

## Decreased L-tryptophan concentration in distinctive brain regions of mice treated repeatedly with phencyclidine

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**Abstract** It has been reported that repeated phencyclidine (PCP) treatment induces schizophrenia-like behavior in mice. L-Tryptophan (Trp) concentrations in brain tissues of control ( $n=8$ ) and PCP-treated mice (10 mg/kg/day, s.c., 14 days,  $n=10$ ) were determined using high-performance liquid chromatography (HPLC) with fluorescence detection. The HPLC method involved pre-column fluorescence derivatization with (*R*)-(-)-4-(*N,N*-dimethylaminosulfonyl)-7-(3-

isothiocyanatopyrrolidin-1-yl)-2,1,3-benzoxadiazole (DBD-PyNCS). Eight different parts of the brain, namely, the frontal cortex, nucleus accumbens, striatum, hippocampus, amygdala, thalamus, hypothalamus, and cerebellum, of both groups were investigated. A significant decrease in the L-Trp concentration in the nucleus accumbens ( $p=0.024$ ) and hippocampus ( $p=0.027$ ) was observed in PCP-treated mice, suggesting that alteration of the L-Trp metabolism might occur in these brain parts.

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### Abbreviations

DBD-PyNCS (*R*)-(-)-4-(3-Isothiocyanatopyrrolidin-1-yl)-7-*N,N*-dimethylaminosulfonyl-2,1,3-benzoxadiazole  
L-Trp L-Tryptophan  
PCP Phencyclidine

### Introduction

1-(1-Phenylcyclohexyl) piperidine (phencyclidine, PCP), a noncompetitive antagonist of the *N*-methyl-D-aspartate (NMDA) receptor, has been used for the production of a rodent model of schizophrenia, one of the severe psychiatric diseases [1]. Acute and chronic PCP administrations to mice induced abnormal behaviors such as hyperlocomotion, social interaction deficits, and sensorimotor gating deficits [1]. These changes are regarded as schizophrenia-like behaviors and have been useful for screening assays of drug candidates to treat schizophrenia.

Accompanied with abnormal behaviors, changes of the several neurotransmitters (dopamine, serotonin, glutamate, and  $\gamma$ -aminobutyric acid) in brain tissues of PCP-treated mice have been suggested [1]. These concentration changes of endogenous substances related to neurotransmission could be closely involved in the etiology of abnormal behaviors induced by PCP.

Thus far, neuroactive amino acids, e.g., glutamate, aspartic acid, and glycine, in the central nervous system have been mainly targeted for the research on schizophrenia. On the other hand, L-tryptophan (L-Trp), one of the essential amino acids, is metabolized to several neuroactive metabolites such as quinolinic acid (QA), kynurenic acid (KYNA), and serotonin by two metabolic pathways, namely, the kynurenine and serotonin pathways [2, 3]. Therefore, L-Trp is a precursor for neuroactive substances. It is likely that changes in the L-Trp concentration could affect regular neurotransmission, and disturbance of the normal L-Trp concentration in the brain may contribute to psychiatric diseases such as schizophrenia. This assumption also implies that alterations in the brain L-Trp concentration may occur in an animal model of schizophrenia. In PCP-treated mice, however, there have so far been no reports on altered L-Trp concentrations. Thus, in the present study, the L-Trp concentration in eight brain regions of mice chronically treated with PCP was investigated by high-performance liquid chromatography (HPLC) with fluorescence detection method using (*R*)-(-)-4-(*N,N*-dimethylaminosulfonyl)-7-(3-isothiocyanatopyrrolidin-1-yl)-2,1,3-benzoxadiazole [(*R*)-(-)-DBD-PyNCS] [4] as a pre-column derivatization reagent.

## Materials and methods

### Chemicals

PCP was synthesized by Prof. Furukawa, Meijo University according to the method by Maddox et al. [5]. L-Trp and bovine serum albumin were purchased from Sigma (St. Louis, MO, USA). (*R*)-(-)-, (*S*)-(+)-DBD-PyNCS, and 4-*N,N*-dimethylaminopyridine (DMAP) were purchased from Tokyo Chemical Industries Co. Ltd. (Tokyo, Japan). Coomassie Blue was purchased from Bio-Rad Laboratories, Inc. (Hercules, CA, USA). Water was purified using a Milli-Q Labo system (Nihon Millipore Co. Ltd., Tokyo, Japan). Acetonitrile (CH<sub>3</sub>CN) and acetic acid (AcOH) of HPLC grade were obtained from Kanto Kagaku Co., Ltd (Tokyo, Japan) and Wako Pure Chemicals Industries, Ltd (Osaka, Japan), respectively.

