Neurochemistry International 124 (2019) 193–199

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/neuint

Chronic methylphenidate preferentially alters catecholamine protein targets in the parietal cortex and ventral striatum

Emmanuel Quansah, Tyra S.C. Zetterström*

Pharmacology and Neuroscience Research Group, Leicester School of Pharmacy, Faculty of Health and Life Sciences, De Montfort University, The Gateway, Leicester, LE1 9BH, UK

ARTICLE INFO

Keywords:
Methylphenidate
ADHD
DAT
NET
VMAT2

ABSTRACT

The psychostimulant methylphenidate (MPH) is the primary drug treatment for attention deficit hyperactivity disorder (ADHD) in children. MPH is well known to acutely block the dopamine (DAT) and noradrenaline (NET) transporters. Its effect on additional catecholamine targets is however less known. This study was aimed at comparing the effects of acute (2 mg/kg, i.p.) and chronic (2 mg/kg twice daily for 2 weeks) MPH treatment to young rats on key catecholamine protein targets in brain regions implicated in the symptoms and treatment of ADHD. For this purpose, the density of DAT, NET, the vesicular monoamine transporter 2 (VMAT2), the rate limiting enzyme for catecholamine synthesis tyrosine hydroxylase (TH) and the dopamine D_1 receptor were measured in frontal (FC), parietal cortex (PCx) and the dorsal (DS) and ventral (VS) striatum. The data demonstrate that the effects of MPH depend on duration of treatment and brain region investigated. With the exception of DAT in the VS our results indicate that chronic but not acute administration of MPH increases levels of DAT, NET, TH, VMAT2 and D_1. These effects were further more prominent in the VS over DS and in the PCx compared to the FC. In addition, chronic MPH enhanced DAT levels in the left DS but not in right side. To summarize, this study shows new evidence that chronic MPH to young rats preferentially alters catecholamine targets in PCx and VS over DS and FC. The effect of chronic MPH to increase levels of DAT, NET and VMAT2 suggests that the drug might long-term loose some of its acute action to increase extracellular levels of dopamine and noradrenaline. In conclusion, these findings provide novel insights into the mechanism of action by MPH in the treatment of ADHD and further suggest that the long-term effectiveness of the stimulant drug could be limited.

1. Introduction

Attention deficit hyperactivity disorder (ADHD) is a prevalent childhood neuropsychiatric disorder and twin studies have established that there is a strong genetic component of the condition (Shastry, 2004). Specifically, the latrophilin 3 (LPHN3) gene has been associated with ADHD genetic susceptibility and the pharmacogenetics of the condition (Labbe et al., 2012); Bruxel et al. (2015). The key symptoms of ADHD are inattention, impulsivity, hyperactivity, and deficits in catecholamines (i.e. dopamine and noradrenaline) have often been associated with these symptoms of the disorder (Volkow et al., 2007; Del Campo et al., 2011). Acute administration of the psychostimulant methylphenidate (MPH), which is the first-line drug for ADHD, is suggested to increase catecholamine signalling by blocking both dopamine (DAT) and noradrenaline (NET) transporters. This has been associated with symptom improvements in young and adolescent individuals diagnosed with ADHD (Vles et al., 2003; Rosa-Neto et al., 2005). However, the effects of chronic MPH treatment on catecholamine signalling including, DAT, NET, and other catecholaminergic markers in brain areas associated with the symptoms of ADHD are not fully known.

The underlying reasons for a dysfunctional dopamine and noradrenaline activity in ADHD have been suggested to be mediated via altered expression of some key proteins for dopamine and noradrenaline neurotransmission including, the rate limiting enzyme tyrosine hydroxylase (TH), the catecholamine transporter proteins DAT and NET, as well as the vesicular monoamine transporter protein 2 (VMAT2) (Volkow et al., 2007, 2009; Calipari et al., 2014). Dopamine regulates cortical and striatal pathways through the activation of five distinct receptors, D_1-D_5 (Beaulieu and Gainetdinov, 2011). Together with the transporter proteins (DAT, NET, and VMAT2) and the rate limiting enzyme TH, abnormalities of some dopamine receptors have also been implicated in the pathology of ADHD. In particular, the D_1

* Corresponding author.
E-mail address: tscz@dmu.ac.uk (T.S.C. Zetterström).

https://doi.org/10.1016/j.neuint.2019.01.016
Received 22 November 2018; Received in revised form 15 January 2019; Accepted 16 January 2019
Available online 17 January 2019

type, an abundant brain dopamine receptor with prominent action on cortical cognitive function has been implicated in the symptoms of ADHD and its treatment (Del Campo et al., 2011; Calipari et al., 2014; Narendran et al., 2015).

