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# Dopamine D3 receptor blockade rescues hyper-dopamine activity-induced deficit in novel object recognition memory

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ABSTRACT

Patients afflicted with bipolar disorder demonstrate significant impairments in recognition and episodic memory during acute depressive and manic episodes. These impairments and the related pathophysiology may result from over-activation of the brain dopamine (DA) system. In order to model overactive DA transmission in a well-established novel object recognition (NOR) memory test, we used DA transporter knockdown (DAT-KD) mice, which exhibit reduced DAT expression and display hyperdopaminergic phenotypes. DAT-KD mice exhibited impaired NOR memory compared to wild-type (WT) mice. This impairment was prevented by administration of FAUC365, a DA $D_3$ receptor ($D_3R$) selective antagonist, prior to object learning. Similarly, $D_3R$ knockout (KO)/DAT-KD double mutant mice displayed performance in the NOR test that was comparable to WT mice, suggesting that deficiencies in NOR performance in DAT-KD mice can be compensated by diminishing $D_3R$ signaling. GBR12909, a DAT blocker, also impaired NOR performance in WT mice, but not in $D_3R$ KO mice. Impaired NOR performance in GBR12909-treated WT mice was also prevented by pretreatment with FAUC365. Together, these findings indicate that reduced DAT activity can impair recognition memory in the NOR test, and $D_3R$ appears to be necessary to mediate this effect.



## 1. Introduction

Bipolar disorder (BD) manifests as recurrent alternation of depressive and manic episodes, and associated mania recurrence-induced cognitive and behavioral abnormalities (López-Jaramillo et al., 2010; Martínez-Arán et al., 2004; Robinson et al., 2006). One such abnormality that is frequently associated with manic episodes, is impaired recognition or episodic memory (Martínez-Arán et al., 2000; Quraishi and Frangou, 2002). Excessive synaptic dopamine (DA) availability and the over-activation of brain DA systems may be involved in the pathophysiology of mania-associated recognition and/or episodic memory deficits (Cousins et al., 2009; Swerdlow and Koob, 1987). Since DA transporter (DAT) modulates the level of synaptic DA by mediating DA reuptake, reduced DAT function will result in the accumulation of excess synaptic DA and may drive the emergence of BD symptoms (Horschitz et al., 2005; Pinsonneault et al., 2011). DAT knockout (KO) mice and mice expressing only one copy of the DAT gene exhibit high extracellular DA levels and impaired spatial recognition memory (França et al., 2016; Li et al., 2010; Spielewoy et al., 2000). In another model of DAT deficiency, DAT-knockdown (DAT-KD) mice have decreased expression of DAT (approximately 10% of WT mice) that is associated with a 70% increase in striatal extracellular DA levels (Zhuang et al., 2001). These DAT-KD mice also display aberrant regulation of cortical glutamatergic afferents, which influences the quality of spatial recognition memory upon DA release in the striatum (Wu et al., 2007). Furthermore, DAT-KD mice or mice treated with a selective DAT inhibitor, GBR12909, exhibit mania-like behaviors, including hyperactivity, increased exploration and risk-taking bias in a gambling-like task (van Enkhuizen et al., 2014; Young et al., 2011). These findings all support the relationship between the excess synaptic DA and the pathophysiology of BD symptoms, indicating that DAT-KO or DAT-KD mice may serve as an appropriate animal model to reflect the pathophysiology of BD symptoms.

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Intracellular signaling from synaptic DA transmission is mainly mediated by two subtypes of G-protein-coupled receptors, $D_1$-like ($D_1R$ and $D_5R$) and $D_2$-like ($D_2R$, $D_3R$, and $D_4R$) receptors. Among the receptors in these subtypes, $D_3R$ exhibits the highest binding affinity towards DA, as measured by *Km* (Sokoloff et al., 1992). Functionally, $D_3R$ is known to play an important role in regulating cortical DA neurotransmission related to cognition (Gross and Drescher, 2012; Gross et al., 2012; Nakajima et al., 2013). Schizophrenic patients with a homozygous single nucleotide polymorphism in $D_3R$ (the Gly/Gly genotype) may display relatively poor performance in working memory and executive function, when compared to patients that carry the Ser/Ser genotype of $D_3R$ (Bombin et al., 2008; Szekeres et al., 2004). This difference is possibly due to the fact that Gly-containing $D_3R$ has higher DA binding affinity compared to Ser-containing $D_3R$ (Bombin et al., 2008; Szekeres et al., 2004). ABT-925, a $D_3R$ antagonist, improves executive function and emotion recognition in schizophrenia patients that carry the Gly/Gly genotype of $D_3R$ (Bhathena et al., 2013). Moreover, mice treated with the $D_3R$ agonists, PD128907 or 7-OH-DPAT, exhibit cognitive impairment (Ukai et al., 1997; Watson et al., 2012a). The implications of these findings are two-fold: 1) $D_3R$-related phenotypes may affect executive function; 2) pharmacological stimulation of $D_3R$ may cause cognitive dysfunction, which may potentially be rescued by reducing the binding of $D_3R$ with its natural ligand, DA.

