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Chronic L-tyrosine alters the locomotor activity and brain monoamine levels in Roborovskii hamsters

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ABSTRACT

The Roborovskii hamster (*P. roborovskii*) has high locomotor activity (hyperactivity) and low dopamine levels in the brain compared with the congeneric Djungarian hamster (*P. sungorus*). To clarify the efficacy of dietary L-tyrosine in ameliorating signs of hyperactivity, we investigated the effects of chronic administration of L-tyrosine, the primary precursor of dopamine, on locomotor activity and brain monoamine levels in Roborovskii hamsters. Chronic supplementation of L-tyrosine had no effect on locomotor activity in the open field, but did decrease locomotor activity in the home cage. Tyrosine increased dopamine and norepinephrine turnover rates and decreased in serotonin turnover rate in the brain. These findings suggest that long-term feeding of L-tyrosine may be effective in ameliorating signs of hyperactivity.

Hyperactivity, which is characterized by high locomotor activity, is one of the problematic behaviors seen in children with attention-deficit/hyperactivity disorder (ADHD) [21], as well as hyperactive dogs [16]. ADHD is one of the most common chronic neurobehavioral diseases observed in child development. It is characterized by hyperactivity together with lack of attention and impulsivity [4, 21], with approximately 8 to 12% of children exhibiting these symptoms worldwide [4]. Furthermore, these symptoms secondarily induce learning dysfunction [21]. Similarly, hyperactive dogs have high activity levels together with features such as lack of trainability and failure to habituate to external stimulation, and their mechanism of hyperactivity is thought to be similar to that of ADHD. Moreover, these changes aggravate relationships between dogs and their guardians. Hyperactivity can thus decrease the quality of life (QOL) of both humans and companion animals.

Although the neurobiological basis of hyperactivity remains poorly understood, it appears that the cause of hyperactivity involves not only environmental factors such as lack of discipline but also biological disorders such as dysregulation of monoamine neurotransmission [2, 4]. Dopaminergic (DA) neurotransmission, in particular, is believed to play important roles in the pathology of hyperactivity [9, 15, 25]. In fact, children with ADHD are clinically treated with DA-based psychostimulants. To determine the pathology of hyperactivity we have examined the Roborovskii hamster (*Phodopus roborovskii*) as a potential animal model of hyperactivity, which exhibits markedly increased locomotor activity compared with the congeneric Djungarian hamster [13]. Roborovskii hamsters also have low DA levels in the brain, and it was hypothesized that such levels play a role in the pathogenesis of hyperactivity. To test this hypothesis, we administered 3,4-dihydroxyphenylalanine (L-DOPA), a precursor in DA synthesis, to Roborovskii hamsters and observed a dose-dependent increase in DA levels in the brain and decreased locomotor activity [11].

L-Tyrosine, an aromatic amino acid, is also a precursor in DA synthesis. There have been inconsistent findings that L-tyrosine increases the release of DA [1, 8] or that it has no effect on DA release in normal animals [5]. DA is also the precursor to norepinephrine. Few studies have examined whether L-tyrosine affects DA-related disorders associated with hyperactivity. The effects of dietary L-tyrosine on behavioral alteration induced by chronic stress were investigated by employing a social isolation stress model in mice [12]. Social isolation stress increased locomotor activity in both the home cage and open field. These increases in locomotor activity were suppressed by dietary L-tyrosine. Moreover, L-tyrosine increased both the concentration and turnover rate of norepinephrine metabolites [12]. These findings suggest a possible role of dietary L-tyrosine for

psychic dysfunctions induced by chronic stress.   However, the acute and single feeding of L-tyrosine may be effective in modifying brain monoamine metabolism, but not ameliorating signs of hyperactivity in the Roborovskii hamster [14].   This study, therefore investigated whether chronic L-tyrosine affects the hyperactive behavior and brain monoamine levels of Roborovskii hamsters was.