Given the role of cortico-striatal networks in cognition and motor behaviour, current neurobiological theories of ADHD focus on morphological and functional abnormalities in these two major brain networks as they are likely mediating the core symptoms of ADHD (Ashtari et al., 2005; Bush, 2011; Castellanos et al., 1996; Semrud-Clikeman et al., 2000). A deviation from the common pattern of cerebral lateralisation is also suggested to contribute to the symptoms of ADHD including the executive function deficits (Ribases et al., 2009; Liu et al., 2013). Indeed, some studies have found increased involvement of the right cerebral hemisphere in ADHD. For instance, a previous study observed significant reductions of the right caudate and the right cerebral cortex in ADHD patients compared to individuals not diagnosed with the condition (Valera et al., 2007). Moreover, functional magnetic resonance imaging studies have detected significant under-activation of the right frontal and parietal cortices in individuals with ADHD during motor and attentional tasks (Rubia et al., 1999; Vance et al., 2007). However, not much is known about the lateralised effects of psychostimulants used in ADHD treatment.

In this study, we sought to compare the acute and chronic effects of MPH treatment on cortical and striatal tissue levels of DAT, NET, VMAT2, TH and D_1 receptors in the juvenile rat brain. The effect of acute and chronic MPH treatment on striatal DAT levels were further compared in the left and right hemispheres.

2. Materials and methods

2.1. Animals and drug treatment

All animal experiments were conducted in strict accordance with the UK Home Office guidelines and the Animal Scientific Procedures Act (1986). Young and adolescent male Sprague-Dawley rats at postnatal day (PND) 20 and 35 respectively were purchased from Charles River (UK) and were housed six per cage with access to food and water *ad libitum*. The rats were maintained under controlled conditions of light (12 h light/dark cycles) and temperature (22–25 °C, 45% humidity). The animals were allowed to acclimatise in a five-day period prior to the start of the experiments when they were injected intraperitoneally (i.p.) with MPH. Following acclimatisation, rats were randomly assigned to acute (single 2 mg/kg MPH or 1 ml/kg saline at PND 39) or chronic (2 mg/kg MPH or 1 ml/kg saline; twice daily [9am and 5pm] for 2 weeks starting from PND 25) treatment groups. The 2 mg/kg MPH dose used in this study has been shown to reach peak plasma levels similar to that observed in humans receiving therapeutic MPH doses (Schiffer et al., 2006; Balcioglu et al., 2009; Kuczenski and Segal, 2005). There were 6 rats per treatment group (n = 6 rats/group) and the animals were sacrificed 24 h after the last MPH or saline injection (at PND 40). The animals were sacrificed via a rapid dislocation of the neck and brain regions corresponding to the frontal and parietal cortices, as well as the dorsal and ventral striatum were rapidly dissected out on ice and snap-frozen in isopentane on dry ice. The brain samples were subsequently stored at −80 °C until required for analysis.

2.2. Western blot analysis

The brain tissues were homogenised in RIPA lysis buffer (Sigma Aldrich, UK) containing protease inhibitor at 4 °C, as previously described (Quansah et al., 2017b). After centrifugation for 10 min at 13,000 *g*, total protein concentration was determined via Bradford assay (Sigma Aldrich, UK). The protein extracts were incubated with electrophoresis buffer (5% β-2-mercaptoethanol, 20% glycerol, 0.5 M Tris-HCl of pH 6.8, 0.006% w/v bromophenol blue and 10% sodium dodecyl sulphate) for 3 min at 90 °C. 20 μg/μl (~15 μl per lane) of the protein extracts were loaded and separated on 12% sodium dodecyl sulphate-polyacrylamide gels (SDS-PAGE) and the proteins transferred onto a 0.45 μm nitrocellulose membrane (GE Healthcare, UK) using a BioRad Trans-blot instrument. All blue precision standard markers (BioRad, UK) were loaded in the first lane to aid in determining the molecular weight range of the proteins of interest. Following transfer, the membranes were blocked with 3% milk in TBST buffer (50 mM Tris; 150 mM NaCl; 0.05% Tween-20; pH 7.5) and incubated overnight with the indicated primary antibodies at 4 °C. The primary antibodies used included: DAT (1:1000 dilution; Santa Cruz, USA), NET (1:1000 dilution; OriGene, USA), TH (1:1000 dilution; Abcam, UK), VMAT2 (SLC18A2; 1:1000 dilution; OriGene, USA) and D_1 receptor (1:1000 dilution, Millipore, UK). β-actin (1:2000 dilution; Santa Cruz, USA) was used as a loading control in this study. Following primary antibody incubation, the membranes were washed three times in TBST buffer and incubated for 1 h with either an anti-rabbit or anti-mouse secondary antibody (IgG-HRP at 1:2000 dilution; Santa Cruz Biotechnology, USA). The membranes were washed three times in TBST buffer following secondary antibody incubation and developed with enhanced chemiluminescent HRP substrate (Amersham Biosciences, UK) and the signal detected with autoradiography film (Kodak™, UK) and then quantified using MCID™ image analysis software (version 7.0). The data was analysed using GraphPad Prism (version 5.0, Graph Pad software Inc., La Jolla, CA) and SPSS (version 22, IBM Statistics, Illinois, USA). Statistical analyses of all data were performed using separate two- and three-way ANOVA designs with the factors 'drug' (MPH *vs.* saline) and 'duration' (acute *vs.* chronic), as well as 'brain hemisphere' (left *vs.* right; in the case of DAT protein expression analysis), along with Bonferroni *post-hoc* tests. Differences were considered statistically significant if $p < 0.05$.

