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Attentional deficits and altered neuronal activation in medial prefrontal and posterior parietal cortices in mice with reduced dopamine transporter levels

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ABSTRACT

The executive control function of attention is regulated by the dopaminergic (DA) system. Dopamine transporter (DAT) likely plays a role in controlling the influence of DA on cognitive processes. We examined the effects of DAT depletion on cognitive processes related to attention. Mice with the DAT gene genetically deleted (DAT +/− heterozygotes) were compared to wild type (WT) mice on the Attentional Set-Shifting Task (ASST). Changes in neuronal activity during the ASST were shown with early growth response genes 1 and 2 (egr-1 and egr-2) immunohistochemistry in the medial prefrontal cortex (mPFC) and in the posterior parietal cortex (PPC). Heterozygotes were impaired in tasks that tax reversal learning, attentional-set formation and set-shifting. Densities of egr-2 labeled cells in the mPFC were lower in mutant mice when compared with wild-types in intradimensional shift of attention (IDS), extradimensional shift of attention and extradimensional shift of attention-reversal phases of the ASST task, and in PPC in the IDS phase of the task. The results demonstrate impairments of the areas associated with attentional functions in DAT +/− mice and show that an imbalance of the dopaminergic system has an impact on the complex attention-related executive functions.

1. Introduction

Attentional processes facilitate cognitive and behavioral performance in several ways, such as by maintaining alertness and vigilance, as well as by orienting towards salient sensory input and then selecting and integrating it, and finally through executive control of undertaken actions (Cohen, 2013). The brain dopamine (DA) system, which targets the prefrontal cortical and anterior cingulate areas, is implicated in attentional processes that require executive control, such as attentional set-shifting (Robbins and Roberts, 2007; Ranganath and Jacob, 2016). It is believed that the dopamine transporter (DAT) provides the most important regulatory control of temporal and spatial activity of released dopamine *via* removal of DA from extracellular space (Vaughan and Foster, 2013). Given that the DA system plays an important role in cognition, the DAT also likely plays a role in controlling the influence of DA on these processes.

Changes in dopaminergic control of frontocorticostriatal circuits are tightly associated with attention deficit hyperactivity disorder (ADHD) symptoms (Biederman and Faraone, 2005; Arnsten, 2006) and altered levels of DAT availability in the striatum have been found across studies of patients with ADHD (Krause, 2008). An association between ADHD and polymorphisms in the human DAT gene (*DAT1*) has been reported (Cook et al., 1995; Gill et al., 1997; Waldman et al., 1998), and *DAT1* was postulated to be the most useful biomarker for ADHD diagnosis (Faraone et al., 2014). It has been suggested that all symptoms of ADHD can be attributed to a primary deficit in cognition, particularly impairment of executive functions (Lawrence et al., 2004). One of the measures of human fronto-executive function is the attentional set-shifting task and indeed, deficits of attentional set-shifting have been reported in ADHD patients (Martinussen et al., 2005; Chamberlain et al., 2010). The search for the underlying neurochemical hallmarks associated with ADHD symptoms has largely relied on the development of animal models, one of which is the DAT-knockout (DAT-KO) mouse (Giros et al., 1996; van der Kooij and Glennon, 2007). DAT-gene knockout leads to extremely high levels of DA synthesis and turnover with a persistent increase in extracellular DA levels in the striatum and nucleus accumbens, accompanied by reduced tissue DA levels (Giros et al., 1996; Jones et al., 1998; Gainetdinov and Caron, 2003). Profound impairments such as growth retardation, impaired gut motility, respiratory control and others (Rodriguiz et al., 2004) have made DAT-KO mice less suitable for studying the role of dopamine in controlling and regulating complex cognitive functions. The heterozygous

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knockout mice (DAT +/− mice), with one copy of the gene and 50% of the DAT protein, exhibit significant yet less pronounced DA depletion in tissue stores (Jones et al., 1998; Gainetdinov et al., 1998) than do homozygotes, and no significant deficits were observed in their basic behaviors and learning (Morice et al., 2007; Li et al., 2010).

In the present study we sought to determine the functional consequences of DAT depletion in DAT heterozygotes on performance in tasks which measure attention-related executive functions. The results of animal research demonstrate the important role of the rodent medial prefrontal cortex (mPFC) in attention, including executive functions (Muir et al., 1996; Birrell and Brown, 2000). The posterior parietal cortex (PPC), which has dense reciprocal connections with frontal cortical regions, shares many of its functional properties (Reep et al., 1994). Bilateral lesions of the mPFC or PPC of rats have been shown to selectively impair their performance on the attention-set-shifting task (ASST), a test that enables the investigation of cognitive processes related to attention as well as the ability of an animal to alter behavior under reversal conditions (Birrell and Brown, 2000; Fox et al., 2003). We tested the performance of WT and DAT +/− mice in the ASST and compared neuronal activation in the infralimbic (IL) and prelimbic (PrL) cortices of the mPFC and PPC, measuring the magnitude of the test-induced expression of egr-1 (Zif-268) and egr-2 (Krox-20). The expression of individual members of the EGR family is differentially induced during different cognitive processes. Expression of the egr-1 depends on neuronal activity and is thought to be involved in neuronal plasticity (Cole et al., 1989), learning and memory (e.g. Hall et al., 2001; Bozon et al., 2003). The magnitude of egr-2 induction in the medial prefrontal cortex (mPFC) correlates with the magnitude of attentional demand/cognitive control (DeSteno and Schmauss, 2008).