Male Roborovskii hamsters, 3 weeks-of–age, purchased from a local pet shop (Nomura, Fukuoka, Japan), were reared in a controlled environment.   The hamsters were housed individually in plastic cages (22 cm × 15 cm × 12 cm) and allowed *ad libitum* access to a mash diet (MF; Oriental Yeast, Tokyo, Japan) and water.   A 12-h light/dark cycle was maintained throughout the experiments with lights on at 0800 and off at 2000. Room temperature was maintained at 23 ± 1℃.   After a one-week acclimation period, the animals were subjected to the experimental procedures described below.   The experimental procedures followed the Guidelines for Animal Experiments of the Faculty of Agriculture and the Graduate School of Kyushu University, as well as Japanese Law (No.105) and a Notification (No. 6) by the Japanese Government.

L-Tyrosine was purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan.   The animals were weighed and randomly assigned to two feeding groups (n = 8 each): a control group receiving a standard mash diet, and a group receiving a high L-tyrosine (4%) diet.   After a 10-day feeding regimen, the animals were subjected to two behavioral tests (home cage and open field tests).

General locomotor activity was measured during the dark period (12 h), when hamsters are active in their home cages.   Activity was counted with an infrared beam sensor (NS-AS01; Neuroscience Inc., Tokyo, Japan) placed about 15 cm above the center of the cage, and analyzed using DAS-008 software (Neuroscience Inc.).

Locomotor activity in a novel environment was determined using the open field test.   Briefly, animals were individually transferred to an open field arena from the home cages.   The arena was circular (diameter 60 cm and height 35 cm), and made of black Takiflex.   The test was begun by placing the animal at the center of the arena.   The behavior of animals was then observed for 5 min under dim light (100 lux).   After each test, the field was cleaned with an ethanol-water solution.   The following behavioral categories were examined: distance of path, time the animal spent moving, speed of movement, and frequency of defecation.   All behaviors except defecation were automatically analyzed with a computer-based video tracking system (AXIS-90, Neuroscience, Inc., Japan).   Frequency of defecation was manually recorded.   The locomotor activity in a novel environment and habituation were performed employing the open field test for 4 consecutive days, and all animals were killed by cervical dislocation and decapitated immediately following completion of the open field test.   Whole brains were immediately removed, weighed, and kept at -80℃ until analyzed.

Levels of monoamines and their metabolites (contents/g wet tissue) were analyzed using a previously described method [24] with some modifications. Briefly, the tissue was homogenized and deproteinized in 0.2 M perchloric acid containing 100 μM EDTA disodium. The homogenate was left for 30 min for deproteinization. Then, the homogenate was centrifuged at 10,000 x g for 15 min at 0ºC. After centrifugation, the pH of supernatant was adjusted to approximately 3.0 by adding 1 M sodium acetate. The supernatant was then centrifuged with a centrifuge-filtration unit (Ultra Free C3-GV Millipore, Bedford, MA, USA) at 10,000 x g for 5 min at 0ºC. A 30 μl portion of filtrate was applied to a high performance liquid chromatography (HPLC) system (Eicom, Kyoto, Japan) with a 150 x 2.1 mm octadecyl silane (ODS) column (SC-5ODS, Eicom) and an electrochemical detector (ECD-300, Eicom) at an applied potential of +0.75 V versus Ag/AgCl reference analytical electrode. Changes in electric current (nA) were recorded in a computer using an interface system (Power Chrom ver 2.3.2.j; AD Instruments, Tokyo, Japan). The mobile phase consisted of 0.1 M aceto-citric acid buffer (pH 3.5), methanol, 0.46 M sodium 1-octane sulfonate, and 0.015 mM disodium ethylenediaminetetraacetic acid (830:170:1.9:1) at a flow rate of 0.2 ml/min. The concentrations of monoamines and metabolites including DA, norepinephrine (NE), serotonin (5-HT), the DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC), NE metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG), and 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) were determined, and their levels in brain were calculated. Turnover rates (DOPAC/DA, MHPG/NE, and 5-HIAA/5-HT) were also calculated. The limit of detection of the system for all monoamines was 0.1 pg/sample.