3. Results

3.1. Chronic MPH induces striatal DAT overexpression

DAT abnormalities have been reported in ADHD, along with increased right hemisphere dysfunctions (Rubia et al., 1999; Valera et al., 2007; Del Campo et al., 2011). Indeed, DAT is the major target of MPH and other psychostimulants used in ADHD treatment. We therefore hypothesized that acute and chronic MPH may induce a modified expression of this transporter, possibly in a hemisphere-specific manner. Using the Western blot technique, we evaluated DAT expression in the left and right ventral and dorsal striatum following acute and chronic MPH treatment. We found that acute MPH 24 h after the injection increased the expression of DAT in the ventral striatum (effect of drug: $F_{(1,20)} = 19.1$, $p < 0.0001$; no effect of hemisphere: $p = 0.09$; no hemisphere x drug interaction: $p = 0.11$), with the effect being more prominent in the left side ($p < 0.05$) rather than the right side ($p = 0.06$) (Fig. 1). Chronic MPH also increased DAT in the ventral striatum (effect of drug: $F_{(1,20)} = 21.2$, $p = 0.009$; no effect of hemisphere: $p = 0.13$; no hemisphere x drug interaction: $p = 0.08$), with the effect being prominent in both the left ($p < 0.05$) and right ($p < 0.01$) sides (Fig. 1). In the dorsal striatum however acute MPH did not significantly alter DAT density but chronic MPH significantly increased dorsal striatal DAT (effect of drug: $F_{(1,20)} = 19.1$, $p < 0.0001$; effect of hemisphere: $p < 0.0001$; hemisphere x drug interaction: $p < 0.0001$). This effect was detected in the left dorsal striatum ($p < 0.001$) but not in the right (Fig. 1). In summary, acute MPH increased DAT density in the left VS but had no significant effect on DAT in the DS. In comparison, chronic MPH increased DAT density in both the left and right VS, as well as in the left DS.

3.2. Chronic but not acute MPH increases NET in PCx and VS

In addition to dopamine, MPH has also been shown to increase extracellular noradrenaline levels (Kuczenski and Segal, 2002). Thus,

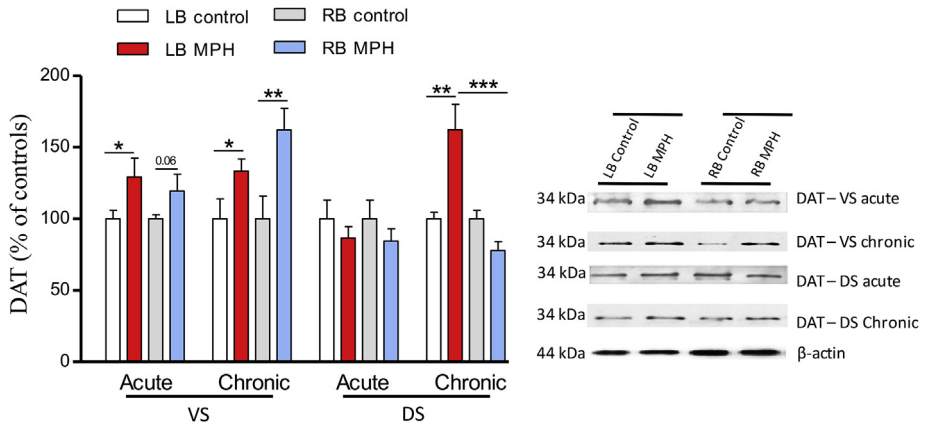

Fig. 1. Effect of MPH treatment on DAT protein density in the left (LB) and right (RB) hemispheres of ventral striatum (VS) and dorsal striatum (DS) of adolescent rats. Values represent percentage ± S.E.M (n = 6 rats/group); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Representative blots are shown to the right of the plot.

