behavioral study demonstrated that the spontaneous locomotor activity in beige-J mice was
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Greater than that in ddY mice. Similarly, the DA turnover ratio in the limbic forebrain of beige-J mice was greater than that in ddY mice. It is well known that an increase in locomotor activity is critically linked to activation of the dopaminergic, and especially the mesolimbic, system. These results indicate that beige-J mice have a hyperactive mesolimbic dopaminergic system due to an increase in the release of dopamine from nerve terminals. On the other hand, the dopamine-receptor agonist apomorphine, which increases in locomotor activity mediated by activation of postsynaptic dopamine receptors, induced hyperlocomotion in beige-J mice was greater than that in ddY mice. Our findings indicate that the supersensitivity of the mesolimbic dopaminergic system as presynaptic as well as postsynaptic events may also be involved in the expression of hyperlocomotion in beige-J mice.

Beige-J mice have been shown to be less responsive than their normal littermates to the antinociceptive effects of \( \mu \)-opioid-receptor agonists, and splenectomy restored the antinociceptive responsiveness of \( \mu \)-opioid agonists in beige-J mice to close to normal (11). The present study demonstrated that splenectomy reduced the spontaneous hyperlocomotion and high levels of DA turnover in the limbic forebrain of beige-J mice. However, splenectomy did not alter apomorphine-induced hyperlocomotion in beige-J mice. These results suggest that some circulating substances originating from the spleen may modulate the release of dopamine from the nucleus accumbens.

The DA theory, which proposes an excess of dopaminergic stimulation in schizophrenia, has been established. Interestingly, immunological dysfunction has been described as an etiology of schizophrenia (16). For example, NK cell activities were shown to be potently reduced in schizophrenic patients (10). It is unclear...
whether the augmentation of DA turnover in the limbic forebrain is due to immune dysfunction in beige-J mice. However, the present data indicate that beige-J mice could be a new experimental animal model for investigating the hyperactive states of dopaminergic systems as regulated by the immune system. On the other hand, tyrosine hydroxylase activity in the cell body of the dopaminergic neurons were correlated to the dopamine concentration of nerve terminals (17). Even though further studies will be needed to investigate how splenectomy regulates the hyperactive states of dopaminergic systems, and synthesis of dopamine in beige-J mice, our results suggest that circulating substances originating from the spleen and/or immune dysfunction may participate in a dopamine hyperactive state–related disorder like schizophrenia.

References

13 Kamei J, Saitoh A. Evidence for the modulation of spontaneous locomotor activity by higher serum glucose levels and/or spleen-derived factor(s) in diabetic mice. Life Sci. 1997;60:1699–1708.
15 Goodrick CL. Body weight change over the life span and longevity for C57BL/6J mice and mutations which differ in maximal body weight. Gerontology. 1977;23:405–413.