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Pharmacological assessment of methamphetamine-induced behavioral hyperactivity mediated by dopaminergic transmission in planarian *Dugesia japonica*

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ABSTRACT

The freshwater planarian *Dugesia japonica* has a simple central nervous system (CNS) and can regenerate complete organs, even a functional brain. Recent studies demonstrated that there is a great variety of neuronal-related genes, specifically expressed in several domains of the planarian brain. We identified a planarian *dat* gene, named it *D. japonica dopamine transporter* (*Djdat*), and analyzed its expression and function. Both *in situ* hybridization and immunofluorescence revealed that localization of *Djdat* mRNA and protein was the same as that of *D. japonica* tyrosine hydroxylase (DjTH). Although, dopamine (DA) content in *Djdat(RNAi)* planarians was not altered, *Djdat(RNAi)* planarians showed increased spontaneous locomotion. The hyperactivity in the *Djdat(RNAi)* planarians was significantly suppressed by SCH23390 or sulpiride pretreatment, which are D_1 or D_2 receptor antagonists, respectively. These results suggest that planarians have a *Djdat* ortholog and the ability to regulate dopaminergic neurotransmission and association with spontaneous locomotion.

1. Introduction

Dopamine transporter (DAT) is a presynaptic protein, which plays an important role in regulating extracellular dopamine (DA) concentration by reuptaking into presynaptic terminals after release [1–3]. DAT is also a target for psychoactive drugs, such as methamphetamine and cocaine [4,5]. Because *DAT*-knockout mice show hyperactivity, they have been used as a disease animal model of attention-deficit/hyperactivity disorder (ADHD), which is characterized by attention deficit, inappropriate hyperactivity, and impulsivity [6]. Many investigations have used a rodent model, but many issues remain unclear. Thus, planarians provide unique opportunities to investigate such issues, considering they have relatively simple nervous systems.

The freshwater planarian *Dugesia japonica* has a simple central nervous system (CNS) consisting of a brain and a pair of ventral nerve cords (VNCs) (Fig. 1D and E). After artificial amputation, planarians can regenerate into complete animals, including a functional brain [7–9]. This high regenerative capacity is maintained by pluripotent stem cells that are present in the mesenchymal space throughout the planarian body [10–13]. In our laboratory, some neurotransmitters such as DA, serotonin, γ-aminobutyric acid (GABA), octopamine, and acetylcholine, and genes coding these synthesizing enzymes were identified in planarians [14–18]. Thus, it is believed that planarians can regenerate a functional brain from adult pluripotent stem cells [19–21]. In this study, we isolated a planarian *dat* gene, named it *D. japonica dat* (*Djdat*), and analyzed its function by an RNA interference (RNAi) method and pharmacological approaches.

2. Materials and methods

2.1. Animals

In this study, planarians (*D. japonica*) of the SSP strain were used. They were maintained in autoclaved tap water at 24 °C, and fed chicken liver twice a week.