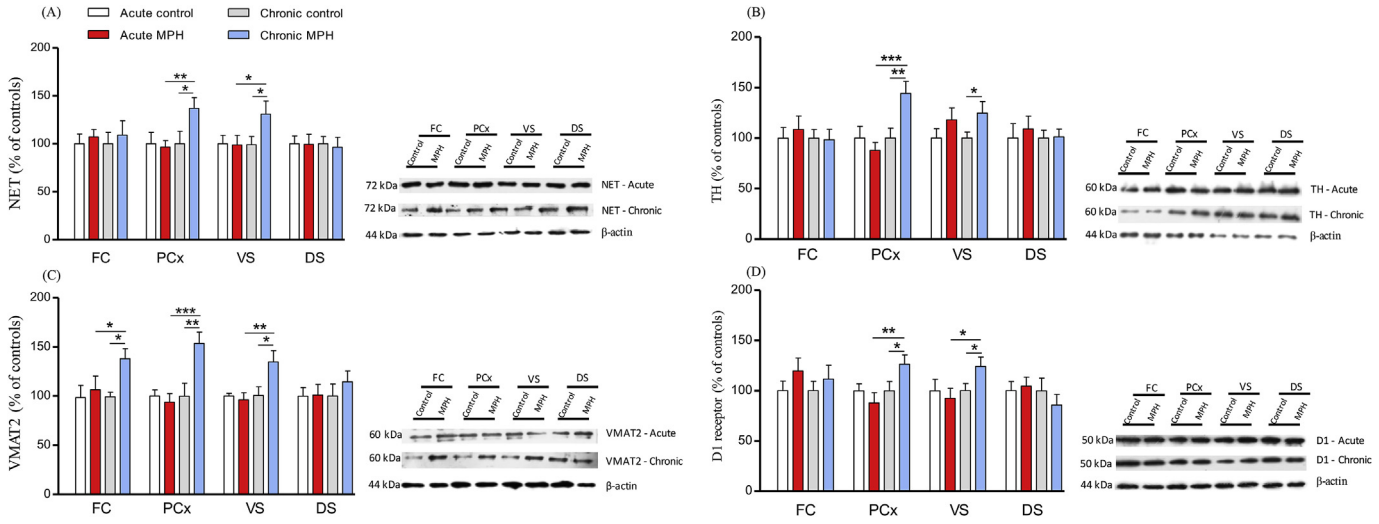

Fig. 2. Effect of MPH treatment on: (A) Noradrenaline transporter [NET], (B) Tyrosine hydroxylase [TH], (C) Vesicular monoamine transporter [VMAT2], and (D) D_1 receptor densities in the frontal cortex [FC], parietal cortex [PCx], ventral striatum [VS], and dorsal striatum [DS] of adolescent rats. Representative blots are shown to the right of each plot. Values represent percentage ± S.E.M (n = 6 rats/group but 12 data points/group: 2 data points from each animal, one from the left and one from right side); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

we tested whether acute and chronic MPH modify the expression of NET. We assessed the effect of the drug on NET expression in both the FC and PCx, as well as the VS and DS (no hemisphere effect was evaluated). We found NET density to be significantly increased by chronic MPH (but not acute) in the PCx (effect of drug: $F_{(1,44)} = 18.4$, $p = 0.02$; effect of duration: $p = 0.003$; duration x drug interaction: $p < 0.001$) and the VS (effect of drug: $F_{(1,44)} = 10.1$, $p = 0.04$; effect of duration: $p = 0.02$; duration x drug interaction: $p = 0.0008$) (Fig. 2A). In summary, chronic MPH induced NET overexpression in the PCx and VS but not in the FC or DS.

3.3. Chronic but not acute MPH increases TH and VMAT2 levels

Next, we examined whether acute and chronic MPH alter the density of TH, the rate limiting enzyme of dopamine and noradrenaline synthesis. The effect of MPH on TH density was investigated in the FC and PCx, as well as in the VS and DS (Fig. 2B). We found that MPH enhanced TH expression in the VS (effect of drug: $F_{(1,44)} = 6.6$, $p = 0.02$; no effect of duration: $p = 0.12$; no duration x drug interaction: $p = 0.51$) following chronic ($p < 0.05$) but not the acute treatment. MPH treatment also increased TH levels in the PCx (effect of drug: $F_{(1,44)} = 4.5$, $p = 0.01$; effect of duration: $p = 0.0008$; duration x drug interaction: $p = 0.02$) following chronic MPH ($p < 0.01$) but not acute (Fig. 2B).