Levels (contents/g wet tissue) of L-tyrosine were determined using a previously described method [10] with some modifications. Briefly, brains were homogenized, deproteinized, and centrifuged by the same process as for the analysis of monoamines. After centrifugation, the pH of supernatant was adjusted to approximately 7.0 by adding 1 M sodium hydroxide. A sample (20 μl) was then completely dried under reduced pressure. Dried residue was dissolved with 10 μl of a 1 mol/l sodium acetate-methanol-triethylamine (2:2:1) solution. The sample was re-dried, and dissolved in 20 μl of deprivatization solution (methanol-water-triethylamine-phenylisothiocyanate [7:1:1:1]). The sample was maintained at room temperature for 20 min to allow phenylisothiocyanate to react with the amino groups to produce phenyltiocarbamyl amino acid residues. The sample was dried again, and was disssolved with 100 μl of Pico-Tag Diluent (Waters, Milford, MA., USA). This diluted sample was filtered through a 0.45 μm filter (Millipore). The same method was applied to standard solutions prepared by diluting L-tyrosine with distilled water. These derivatized samples (5

µl) were applied to a Waters HPLC system (Pico-Tag free amino acid analysis column [3.9 x 300 mm], Alliance 2690 separation module, 2487 dual-wavelength UV detector, and Millennium 32 chromatography manager; Waters). Samples were equilibrated with buffer A (70 mmol/l sodium acetate [pH 6.45 with 10% acetic acid]-acetonitrile [975:25]) and eluted with a linear gradient of buffer B (water-acetonitrile-methanol [40:45:15]) (0, 3, 6, 9, 40, and 100%) at a flow rate of 1 ml/min at 46ºC. The absorbance at 254 nm was measured, and concentration of L-tyrosine was determined and its level in the brain calculated.

The statistical significance of differences in locomotor activity in the home cage was analyzed using Mann–Whitney U test. The levels of monoamines and L-tyrosine in the brain between the groups were analyzed using a Student's t-test. Locomotor activity in the open field over 4 consecutive days was analyzed using a repeated measure two-way ANOVA.

Fig. 1 shows locomotor activity in the home cage in the dark period. L-Tyrosine significantly decreased the locomotor activity of Roborovskii hamsters in their home cages.

Fig. 2 shows locomotor activity (distance of path) in the open field test on 4 consecutive days. There were no tyrosine-induced differences in locomotor activity in a novel environment (day 1). Similarly, regarding habituation, there were no significant interactions ($F_{(3,39)}=1.456$, $P>0.05$) between treatment groups and day, and no significant differences were observed among treatment groups ($F_{(1,13)}=0.049$, $P>0.05$). Likewise, no significant differences in other measurements (time spent moving, speed of movement, and frequency of defecation) between groups (data not shown) were observed.

Tables 1 and 2 show the level of monoamines, their metabolites, and their turnover rates in the cerebral cortex and hypothalamus. L-Tyrosine significantly increased DA turnover rate in the cerebral cortex. Furthermore, L-tyrosine significantly increased MHPG level and NE turnover rate in both the cerebral cortex and hypothalamus. On the other hand, 5-HT turnover rate was significantly decreased in both the cerebral cortex and hypothalamus.

Fig. 3 shows the levels of L-tyrosine in the cerebral cortex and hypothalamus. L-Tyrosine significantly increased L-tyrosine levels in both the cerebral cortex and hypothalamus.

In the present study, we investigated whether chronic dietary L-tyrosine could alter the locomotor activity and brain monoamine levels in Roborovskii hamsters as a potential animal model of hyperactivity. Chronic L-tyrosine significantly decreased the locomotor activity of Roborovskii hamsters in their home cages. In the open field, no significant effect of L-tyrosine on locomotor activity was observed on the first day (when the testing environment was novel). This finding agreed with a previous report for mice [22,

23]. The locomotor activity in the open field over 4 consecutive days was measured to determine the effects of L-tyrosine on habituation, one of the most elementary types of learning phenomena [19]. Learning behavior is in general impaired by hyperactivity [21]. In particular, it was observed in preliminary experiments that Roborovskii hamsters had difficulty acclimating to a novel environment. In general, less activity is observed when an animal is exposed to a known exploratory situation than in a novel environment. In this study, no difference in habituation was observed between the feeding groups. Following a single feeding of L-tyrosine, brain monoamine metabolism was modified, but did not ameliorate signs of hyperactivity in the open field in the Roborovskii hamster [14]. Thus, L-tyrosine may have no effect on habituation of the Roborovskii hamster.