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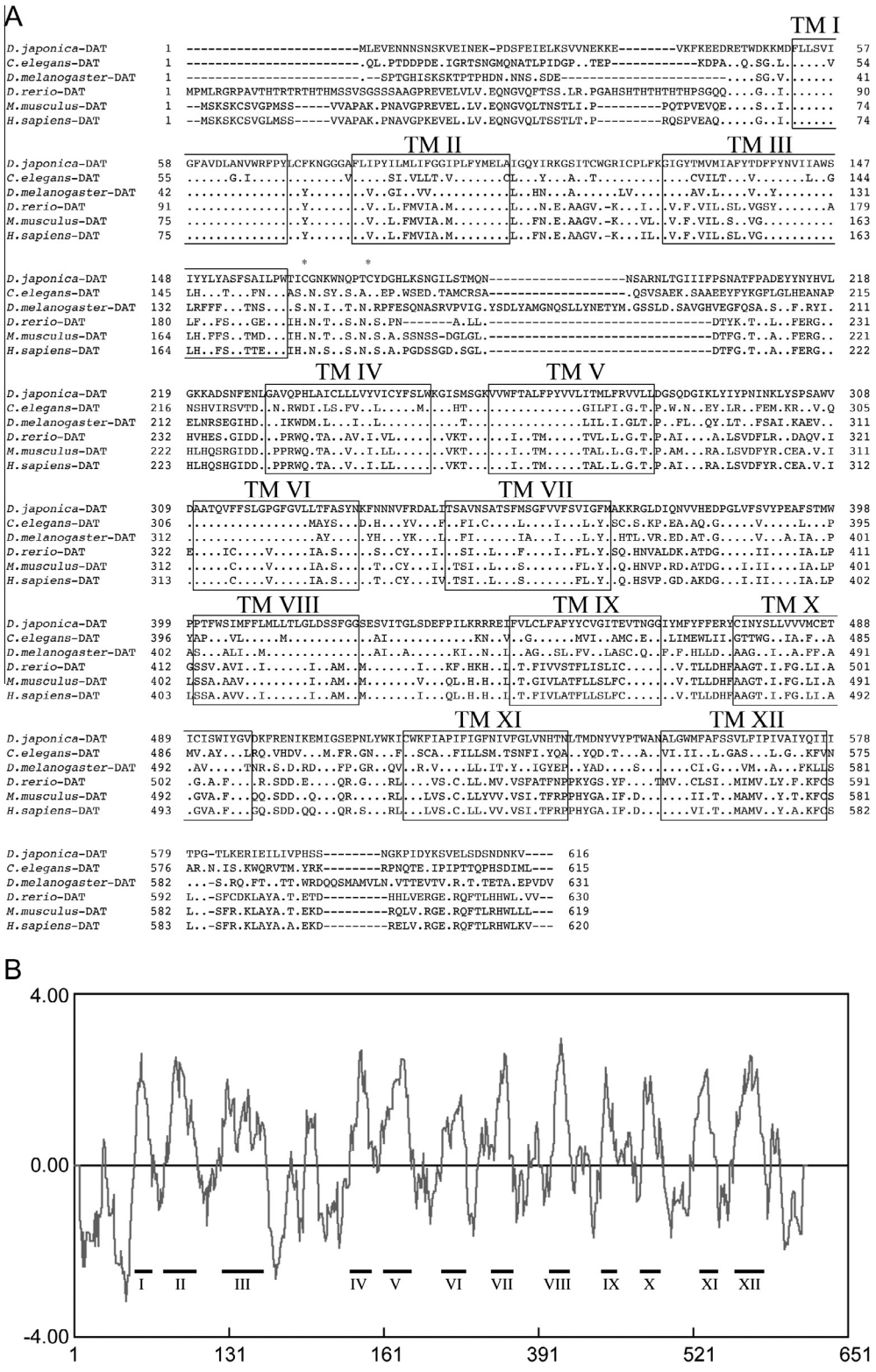

Fig. 1. Sequence analysis of DjDAT. (A) Comparison of amino acid sequences of DjDAT with DAT in other species, corresponding to DAT of *Caenorhabditis elegans* (Q03614), *Drosophila melanogaster* (AAF76882), *Danio rerio* (NP_571830), *Mus musculus* (CAB51926), and *Homo sapiens* (AAC50179). Twelve transmembrane domains are numbered (TM I–XII). Asterisks indicate the 164th and 173th Cys residue. (B) Hydrophobicity analysis of DjDAT. Hydropathy calculations were conducted according to the method by Kyte and Doolittle using GENETYX-MAC Ver.12.2.0. The 12 transmembrane domains are underlined and numbered with Roman numerals I–XII.

2.2. Cloning of Djdat cDNA

Total RNA was extracted from 100 planarians using Isongen-LS (Nippon Gene, Toyama, Japan). mRNA was purified from the total RNA using an Oligotex-dT30 <Super> mRNA purification kit (Takara, Kyoto, Japan). cDNA was synthesized from mRNA using a first-strand cDNA synthesis kit (Amersham Biosciences, Arlington Heights, IL, USA). Degenerate oligonucleotides were designed for

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