Further, we tested whether MPH induces changes in the vesicular transporter VMAT2. Here, we found that chronic (but not acute MPH) increased VMAT2 in the FC (effect of drug: $F_{(1,44)} = 3.7$, $p = 0.013$; effect of duration: $p = 0.04$; no duration x drug interaction: $p = 0.10$), PCx (effect of drug: $F_{(1,44)} = 8.2$, $p = 0.01$; effect of duration: $p = 0.0005$; duration x drug interaction: $p = 0.005$) and in the VS (effect of drug: $F_{(1,44)} = 3.5$, $p = 0.02$; effect of duration: $p = 0.009$; duration x drug interaction: $p = 0.003$) but not the DS (Fig. 2C). In summary, chronic but not acute MPH increased both TH and VMAT2 in the PCx and VS. Chronic but not acute MPH also increased VMAT2 in FC.

3.4. Chronic MPH treatment upregulates D_1 receptor expression in PCx and VS

The D_1 receptor plays an important role in cognition and some ADHD patients show deficits in cognitive functions (Del Campo et al., 2011; Calipari et al., 2014; Narendran et al., 2015).

Here, we tested whether MPH treatment influences D_1 receptor levels. Chronic MPH (but not acute) increased D_1 receptor density in the PCx (effect of drug: $F_{(1,44)} = 3.7$, $p = 0.02$; effect of duration: $p = 0.004$; duration x drug interaction: $p = 0.01$) and VS (effect of

drug: $F_{(1,44)} = 4.9$, $p = 0.03$; effect of duration: $p = 0.04$; duration x drug interaction: $p = 0.03$) but failed to alter D_1 receptor density in the FC and DS (Fig. 2D). In summary, similar to TH and VMAT2 the effect of chronic (but not acute) MPH administration on D_1 receptor expression was only detected in PCx and VS.

4. Discussion

In the first part of this study, we show that MPH increases DAT expression in striatum and that such effect was dependent on both duration of treatment and brain hemisphere investigated. This study also provides new evidence that chronic (2 mg/kg, twice daily for 2 weeks) but not acute MPH administration has a preferential action on key catecholamine protein targets: NET, VMAT2, TH and D_1 receptors in the PCx and VS over the FC and DS.

DAT is the main target of MPH and abnormalities of this transporter have been widely associated with ADHD (Dougherty et al., 1999; Volkow et al., 2007; Del Campo et al., 2011). However, the direction of DAT abnormality in ADHD is still under intense debate with some studies suggesting increased striatal DAT expression in individuals diagnosed with ADHD (Dougherty et al., 1999; Cheon et al., 2003), while other reports indicate decreased DAT levels in the striatum (Volkow et al., 2007, 2009; Hesse et al., 2009), and yet some others report no differences in DAT density between ADHD patients and healthy controls (van Dyck et al., 2002; Jucaite et al., 2005). Interestingly, a similar confusion exists in studies using animal models of ADHD. Specifically, studies using the spontaneously hypertensive rat (SHR), a validated model for some of the symptoms of ADHD (Sagvolden and Johansen, 2012), have shown increased striatal DAT gene and protein expression, as well as reduced DAT expression in cortical and striatal areas compared to normal rats (Roessner et al., 2010; Simchon et al., 2010; Somkuwar et al., 2013). It is however, possible that the selection of different reference strains used as control animals could explain these differences (Sagvolden et al., 2009). Considering the inconsistency of DAT expression using animal models of ADHD and the lack of a defined pathogenesis and reliable biomarkers for ADHD (Faraone et al., 2014), this study used normal juvenile rats to investigate the action of MPH on protein markers for dopamine and noradrenaline function. While the diagnosis of ADHD is acknowledged in both children and adults, age-dependent action of MPH has been identified in both human and animal studies (Schrantee et al., 2016). The aim of the current study was to investigate the action of MPH on the still developing brain. Hence, we used rats at the developmental stages of PND 25 and 39 which could be approximated to human ages of 2–4 years and 12–13 years respectively (Andreollo et al., 2012).