2. Materials and methods

2.1. Animals

Female DAT +/− (n = 39), wild-type (n = 40) and DAT-KO (n = 3) littermate mice aged 10–15 weeks old were used in this study. This line of animals has been described previously (Giros et al., 1996). Briefly, homozygous DAT-KO, heterozygous DAT +/−, and WT mice were obtained by homologous recombination as previously described (Giros et al., 1996), and the two B6-DAT and D2-DAT congenic strains were maintained by consistently backcrossing for > 12 generations onto the B6 and D2 inbred strains as previously described (Morice et al., 2007). The cohort of mice was bred from the couples of founders, which were a generous gift obtained from Prof. B. Giros of the CNRS, Paris. The mice were bred in the Animal Facility, Faculty of Biology, University of Warsaw, weaned at 4 weeks and then housed by sex and litter under standard conditions, on a 12 h light/dark cycle in the Animal House, Nencki Institute. The genotypes of the mice were determined by PCR analysis (Carboni et al., 2001) with the use of the primers DAT-1 (CCCGTC TACCCATGAG-TAAAA), DAT-2 (C TCCACC TTCC TAGCAC TAAC), and NEO2 (TGACCGC TTCC TCGTGC).

It was not possible to collect sufficient number of DAT-KO mice to run the experiment, because of their high level of premature death and/or bad physical condition which did not allow for behavioral testing. Three DAT-KO mice were trained in the Làt-maze and in the ASST apart from WT and DAT +/− mice (Supplementary Information - Supplementary Table 1). The results of these DAT-KO mice in the Làt-maze were highly variable; they could not complete the compound discrimination (CD) phase of the ASST experiment, therefore the data are not presented.

All work was conducted in accordance with the European Community Council Directive (86/609/EEC) and was approved by the Animal Care and Use Committee of the Polish Academy of Sciences.

2.2. Làt-maze

Mice were tested for horizontal and vertical activity (Aspide et al., 1998; Aspide et al., 2000). The experimental apparatus was a Làt-maze: a squared wooden box (interior dimensions: 30 cm × 30 cm × 21 cm) with a smaller (16 cm × 16 cm × 21 cm) plastic transparent box in the middle (slightly modified in comparison with the one described in Ruocco et al., 2008). Mice were allowed to explore the resulting corridor (30 cm long, 7 cm wide and 21 cm high) during a 10-min session. At the end of the test the floor was cleaned with 50% alcohol. The number of corner crossings (horizontal activity), being a measure of locomotor activity, and also the number and duration of rearings on hindlimbs (vertical activity), were monitored by a camera and analyzed off-line according to methods adapted from Aspide et al. (1998, 2000). Additionally, the number of fecal boluses was counted as a simple emotionality index (Broadhurst, 1960).

2.3. Attentional set-shifting task

The ASST (Birrell and Brown, 2000) is the rodent version of the Wisconsin Card Sorting Task (Berg, 1948; Monchi et al., 2001) or, more precisely, rather the version of the intradimensional-shift/extradimensional-shift task (ID/ED task in the Cambridge Neuropsychological Automated Testing Battery; Sahakian and Owen, 1992), that tax executive functions (Tait et al., 2014). The ASST is a measure of attention and cognitive flexibility in animals. In that task animals learn to discriminate between two perceptual dimensions: odor and texture.

2.3.1. Apparatus

The test apparatus was a wooden cage (30 cm × 38 cm × 21 cm) with Plexiglas panels used to divide half of the length of the cage into two “choice” sections. A removable divider separated the “start” section from the two “choice” sections in which the digging bowls were placed. The digging bowls were glass pots (interior diameter 4.5 cm, depth 5 cm). The bowls were filled with digging media of different textures and the media were scented with different flavoring essences (Dr. Oetker®, Poland; see Supplementary Information - Supplementary Table 2 for examples). The bowls were baited with DottyChrups (Otmuchow, Poland). During testing the pieces of reward (~3 mm in diameter) were placed on the bottom of the glass pot and buried under ~2–3 cm of digging media (depending on the type of digging media: ~2 cm for gravel and ~3 cm for wool). Only one of the bowls was baited and mice were required to determine which of the two bowls was baited using either the texture of the digging medium or the odor of the medium as cues.

2.3.2. Habituation

Before testing mice were habituated with the bowl filled with the reward in their home cages for 24 h. Then mice were habituated to consume the reward presented in the bowls filled with the digging media and placed in the testing apparatus for 10 min per day for 2 days, or as many days as they needed to be habituated and to consume the reward. The digging media were not scented during habituation. The reward was placed in both bowls filled with the digging media. A piece of the food reward was placed on top of the digging media during the first day of habituation and covered with a thin layer of the medium on the second day. Once the mice retrieved the food reward, they were allowed to consume it before being returned to the home cage. After habituation mice were moved to the first phase of the ASST - simple discrimination (SD).

2.3.3. Testing paradigm

Food availability was restricted prior to testing; the animals had no access to food for ~12 h before the test, with water freely available all the time in the home cage. All mice in one group (groups of animals for the egr immunohistochemical analysis) were trained on the same