L-Tyrosine increased DA turnover rate in cerebral cortex, where mesocortical DA projections terminate. This finding suggests that L-tyrosine may have enhanced mesocortical DA neurotransmission. It has been suggested that DA mesocortical neurotransmission plays a role in hyperactivity, and attenuation of such neurotransmission is one candidate for pathological changes in hyperactivity [20]. Thus, an increase in DA turnover rate in this region might play a role in decreasing locomotor activity observed in the home cage. Levels of MHPG and turnover rate of NE were also increased by L-tyrosine in both the cerebral cortex and hypothalamus, indicating that L-tyrosine enhanced NE neurotransmission. These findings suggest that NE transmission may play a role in hyperactivity. Atomoxetine, a NE transporter inhibitor, was reported to be effective in treating hyperactivity [6, 17]. In particular, NE neurotransmission is related to impulsive behavior and poor attention, which are signs of hyperactivity together with high locomotor activity. Indeed, enhancement of NE neurotransmission decreased impulsive behavior although the brain region modulating impulsivity remains unknown [18]. In this study, L-tyrosine might have decreased impulsive behavior through increasing NE turnover, which might play a role in decreasing locomotor activity. Furthermore, L-tyrosine decreased the turnover rate of 5-HT in the cerebral cortex and hypothalamus. Since an increase in 5-HT neurotransmission has been suggested to cause hyperactivity [3], a decrease in 5-HT caused by L-tyrosine might be related to decrease in locomotor activity.

A variety of types of pathology have been suggested to play roles in hyperactivity [7]. Our findings suggest that L-tyrosine may be efficacious in treating some types of hyperactivity. However, further studies are needed to determine the mechanism by which L-tyrosine decreases locomotor activity of Roborovskii hamsters in their home cages, since marked interaction exists among the various types of monoamine neurotransmission.

In conclusion, chronic supplementation of L-tyrosine ameliorated the locomotor activity of Roborovskii hamsters in their home cages while chronic supplementation had no effect in open field testing. Furthermore, L-tyrosine altered levels of monoamine metabolites and turnover rates in the brain. The decreased home cage locomotor activity caused by tyrosine may have been due to alternations in monoamine neurotransmission. These findings suggest the possibility that dietary L-tyrosine may be efficacious in treating hyperactivity.

Acknowledgments

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LEGENDS OF FIGURES

Fig. 1

Effects of chronic supplementation of L-tyrosine on locomotor activity in the home cage. Values are means ± S.E.M. *Significantly different from the control group ($P<0.01$).

Fig. 2

Effects of chronic supplementation of L-tyrosine on locomotor activity (distance of path) in the open field for 4 consecutive days. Values are means ± S.E.M.