The findings of the present study that chronic MPH increases striatal DAT expression (Fig. 1) suggest that the previous findings showing overexpressed levels of DAT in ADHD patients could be due to the effects of long-term MPH treatment rather than an assumed pathology of the condition itself. Indeed, the present findings on DAT is consistent with a previous clinical study which demonstrated that chronic exposure to MPH increased ventral striatal DAT density in ADHD patients (Wang et al., 2013). MPH blocks DAT, which increases the extracellular levels of dopamine (Berridge et al., 2006; Kuczenski and Segal, 2002). However, presynaptic DAT density has in turn been shown to be regulated by extracellular dopamine levels, with DAT density declining when extracellular dopamine level is low and increasing when extracellular levels are high (Zahniser and Sorkin, 2004). Thus, it is possible that the increased DAT density observed in the VS and DS of this study, following chronic MPH injections reflects an adaptive response to prolonged MPH treatment. Indeed, several studies have previously shown that both acute and chronic MPH treatment increase extracellular dopamine levels in VS and DS, as well as in other brain areas such as the cortex and midbrain (Calipari et al., 2014; Calipari and Jones, 2014; Koda et al., 2010; Wagner et al., 2009; Berridge et al., 2006; Kuczenski and Segal, 2002; Volkow et al., 2001). A sustained MPH-induced increase of extracellular dopamine, in spite of an upregulation of DAT the main target of the drug, could partly be explained in the light of a recent finding that chronic MPH administration to rats at a similar age (PND 28) as used in the present study (PND 25) causes a partial down-regulation of D_2 auto-receptor function in the ventral tegmental area (Di Miceli et al., 2018). The idea of a neuroadaptive increase of DAT is however, consistent with previous studies which have shown that MPH may be effective in the short-term but that its long-term clinical effectiveness may be limited, as larger doses may be required to ensure clinical effectiveness (Hazell, 2011; Wang et al., 2013). In line with MPH causing neuroadaptive changes, we have recently shown that chronic administration of MPH to young rats enhances the expression of genes and proteins mediating neuroplasticity (Quansah et al., 2017b). In addition, chronic MPH also increased NET density in the VS (Fig. 2A) and it is conceivable that similar to striatal DAT, the levels of NET could also vary as a function of its substrate noradrenaline. It is however, worth noting that additional factors (other than MPH-induced elevation of extracellular catecholamine levels) may also underlie the increased expression of DAT and NET detected in the present study. Thus, considering the high affinity for MPH on the DAT and NET, as well as studies suggesting that these transporters exhibit some of the functional qualities similar to G-protein- coupled receptors, the direct binding of MPH to DAT or NET may also contribute to the upregulation of their expression (Schmitt et al., 2013).

Notably, the MPH-induced upregulation of DAT following chronic administration showed a hemispheric effect in the DS but not in the VS. In the DS, chronic MPH only increased DAT levels in the left side (Fig. 1). This left hemisphere effect of MPH in the DS is not supportive of clinical reports showing greater right hemisphere abnormalities in ADHD (Heilman and Van Den Abell, 1980; Heilman et al., 1986; Castellanos et al., 1996; Vance et al., 2007). However, in support of our preclinical study, a previous clinical investigation has also demonstrated more pronounced left hemisphere (i.e. left cortical and left striatal) effect of MPH on several brain neurochemicals in ADHD children (Benamor, 2014).

The elevated density of TH in the PCx and VS (Fig. 2B) suggests an enhanced catecholamine synthesis following chronic but not acute MPH administration. Similar to our findings on TH in the FC and DS, a previous study using normal and SHR rats, failed to show MPH-induced changes in TH levels in FC (Pardey et al., 2012). In contrast, an additional study using normal rats and beginning the chronic MPH treatment at an earlier stage of development (PND 7) as well as with a longer duration (4 weeks) compared to the present study (PND 25 and 2 weeks respectively), detected increases in TH-immunoreactive fibre density in FC (Gray et al., 2007). The discrepancies between these studies highlight the influence of the developmental stage on the action of MPH. Thus, our previous studies have shown that MPH induced effects on gene expression as well as firing of prefrontal cortical neurons are both age dependent (Banerjee et al., 2009; Gronier et al., 2010). In addition, and more recently Di Miceli et al. showed; decreased D_2 auto-receptor function in young rats (PND 28) chronically treated with MPH but not in adolescent rats (PND 42), (Di Miceli et al., 2018). In support, of an increased TH activity as shown here in the PCx and VS, the present study also shows increased levels of VMAT2 in these two brain regions (Fig. 2C). In this respect, previous studies have shown that MPH acutely increases vesicular [^{3}H]DA uptake and binding to the VMAT2, as well as promoting cellular redistribution of VMAT2 from the plasma membrane to the subcellular vesicular fraction (Sandoval et al., 2002). Together with the results of the present study, this suggests that chronic MPH treatment increases synthesis and vesicular storage of dopamine and noradrenaline in the PCx and VS. In this respect, it is interesting to note that we have shown in a recent study that a single MPH injection to adolescent rats increases levels of the catecholamine precursor tyrosine in tissue samples of the cerebrum (Quansah et al., 2017a). In the FC, we also measured an increase of VMAT2 this effect however, did not coincide with an enhanced expression of TH. Given that the magnitude

of MPH-induced increase of locomotor activity and extracellular catecholamine levels following chronic administration has been shown to be closely matched by acute administration (Koda et al., 2010; Kuczenski and Segal, 2002), simultaneous increases of TH, VMAT2 and tyrosine could compensate for the continuous blockade of DAT and NET (Figs. 1 and 2A) and thereby help to sustain high levels of the two catecholamines in the synaptic cleft.

