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The effects of reduced dopamine transporter function and chronic lithium on motivation, probabilistic learning, and neurochemistry in mice: Modeling bipolar mania

Morgane Milienne-Petiot [a, b], James P. Kesby [a], Mary Graves [a], Jordy van Enkhuizen [a, b], Svetlana Semenova [a], Arpi Minassian [a], Athina Markou [a], Mark A. Geyer [a, c], Jared W. Young [a, c, *]

[a] Department of Psychiatry, School of Medicine, University of California San Diego, 9500 Gilman Drive MC 0804, La Jolla, CA 92093-0804, USA
[b] Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands
[c] Research Service, VA San Diego Healthcare System, San Diego, CA, USA

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ABSTRACT

Background: Bipolar disorder (BD) mania patients exhibit poor cognition and reward-seeking/hypermotivation, negatively impacting a patient's quality of life. Current treatments (e.g., lithium), do not treat such deficits. Treatment development has been limited due to a poor understanding of the neural mechanisms underlying these behaviors. Here, we investigated putative mechanisms underlying cognition and reward-seeking/motivational changes relevant to BD mania patients using two validated mouse models and neurochemical analyses.

Methods: The effects of reducing dopamine transporter (DAT) functioning via genetic (knockdown vs. wild-type littermates), or pharmacological (GBR12909- vs. vehicle-treated C57BL/6J mice) means were assessed in the probabilistic reversal learning task (PRLT), and progressive ratio breakpoint (PRB) test, during either water or chronic lithium treatment. These tasks quantify reward learning and effortful motivation, respectively. Neurochemistry was performed on brain samples of DAT mutants ± chronic lithium using high performance liquid chromatography.

Results: Reduced DAT functioning increased reversals in the PRLT, an effect partially attenuated by chronic lithium. Chronic lithium alone slowed PRLT acquisition. Reduced DAT functioning increased motivation (PRB), an effect attenuated by lithium in GBR12909-treated mice. Neurochemical analyses revealed that DAT knockdown mice exhibited elevated homovanillic acid levels, but that lithium had no effect on these elevated levels.

Conclusions: Reducing DAT functioning recreates many aspects of BD mania including hypermotivation and improved reversal learning (switching), as well as elevated homovanillic acid levels. Chronic lithium only exerted main effects, impairing learning and elevating norepinephrine and serotonin levels of mice, not specifically treating the underlying mechanisms identified in these models.

1. Introduction

Bipolar disorder (BD) is a debilitating life-long illness, affecting approximately 2% of the global population (Merikangas et al., 2011). Mania is a cardinal feature of BD and is characterized by complex and multifaceted symptoms. Symptoms of BD mania include heightened risk-taking, impaired decision-making, and increased hedonistic (reward-directed) behavior (DSM-V, 2013). Patients with BD exhibit impaired cognitive functioning including impaired decision making in the Iowa Gambling Task (IGT) (Christodoulou et al., 2006; Jollant et al., 2007; van Enkhuizen et al., 2014b), a probabilistic reversal learning task (PRLT) that also includes reward and punishments. There is also evidence of impaired simplistic probabilistic reversal learning in youths at risk of BD (Dickstein et al., 2010), in addition to other learning deficits (Duek et al., 2014; Pizzagalli et al., 2008). Increased reward-directed behaviors have not been as readily quantified in patients but are commonly

* Corresponding author. Department of Psychiatry, University of California San Diego, 9500 Gilman Drive MC 0804, La Jolla, CA, 92093-0804, USA.
E-mail address: jaredyoung@ucsd.edu (J.W. Young).

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measured in rodents using a progressive ratio breakpoint (PRB) schedule of reinforcement (Young and Markou, 2015). These dysfunctional behaviors negatively affect a patient's quality of life and no medications have been approved for their treatment. Currently approved BD treatments are ineffective at treating such cognitive deficits (Burdick et al., 2012; Joffe et al., 1988; Mora et al., 2013). In fact, one of the most commonly approved treatments, the mood stabilizer lithium, adversely affects certain aspects of cognition including learning and memory in healthy subjects (Stip et al., 2000). Identifying treatments that can improve, or at least not impair, cognition in patients is, therefore, an important target for therapeutic development. These targets would more readily be identified with a better understanding of underlying mechanisms and the effects of current treatments on such abnormal behavior.

Some neurobiological mechanisms for BD have been proposed. For example, altered dopaminergic homeostasis may contribute to mania symptoms (van Enkhuizen et al., 2014a). The dopamine transporter (DAT) regulates synaptic dopamine levels and polymorphisms in this gene have been associated with BD (Greenwood et al., 2006; Pinsonneault et al., 2011). This polymorphism may result in reduced DAT levels (Horschitz et al., 2005), as is observed in positron emission tomography imaging of unmedicated BD patients (Anand et al., 2011). Reduced DAT levels slow the clearance of synaptic dopamine, allowing for greater metabolism of dopamine to HVA by monoamine oxidase (MOA) and catechol-O-methyl transferase (COMT) (Best et al., 2009). This mechanism likely drives the elevated homovanillic acid (HVA) levels seen in the cerebrospinal fluid (CSF) of mania patients (Gerner et al., 1984). To-date, it is unclear what effect chronic lithium has on neurotransmitter levels of organisms with reduced DAT function exhibiting mania-like behaviors. Although, in studies of rats with normal levels of DAT expression, lithium did not affect basal dopamine levels, but impaired dopamine release (Ferrie et al., 2005, 2006).