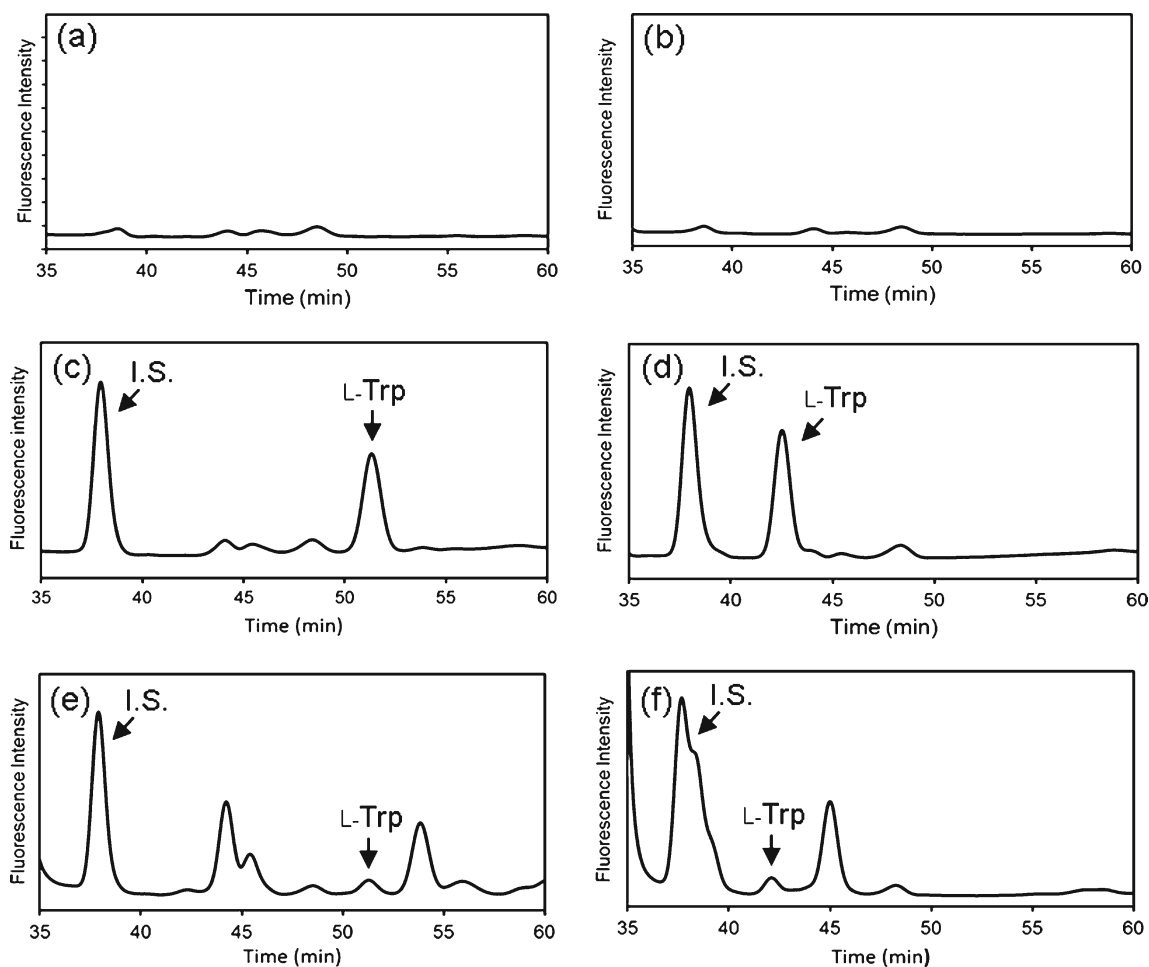
### Animal experiments

Male ICR mice (6 weeks old) were purchased from Japan SLC, Inc. (Shizuoka, Japan) and housed in an environmentally

controlled room (24±1 °C, 55±5 % humidity) with a 12-h light/dark cycle (lights on at 08:00 hours). All animal experiments were approved by the Institutional Animal Care and Use Committee of Meijo University, and all animal care and use was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and Experimental Animal Care issued by the Japanese Pharmaceutical Society. PCP (1.0 mg/mL in saline) was administered (s.c.) at a dose of 10 mg/kg for 14 days (*n*=10) [6]. As a control, vehicle solution (saline, 10 mL/kg) was similarly administered (*n*=8). At 24 h after the last administration, the mice were decapitated without anesthesia, and the brains were removed and separated into the required parts: frontal cortex, nucleus accumbens, striatum, thalamus, hypothalamus, amygdala, hippocampus, and cerebellum according to the mouse brain atlas. The isolated brain regions were immediately homogenized using an Astrason Ultrasonic Processor XL (Misonix Inc., Farmingdale, NY, USA) with ten volumes of phosphate-buffered saline under ice-cold conditions and then centrifuged at 3,000×*g* for 10 min at 4 °C. The resulting supernatant was stored at -80 °C until use.