Psychostimulant induced activation of the dopamine D_1 receptor is associated with the reduction of ADHD symptoms, including hyperactivity and cognitive defects (Gamo et al., 2010; Napolitano et al., 2010; Wu et al., 2012). In the present study, chronic MPH increased D_1 receptor density in the PCx and VS suggesting that the treatment increases D_1 receptor-mediated dopamine signalling in these brain areas. Dysfunction of: cingulate, frontal and parietal cortical regions have been implicated in the pathophysiology and symptoms of ADHD including impairment of cognitive performance (Bush, 2011). In this context, the enhancement of D_1 receptor expression in the PCx shown here could contribute to a sustained therapeutic action following long-term MPH treatment (Mehta et al., 2000). The potential beneficial action of enhanced D_1 function in the VS is however questionable, as D_1 receptor activation in this part of the brain is known to stimulate reward-related behaviour following psychostimulant administration and could as such influence/promote drug-seeking behaviour (Self, 2014). Moreover, the finding of this study that MPH-induced elevation of protein targets for catecholamine function was particularly marked in VS and PCx, two brain areas implicated in arousal and attention is supportive of the “arousal theory of ADHD” (Gutierrez et al., 2011). A theory which states that ADHD patients have abnormally low arousal (James et al., 2016). Further when considering the role of catecholamines in promoting arousal (Berridge, 2006) the findings of the present study could help to explain the underlying clinical benefit of MPH to patients suffering from ADHD.

5. Conclusion

In summary, the results of this study highlight the possibility that chronic MPH administration has the potential to induce plasticity of the developing brain. Specifically, the present study shows that chronic MPH administration to young animals induces marked changes in the expression of some key proteins mediating catecholamine function. Some of these changes were particularly prominent in the PCx and the VS. Considering the relatively small number of rats (n = 6 rats/group) and the limited time after cessation of MPH treatment (24 h) further studies should be performed to evaluate the clinical significance of our findings. Notably, we report upregulations of DAT and NET secondary to long-term MPH treatment in young animals. It is possibly that this effect contributes to the occasional need for larger doses of the drug during long-term treatment in order to obtain therapeutic efficacy.

Conflicts of interest

The authors declare that there is no conflict of interest.

Authorship contributions

EQ performed the experiments. EQ and TZ designed the research, analysed the data, and drafted the manuscript.

Acknowledgement

The authors are grateful for generous financial support from De Montfort University. EQ was supported by a DMU “High-Flyers” scholarship.

Abbreviations

ADHD	Attention deficit hyperactivity disorder
DAT	Dopamine transporter
NET	Noradrenaline transporter
TH	Tyrosine hydroxylase
VMAT2	Vesicular monoamine transporter 2
DS	Dorsal striatum
VS	Ventral striatum
FC	Frontal cortex; PCx, Parietal cortex

References

Andreollo, N.A., Santos, E.F., Araujo, M.R., Lopes, L.R., 2012. Rat's age versus human's age: what is the relationship? Arq Bras. Cir. Dig. 25, 49–51 S0102-67202012000100011 [pii].

Ashtari, M., Kumra, S., Bhaskar, S.L., Clarke, T., Thaden, E., Cervellione, K.L., Rhinewine, J., Kane, J.M., Adesman, A., Milanaik, R., Maytal, J., Diamond, A., Szeszko, P., Ardekani, B.A., 2005. Attention-deficit/hyperactivity disorder: a preliminary diffusion tensor imaging study. Biol. Psychiatry 57, 448–455 S0006-3223(04)01290-9 [pii].

Balcioglu, A., Ren, J.Q., McCarthy, D., Spencer, T.J., Biederman, J., Bhide, P.G., 2009. Plasma and brain concentrations of oral therapeutic doses of methylphenidate and their impact on brain monoamine content in mice. Neuropharmacology 57, 687–693. https://doi.org/10.1016/j.neuropharm.2009.07.025.

Banerjee, P.S., Aston, J., Khundakar, A.A., Zetterstrom, T.S., 2009. Differential regulation of psychostimulant-induced gene expression of brain derived neurotrophic factor and the immediate-early gene Arc in the juvenile and adult brain. Eur. J. Neurosci. 29, 465–476. https://doi.org/10.1111/j.1460-9568.2008.06601.x.