In support of a possible mechanistic contribution of DAT to mania-relevant behaviors, mice with reduced DAT functioning exhibit abnormal exploratory profiles consistent with BD mania patients (Perry et al., 2009). Specifically, mice treated with the selective DAT inhibitor GBR12909 (GBR) or with a genetic knockdown (KD) of DAT exhibited hyperactivity, increased specific exploration, and straighter movement through space consistent with BD mania patients in the cross-species behavioral pattern monitor (BPM) (Perry et al., 2009; Young et al., 2010a,b). Chronic administration of the standard BD treatments valproate and lithium, at clinically therapeutic levels, to DAT KD and GBR-treated mice attenuated the hyperactivity of mice, without affecting their specific exploration, or straight-line movement (Queiroz et al., 2015; van Enkhuizen et al., 2013a; van Enkhuizen et al., 2015a,b). These effects were similar to treatment-induced reduction of activity of mania patients (Minassian et al., 2011). Furthermore, GBR treatment or genetic reduction of DAT increased risk taking (van Enkhuizen et al., 2013b; van Enkhuizen et al., 2014b), motivation (Young and Geyer, 2010), and motor impulsivity-like behavior (Loos et al., 2010) similar to aberrations observed in BD patients (van Enkhuizen et al., 2014b). Apart from the reduced DAT in BD with links to similar behavioral abnormalities, the construct validity of this model has rarely been assessed. Hence, testing the behavior, treatment-reactivity, and neurochemistry of mice with reduced DAT function could provide predictive, and construct validity for this model of altered cognition and behavior that is relevant to mania. This assessment could then lead to the development of targeted therapeutics.

Here, we assessed the effect of the pharmacological (GBR) and genetic (KD) DAT inhibition on probabilistic reversal learning and effortful motivation in the PRLT and PRB test, respectively. We also determined the effects of chronic high doses of lithium on these behaviors. Additionally, we assessed the neurochemical profile of these mice treated with water or chronic lithium. We hypothesized that: (a) Chronic lithium would impair learning in the PRLT irrespective of DAT inhibition; (b) GBR-treated mice or DAT KD would exhibit impaired performance in the PRLT and increased reward-seeking/motivation-like behavior in the PRB; (c) Lithium would synergistically normalize BD-relevant behaviors induced by DAT reduction; and (d) DAT KD mice would exhibit elevated HVA consistent with mania patients (Gerner et al., 1984), an effect remediated by lithium treatment.

2. Methods

2.1. Animals

C57BL/6J male mice (n = 45) were purchased from Jackson Laboratories at 3 months old and male DAT KD mice and their WT littermates (n = 55) aged 13 months and weighing between 22 and 29 g were used in this study. DAT heterozygous breeders backcrossed onto a C57BL/6 background for more than 10 generations were sent to the University of California San Diego (UCSD) from the University of Chicago (Zhuang et al., 2001). All DAT KD and WT mice used in this study resulted from heterozygous breeding pairs performed at UCSD. Mice were group housed (maximum 4/cage) and maintained in a temperature-controlled vivarium (21 ± 1 °C) on a reversed day-night cycle (lights on at 19:00 h, off at 07:00 h) and tested during the dark phase between 08:00 h and 16:00 h. All mice had *ad libitum* access to water and were food-restricted to 85% of their free-feeding weight during periods of training and testing. All of the behavioral testing procedures were approved by the UCSD Institutional Animal Care and Use Committee. The UCSD animal facility meets all federal and state requirements for animal care and was approved by the American Association for Accreditation of Laboratory Animal Care.

2.2. Drug treatment

GBR12909 dihydrochloride (Sigma-Aldrich, St Louis, USA) was dissolved in 0.9% saline vehicle after sonicating for 2–4 h at 40 °C as described previously (Young et al., 2010a). GBR12909 at 16 mg/kg dose based on previous publications (Loos et al., 2010; van Enkhuizen et al., 2013a; van Enkhuizen et al., 2013c; van Enkhuizen et al., 2015a,b) was administered by intraperitoneal injection 10 min prior to testing in a volume of 10 ml/kg. Lithium chloride (Sigma-Aldrich, St Louis, USA) was dissolved into the drinking water at 0.6 or 1.0 g/l and given for 10 days and 7 or 8 days for the DAT KD study PRLT and PRB respectively based on previous studies generating clinical therapeutic levels (Dehpour et al., 1995; Roybal et al., 2007; van Enkhuizen et al., 2015a,b).

2.3. Training and testing

Training and testing took place in 15 five-hole operant chambers (25 × 25 × 25 cm; Med Associates, St. Albans, USA). During the first training phase (Hab1), mice were required to recognize magazine illumination and delivery of 30 μl strawberry milkshake as a reward and collect it every 15 s for 10 min (criterion was 30 collection responses per session for two consecutive days). During the second training phase (Hab2), mice were trained to holepoke into one of two lit holes to obtain the reward (criterion was 70 correct holepokes per session for two consecutive days). Once responding consistently, mice were baseline-matched on total responses prior to treatment and testing in PRLT or PRB.

2.4. Probabilistic reversal learning test (PRLT)

During the 60 min probabilistic learning test, the same two