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Dopamine transporter (DAT) genetic hypofunction in mice produces alterations consistent with ADHD but not schizophrenia or bipolar disorder

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ABSTRACT

ADHD, schizophrenia and bipolar disorder are psychiatric diseases with a strong genetic component which share dopaminergic alterations. Dopamine transporter (DAT) genetics might be potentially implicated in all these disorders. However, in contrast to DAT absence, the effects of DAT hypofunction especially in developmental trajectories have been scarcely addressed. Thus, we comprehensively studied DAT hypofunctional mice (DAT+/-) from adolescence to adulthood to disentangle DAT-dependent alterations in the development of psychiatric-relevant phenotypes. From pre-adolescence onward, DAT+/- displayed a hyperactive phenotype, while responses to external stimuli and sensorimotor gating abilities were unaltered. General cognitive impairments in adolescent DAT+/- were partially ameliorated during adulthood in males but not in females. Despite this, attentional and impulsivity deficits were evident in DAT+/- adult males. At the molecular level, DAT+/- mice showed a reduced expression of *Homer1a* in the prefrontal cortex, while other brain regions as well as *Arc* and *Homer1b* expression were mostly unaffected. Amphetamine treatments reverted DAT+/- hyperactivity and rescued cognitive deficits. Moreover, amphetamine shifted DAT-dependent *Homer1a* altered expression from prefrontal cortex to striatal regions. These behavioral and molecular phenotypes indicate that a genetic-driven DAT hypofunction alters neurodevelopmental trajectories consistent with ADHD, but not with schizophrenia and bipolar disorders.

1. Introduction

Dopamine dysfunction is believed to be significantly implicated in the pathophysiology of several psychiatric disorders, among these being schizophrenia (SZ), attention deficit hyperactivity disorder (ADHD), and bipolar disorder (BD) (Fusar-Poli and Meyer-Lindenberg, 2013; Gowrishankar et al., 2014; Pinsonneault et al., 2011; Weinstein et al., 2016). These are conceptualized also as diseases of aberrant synaptic function, possibly on a neurodevelopmental basis (Gowrishankar et al., 2014). These psychiatric disorders all share a strong genetic component (Faraone et al., 2005; Greenwood et al., 2006; Moran et al., 2014). However, how dopamine-related genetic variations might differently affect neurodevelopment, giving rise to divergent abnormalities consistent with ADHD-, SZ- or BD-related dimensions is still not clear.

Dopamine pathophysiology, especially in subcortical regions, is highly linked to the function of the dopamine transporter (DAT) (Gowrishankar et al., 2014), whose gene variants have been implicated to different degrees in the above disorders (Faraone et al., 2014; Greenwood et al., 2006). Animal models of DAT disruption have mainly focused on mice with a complete absence of DAT. DAT null mutant (-/-) mice exhibit extreme phenotypes such as lack of ability to re-uptake dopamine from the synaptic cleft, growth retardation, anterior pituitary hypoplasia, dwarfism, early life mortality and exorbitant hyperactivity (Gainetdinov et al., 1999; Giros et al., 1996; Jones et al., 1998; Spielewoy et al., 2000). In

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agreement, DAT-/- have been ascribed as a bona-fide model for the DAT deficiency syndrome, also known as early Parkinson's disease (Kurian et al., 2011; Ng et al., 2014). In contrast, more subtle changes in DAT activity could be more suitable for understanding its contribution to phenotypes relevant to disorders such as ADHD, SZ, and BD, as suggested by human studies (Fuke et al., 2001; Mergy et al., 2014).

Partial DAT hypofunctioning has been studied prevalently for locomotor responses and reactivity to psychostimulants such as cocaine and amphetamine, and only in adult mice (Supplementary Table 1). Thus, in order to gain insight into the impact of partial DAT genetic disruption on disorders such as ADHD, SZ and BD, we characterized DAT hypofunctioning mice (DAT+/-) at different ages. In particular, considering the developmental aspect of these disorders, we performed behavioral investigations in adolescent and adult animals to follow the trajectory of dopamine dysfunction in DAT+/- mice. We also compared male and female DAT+/+ and +/- mice with and without exposure to amphetamine. Indeed, amphetamines may ameliorate symptoms in ADHD (Chan et al., 2016), conversely, these same drugs may precipitate or exacerbate psychotic symptoms in both BD (Koehler-Troy et al., 1986) and SZ patients (Toda and Abi-Dargham, 2007). Moreover, all these psychiatric disorders show sex-dependent differences of the correlated behavioral abnormalities (Biederman et al., 2002; Kawa et al., 2005; Sannino et al., 2014). Finally, in line with the hypothesis that dopamine dysregulation in SZ, BP and ADHD has been associated with the common final pathway of an aberrant synaptic function influencing all dopamine–glutamate physiology (Fusar-Poli and Meyer-Lindenberg, 2013; Rao et al., 2012), we investigated in cortical and subcortical brain regions alterations of key transcripts of the postsynaptic density (*Homer1a, Homer1b, and Arc)*. These genes have been demonstrated to be implicated in the pathophysiology and animal modeling of ADHD, SZ and BP (de Bartolomeis et al., 2015; Hong et al., 2011, 2009; Lominac et al., 2005; Managò et al., 2016).