### Determination of L-Trp

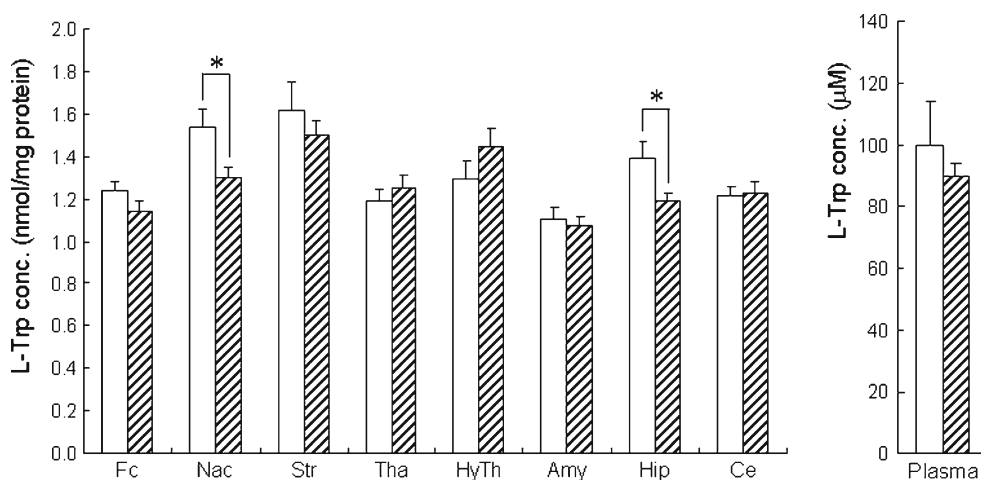
The L-Trp concentration in each brain part was determined by HPLC with fluorescence detection as previously reported [7] with minor modifications. Briefly, 10  $\mu$ L of the supernatant of each homogenate was mixed vigorously with 10  $\mu$ L of CH<sub>3</sub>CN/H<sub>2</sub>O (50/50), 10  $\mu$ L of a 50  $\mu$ M solution of the internal standard (IS), 8-aminocaprylic acid, in CH<sub>3</sub>CN/H<sub>2</sub>O (50/50), and 120  $\mu$ L of CH<sub>3</sub>CN. The mixed solution was centrifuged at 1,300×*g* for 20 min at 4 °C. Next, 10  $\mu$ L of the obtained supernatant was sampled and added to 10  $\mu$ L of a 10 mM solution of (*R*)-(-)-DBD-Py-NCS in CH<sub>3</sub>CN and 10  $\mu$ L of a 30 mM solution of DMAP in CH<sub>3</sub>CN. The resulting solution was heated at a temperature of 55 °C for 20 min, after which 20  $\mu$ L of 39 % CH<sub>3</sub>CN-H<sub>2</sub>O containing 1.1 % AcOH was added to dilute the reacted solution. Thereafter, 5.0  $\mu$ L of the final solution was injected into the HPLC apparatus, which comprised of a Shimadzu LC20AD pump, a Shimadzu CTO-10Avp column oven, a Shimadzu RF-20AXL fluorescence detector (Shimadzu Corporation, Kyoto, Japan), and PC software, CDS ver. 5 (LASoft Ltd., Chiba, Japan). The separation column, Inertsil ODS-3 (250×2.0 mm; i.d., 3  $\mu$ m) (GL Sciences Co., Ltd., Tokyo, Japan), was maintained at 40 °C and the mobile phase, 39 % CH<sub>3</sub>CN-H<sub>2</sub>O containing 1.1 % AcOH, was constantly pumped at 0.2 mL/min. For fluorescence detection, the excitation and emission wavelengths were set at 440 and 565 nm, respectively. Protein concentration of each homogenate sample was



**Fig. 1** Chromatograms of blank sample [(a) and (b)], standard L-Trp [(c) and (d)], and mouse brain (cerebellum) sample [(e) and (f)] using (R)-DBD-PyNCS and (S)-DBD-PyNCS as a pre-column derivatization reagent, respectively

determined by using the Bradford method. With regard to mouse plasma, the pretreatment procedure was the

same as that described previously [7]. The concentrations were expressed as nanomoles per milligram



**Fig. 2** Concentrations of L-tryptophan (in nanomoles per milligram protein or micromolar) in eight different parts of the brain and in the plasma of control (open column, n=8) and PCP-treated mice (shaded column, n=10). Fc frontal cortex, Nac nucleus accumbens, Str striatum,

Tha thalamus, HyTh hypothalamus, Amy amygdala, Hip hippocampus, Ce cerebellum. Data are expressed as mean±S.E. \*p<0.05 versus control group

protein or nanomoles per liter for brain or plasma sample, respectively.