The present study was designed to test whether excessive extracellular DA availability and subsequent $D_3R$ activation may cause impairment in recognition memory. DAT-KD mice and mice treated with a DAT-blocking agent were both shown to mimic hyperdopaminergia. These mice were then used to examine the effect of excess extracellular DA and hyperdopaminergia on recognition memory. The novel object recognition (NOR) task, which requires a visuospatial parameter but belongs to the group of non-maze-based learning and memory tests (Young et al., 2009), was used to quantify recognition memory during hyperdopaminergia. DAT-KD mice pretreated with FAUC365, a selective $D_3R$ antagonist, or vehicle were used to further assess the role of $D_3R$ activation in mediating performance in the NOR task. $D_3R$ knockout ($D_3KO$) mice and their WT littermates were treated with GBR12909, a DAT inhibitor, prior to NOR testing. $D_3KO$/DAT-KD double mutant mice ($D_3$/DAT) were also used to assess the effects of $D_3R$ on DAT deficiency-induced hyperdopaminergia. Overall, we found that recognition memory deficits are present in the DAT-deficient mouse models, and $D_3R$ is required for the appearance of such memory deficits.

## 2. Materials and methods

### 2.1. Animals

$D_3KO$ mice were purchased from the Jackson Laboratory (B6.129S4-Drd3$^{tm1Dac}$/J). DAT-KD mice were obtained from the University of Chicago (Dr. Xiaoxi Zhuang). Both strains were backcrossed onto a C57BL/6 background for >10 generations. $D_3$/DAT double mutant mice were cross-bred from $D_3$-KO and DAT-KD single mutant mice to produce compound heterozygotes ($D_3$-HT/DAT-HT). The compound heterozygotes were then crossed with single mutants ($D_3KO$ or DAT-KD), and the progeny were crossed to produce $D_3$/DAT double mutant mice. $D_3$/DAT double mutant mice used in the experiments were maintained as homozygotes. To produce sufficient numbers of DAT-KD mice for experiments, homozygotes were bred and used in many experiments. Wild-type (WT) mice were either generated as littermates of $D_3$-KO or DAT-KD mice, or age-matched wild-type C57BL/6 were purchased from National Laboratory Animal Center, Taiwan. All experiments were carried out using male mice, weighing 21–30 g (approximately 3 months of age) at the time of testing. Mice were group housed under a 12-h light/12-h dark cycle (light on at 0700, light off at 1900) and at constant temperature (25 °C) and humidity (70%) in the Chang-Gung Animal Core Facility. Mice were allowed free access to food and water throughout the experiments. All the experimental procedures were performed during the light cycle. All procedures were approved by the Animal Care and Use Committee at Chang-Gung University.

### 2.2. Drugs

FAUC365 was purchased from Glixx Laboratories (Hopkinton, MA, USA). GBR12909 dihydrochloride and L-741,626 were purchased from Sigma Aldrich (St. Louis, MO, USA). FAUC365 (1–10 mg/kg) and L-741,626 (0.3–3 mg/kg) were dissolved in $dH_2O$ with 5% DMSO (J. T. Baker/Avantor Performance Materials, PA, USA) and 0.3% Tween 80 (Nacalai Tesque, Kyoto, Japan). These solutions were injected (5 ml/kg, s.c.) 20 min before the training trial. GBR12909 was dissolved in saline after heating up to 40 °C for 60 min. GBR12909 was injected (10 ml/kg, i.p.) 10 min before the familiarization or test, or immediately after the familiarization trial.

### 2.3. Y-maze test

To test the effect of DAT deficiency on spatial working memory performance, DAT-KD mice and WT littermates were used in the Y-maze test (N = 10 per group). Custom-made Y mazes consisted of three symmetrical and identical arms (15 × 5 × 40 cm). Experimental conditions and procedures were described in detail in a previous report (Cherng et al., 2007). Briefly, mice were placed in the end of a randomly-assigned arm and allowed free navigation in the maze for 5 min. The sequence and number of arm entries were recorded using a video camera throughout the testing period. The sequence triads, in which all three arms were explored (including ABC, ACB, BAC, BCA, CAB, and CBA), were considered as successful alternations, with normal perception and short-term memory. The percentage of successful alterations was calculated as the number of successful alterations divided by the number of possible alternations (the total number of arm entries - 2). The total number of arm entries served as an indicator of locomotor activity.

### 2.4. Morris water maze test (MWM)

To test the effect of DAT deficiency on spatial memory, DAT-KD mice and WT littermates were used in the Morris water maze test (N = 10 per group). Spatial learning memory was assessed by mouse performance in the Morris water maze test. The custom-made maze was a circular pool 120 cm in diameter and 40 cm in height, filled with 25 ± 1 °C water, and made opaque with milk. The location of the maze in the laboratory and the laboratory environment were described in a previous study with minor modifications (Ke et al., 2008). In the acquisition session, each mouse was given four trials per day and four days of training in total to find a hidden platform located 1.5 cm below the water surface. Each mouse was placed the pool, facing the wall, with a different starting point for each trial, so that the direct route to the platform differed each time. The time required by the mouse to find and stand on the platform, was recorded for up to 120 s. The mouse was allowed to remain on the platform for 30 s, and was then removed from the maze and placed its home cage. If mice did not find the platform within 120 s, the animal was placed on the platform for 30 s. The inter-trial interval was at least 30 min. In the probe session, on day 5, the platform was removed from the pool, and mice were tested in a probe trial for 60 s. Mouse swimming tracts were recorded by