Fig. 3

Effects of chronic supplementation of L-tyrosine on levels of L-tyrosine in cerebral cortex and hypothalamus. Values are means ± S.E.M. *Significantly different from the control group ($P<0.01$).
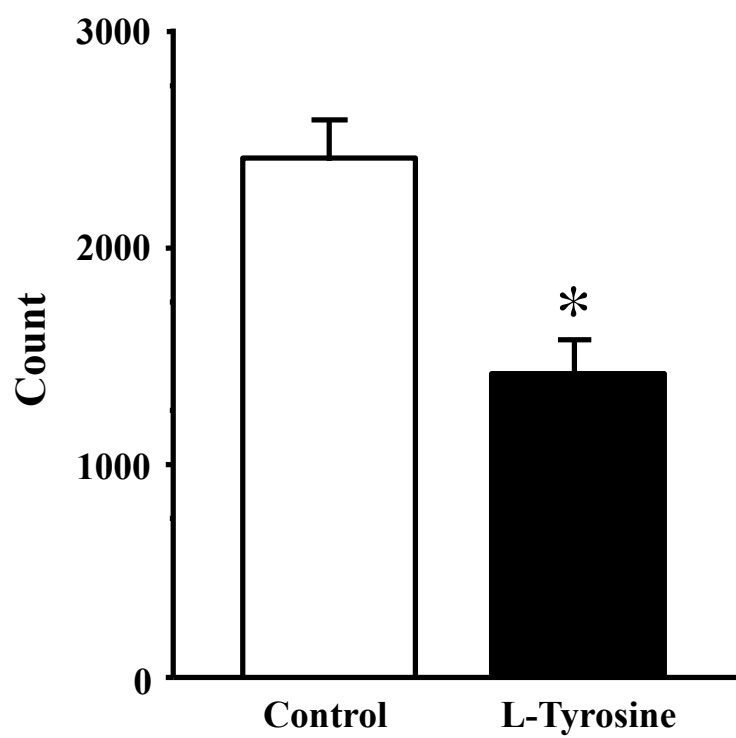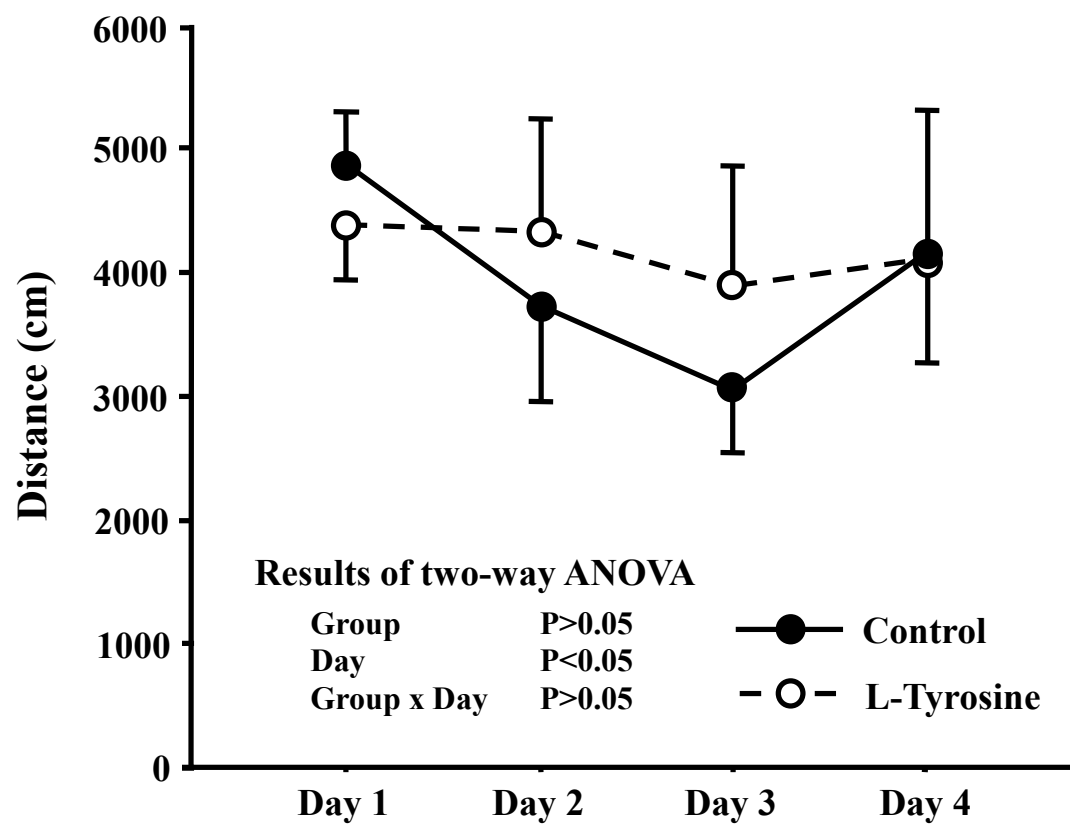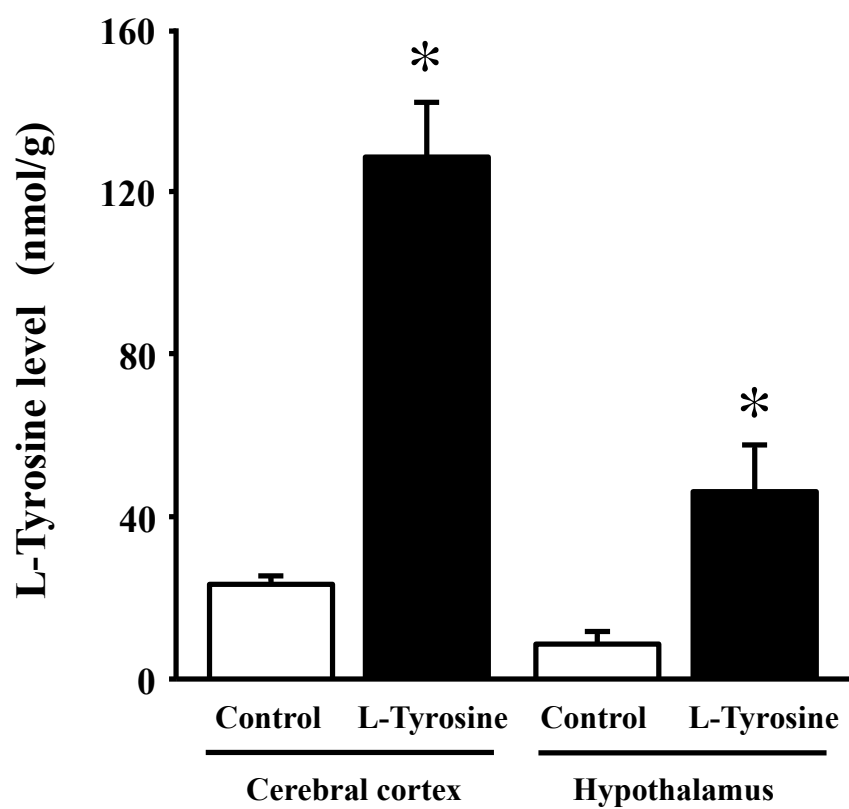
Fig. 1