The procedures for the quantification of L-Trp were performed by analytical researchers (H.I., S.W., M.K., and T.F.), who were blinded to the respective groups, i.e., control and PCP-treated mice.

### Statistical analysis

All quantification data are expressed as mean±S.E. Significant differences between two groups were analyzed by one-way analysis of variance followed by Bonferroni's multiple comparison test. A *p* value below 0.05 was judged as a significant difference.

## Results and discussion

Previously, we reported the determination of the plasma concentrations of D- and L-Trp in rats administered intraperitoneally with D- or L-Trp by HPLC with fluorescence detection using (*R*)-DBD-PyNCS as derivatization reagent [7]. In the present study, the previous HPLC method was applied to determine endogenous L-Trp in the brain of mice administered repeatedly with PCP, which has been used for the production of animal models of schizophrenia [1], because L-Trp is a crucial precursor of neuroactive compounds, e.g., QA, KYNA, and serotonin.

Figure 1 shows representative chromatograms using the proposed HPLC method. Endogenous L-Trp was detected in the mouse brain (cerebellum) sample (Fig. 1(c), (e)) using (*R*)-(-)-DBD-PyNCS as the derivatization reagent. When the enantiomer of the reagent, (*S*)-(+)-DBD-PyNCS, is used, the peak of L-Trp can be detected with different retention time (Fig. 1(c), (d)). Consequently, the endogenous L-Trp peak was also detected in the case of using (*S*)-(+)-DBD-PyNCS (Fig. 1(d), (f)), and the obtained peak area of L-Trp was similar between (*R*)-(-)- and (*S*)-(+)-DBD-PyNCS. However, unknown peak originated from mouse brain sample overlapped the IS peak, when (*S*)-(+)-DBD-PyNCS was used. Therefore, (*R*)-(-)-DBD-PyNCS was chosen in the present study. The mass spectrum of the L-Trp peak in the brain sample revealed an ion peak of *m/z* 556.0286 that corresponded to the pseudomolecular ion [(M-H)<sup>-</sup>], *m/z* 556.1437, of L-Trp tagged with DBD-PyNCS (C<sub>24</sub>H<sub>27</sub>N<sub>7</sub>O<sub>5</sub>S<sub>2</sub>) by using our LC-MS method [8]. Thus, L-Trp can be detected in mouse brain samples by using the proposed HPLC with fluorescence detection method. The validation data were satisfactory, i.e., the obtained working curve for L-Trp, in which 2.5, 5.0, 10, 15, and 20 μM were added to the sample, was linear (*r*<sup>2</sup>=0.999, *n*=3), and the chromatogram of brain (cerebellum) homogenate spiked with L-Trp (20 μM) using (*R*)-DBD-PyNCS is shown in Fig. S1. Relative standard deviation (in percent) and

recovery (in percent) values for L-Trp were 4.86 and 93.4 %, respectively.

The eight brain regions, namely, frontal cortex, nucleus accumbens, striatum, thalamus, hypothalamus, amygdala, hippocampus, and cerebellum, of both control and PCP-treated mice, were dissected and the L-Trp concentration was determined in each sample. Figure 2 shows the L-Trp concentration (in nanomoles per milligram protein) in the brain regions of control and PCP-treated mice. As shown in Fig. 2, significant decreases in the L-Trp concentration were observed in the nucleus accumbens (*p*=0.024) and hippocampus (*p*=0.027) of PCP-treated mice. In the frontal cortex, striatum, and plasma, the L-Trp concentration was also lower in PCP-treated mice; however, the differences did not reach statistical significance. Therefore, it was found that chronic PCP treatment caused a decrease in the L-Trp concentration in distinct brain regions, i.e., nucleus accumbens and hippocampus. It is likely that the concentrations of L-Trp metabolites, QA, KYNA, and serotonin may also be altered, possibly resulting in the dysregulation of neurotransmission in these brain regions. On the basis of the present data, inhibition of L-Trp degradation or supply of L-Trp to mouse brain could affect the PCP-induced behavioral changes as well as the concentrations of neurotransmitters.

In conclusion, L-Trp concentrations in eight different parts of mouse brain, namely, the frontal cortex, nucleus accumbens, striatum, hippocampus, amygdala, thalamus, hypothalamus, and cerebellum were determined by the proposed HPLC with fluorescence detection method using (*R*)-DBD-PyNCS as pre-column derivatization reagent. To our knowledge, this is the first report showing decreased L-Trp concentrations in the brain tissue of PCP-treated mice. The pathophysiological meaning of the significant decrease in the L-Trp concentration in the nucleus accumbens and hippocampus following PCP treatment should be elucidated. Furthermore, determination of the concentration of neuroactive compounds, e.g., QA, KYNA, and serotonin, is the subject of future studies.

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