2. Methods and materials

All procedures were approved by the Italian Ministry of Health (permit n.17 BIS/2014) and Animal Use Committee and were conducted in accordance with guidelines for the care and use of laboratory animals of the NIH and the European Community Council Directives. Original DAT-/- mice (Giros et al., 1996) were backcrossed with C57BL6J mice for at least 8 generations. The breeding scheme used to obtain the experimental mice involved mating DAT hypofunctioning (DAT+/-) male mice with C57BL6J (DAT+/+) females. DAT+/+ mice were used as female breeders in order to avoid altered maternal behavior. Only DAT+/+ and +/- littermates were used for all experiments. Mice were genotyped by PCR analysis of tail DNA. Mice were group-housed (two to four per cage) in a climate-controlled animal facility (22 ± 2 °C) and maintained on a 12 h light/dark cycle (7am-7pm) with *ad libitum* access to food and water, unless specified in particular experiments. All experimental tests were conducted in male and female adolescent (PND 28–45) and adult (3–7 months old) mice during the light phase. Mice were handled by the experimenter on alternate days during the week preceding the test. Experimenters were blind to the genotype during testing.

2.1. Acoustic startle response (ASR) and prepulse inhibition (PPI)

Acoustic startle response (ASR) and prepulse inhibition (PPI) were measured using four SR-Lab System (San Diego Instruments) as previously described (Managò et al., 2016; Papaleo et al., 2012, 2008). Startle experiments test sessions began by placing the mouse in the Plexiglas holding cylinder (5 cm diameter) for a 5 min acclimation period. After the acclimation period, each subject received 36 trials over the 9 min test session. There were six different sound levels (in decibels) presented: 70, 75, 80, 85, 90, and 120. Each stimulus was 40 ms and presented four times in pseudorandom order such that each sound level was presented within a block of six trials. The interval between trials was 10–20 s. The ASR was recorded for 65 ms (measuring the response every 1 ms) starting with the onset of the startle stimulus. The maximum startle amplitude recorded during the 65 ms sampling window was used as the dependent variable. The PPI is an attenuation of the startle response when the startle-eliciting stimulus (pulse), is preceded by a weaker sensory stimulus (prepulse). In this test, mice were presented with each of seven trial types across six blocks of trials for a total of 42 trials. Trial types were presented randomly within each block. The interval between trials was 10–20 s. One trial type measured the response to no stimulus (baseline movement), and another presented the startle stimulus alone (startle), which was a 40 ms, 120 dB sound. The other five were acoustic prepulse plus acoustic startle stimulus trials. Prepulse tones were 20 ms at 70, 75, 80, 85, and 90 dB, presented 100 ms before the startle stimulus (120 dB). The maximum startle amplitude was the dependent variable. A background level of 70 dB white noise was maintained over the duration of the test session.

2.2. Locomotor activity (LMA) and sensitization to psychostimulants

The experimental apparatus consisted of four open field arenas (42 × 42 × 30 cm), illuminated by overhead white lighting (25 ± 5 lux). To quantify exploratory and locomotor activities a video tracking system (ANYMAZE©) was used during 1 h of test. Parameters analyzed were total distance travelled (m) and percentage of time in the internal zone. One week after basal assessment, mice were treated with amphetamine HCl (1.5 mg/kg i.p) immediately before the LMA test and the test was repeated for 5 consecutive days.

2.3. Temporal order object recognition (TOR) task

The test was performed as previously described (Huang et al., 2014; Managò et al., 2016). Mice were tested in an experimental apparatus consisting of an opaque open field box (42 × 42 × 30 cm) with even, overhead white lighting (25 ± 5 lux). Each session was video-recorded using an overhead camera from ANY-maze (Stoelting Co.). Each mouse was monitored for its locomotor activity in the empty open field boxes for 1 h. The next day, in the TOR test, the subjects' ability to differentiate between two objects presented at different intervals was assessed. The objects presented were rectangular boxes (3 × 3 × 6 cm), or two laboratory flasks (4 × 6 cm), each either black or white and too heavy for the animals to displace. The objects were placed in two corners of the open field apparatus, 8 cm from the sidewalls. This task comprised of two sample phases and one test trial. In each sample phase, the subjects were allowed to explore two copies of an identical object for a total of 5 min. Different objects were used for sample phases 1 and 2, with a 1-h delay between the two sample phases. The test trial (5-min duration) was performed 3 h after sample phase 2. During the test trial, a third copy of the objects from both sample phase 1 and sample phase 2 were used. Time spent exploring each object was subsequently scored from the ANY-maze videos as the number of seconds when each subject was facing the object and ≤1 cm away. If temporal order memory is intact, subjects will spend more time exploring the object from sample 1 (i.e., the object presented less recently) compared with the object from sample 2 (i.e., the object