Fig. 2

Fig. 3

Table 1

Effects of chronic supplementation of L-tyrosine on monoamines and their metabolites in cerebral cortex and hypothalamus of Roborovskii hamsters

		DA	DOPAC	NE	MHPG	5-HT	5-HIAA
Cerebral cortex	Control	1303 ± 87	105 ± 7	206 ± 12	38 ± 1	236 ± 11	96 ± 4
	L-Tyrosine	1448 ± 128	115 ± 10	208 ± 17	$188 \pm 11*$	269 ± 16	95 ± 6
Hypothalamus	Control	1563 ± 140	216 ± 15	2053 ± 211	33 ± 2	1229 ± 55	487 ± 23
	L-Tyrosine	2754 ± 675	325 ± 51	1993 ± 253	$183 \pm 25*$	1291 ± 79	469 ± 34

DA: dopamine, DOPAC: 3,4-dihydroxyphenylacetic acid, NE: norepinephrine, MHPG: 3-methoxy-4-hydroxyphenylglycol, 5-HT: serotonin, 5-HIAA: 5-hydroxyindoleacetic acid.

Values are means pmol/g wet tissue $\pm$ S.E.M.

*$P<0.01$, significantly different from the respective control value.

Table 2

Effects of chronic supplementation of L-tyrosine on monoamine turnover rates in cerebral cortex and hypothalamus of Roborovskii hamsters

		DOPAC/DA	MHPG/NE	5-HIAA/5-HT
Cerebral cortex	Control	0.074 ± 0.002	0.193 ± 0.010	0.396 ± 0.005
	L-Tyrosine	0.080 ± 0.004*	0.935 ± 0.081**	0.363 ± 0.005**
Hypothalamus	Control	0.140 ± 0.005	0.017 ± 0.001	0.397 ± 0.008
	L-Tyrosine	0.135 ± 0.011	0.093 ± 0.010**	0.362 ± 0.010*

DA: dopamine, DOPAC: 3,4-dihydroxyphenylacetic acid, NE: norepinephrine, MHPG: 3-methoxy-4-hydroxyphenylglycol, 5-HT: serotonin, 5-HIAA: 5-hydroxyindoleacetic acid.

Values are means $\pm$ S.E.M.

*$P<0.05$, **$P<0.01$, significantly different from the respective control value.