Beaulieu, J.M., Gainetdinov, R.R., 2011. The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol. Rev. 63, 182–217. https://doi.org/10.1124/pr.110.002642.

Benamor, L., 2014. (1)H-Magnetic resonance spectroscopy study of stimulant medication effect on brain metabolites in French Canadian children with attention deficit hyperactivity disorder. Neuropsychiatric Dis. Treat. 10, 47–54. https://doi.org/10.2147/NDT.S52338.

Berridge, C.W., 2006. Neural substrates of psychostimulant-induced arousal. Neuropsychopharmacology 31, 2332–2340 1301159 [pii].

Berridge, C.W., Devilbiss, D.M., Andrzejewski, M.E., Arnsten, A.F., Kelley, A.E., Schmeichel, B., Hamilton, C., Spencer, R.C., 2006. Methylphenidate preferentially increases catecholamine neurotransmission within the prefrontal cortex at low doses that enhance cognitive function. Biol. Psychiatry 60, 1111–1120 S0006-3223(06)00533-6 [pii].

Bruxel, E.M., Salatino-Oliveira, A., Akutagava-Martins, G.C., Tovo-Rodrigues, L., Genro, J.P., Zeni, C.P., Polanczyk, G.V., Chazan, R., Schmitz, M., Arcos-Burgos, M., Rohde, L.A., Hutz, M.H., 2015. LPHN3 and attention-deficit/hyperactivity disorder: a susceptibility and pharmacogenetic study. Genes Brain Behav. 14, 419–427. https://doi.org/10.1111/gbb.12224.

Bush, G., 2011. Cingulate, frontal, and parietal cortical dysfunction in attention-deficit/hyperactivity disorder. Biol. Psychiatry 69, 1160–1167. https://doi.org/10.1016/j.biopsych.2011.01.022.

Calipari, E.S., Ferris, M.J., Melchior, J.R., Bermejo, K., Salahpour, A., Roberts, D.C., Jones, S.R., 2014. Methylphenidate and cocaine self-administration produce distinct dopamine terminal alterations. Addict. Biol. 19, 145–155. https://doi.org/10.1111/j.1369-1600.2012.00456.x.

Calipari, E.S., Jones, S.R., 2014. Sensitized nucleus accumbens dopamine terminal responses to methylphenidate and dopamine transporter releasers after intermittent-access self-administration. Neuropharmacology 82, 1–10. https://doi.org/10.1016/j.neuropharm.2014.02.021.

Castellanos, F.X., Giedd, J.N., Marsh, W.L., Hamburger, S.D., Vaituzis, A.C., Dickstein, D.P., Sarfatti, S.E., Vauss, Y.C., Snell, J.W., Lange, N., Kaysen, D., Krain, A.L., Ritchie, G.F., Rajapakse, J.C., Rapoport, J.L., 1996. Quantitative brain magnetic resonance imaging in attention-deficit hyperactivity disorder. Arch. Gen. Psychiatr. 53, 607–616.

Cheon, K.A., Ryu, Y.H., Kim, Y.K., Namkoong, K., Kim, C.H., Lee, J.D., 2003. Dopamine transporter density in the basal ganglia assessed with [123I]IPT SPET in children with attention deficit hyperactivity disorder. Eur. J. Nucl. Med. Mol. Imaging 30, 306–311. https://doi.org/10.1007/s00259-002-1047-3. ([doi]).

Del Campo, N., Chamberlain, S.R., Sahakian, B.J., Robbins, T.W., 2011. The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. Biol. Psychiatry 69, e145–e157. https://doi.org/10.1016/j.biopsych.2011.02.036.

Di Miceli, M., Omoloye, A., Gronier, B., 2018. Chronic methylphenidate treatment during adolescence has long-term effects on monoaminergic function. J. Psychopharmacol. https://doi.org/10.1177/0269881118805494. 269881118805494.

Dougherty, D.D., Bonab, A.A., Spencer, T.J., Rauch, S.L., Madras, B.K., Fischman, A.J., 1999. Dopamine transporter density in patients with attention deficit hyperactivity disorder. Lancet 354, 2132–2133 S0140-6736(99)04030-1 [pii].

Faraone, S.V., Bonvicini, C., Scassellati, C., 2014. Biomarkers in the diagnosis of ADHD–promising directions. Curr. Psychiatr. Rep. 16https://doi.org/10.1007/s11920-014-0497-1. 497-014-0497-1.

Gamo, N.J., Wang, M., Arnsten, A.F., 2010. Methylphenidate and atomoxetine enhance

prefrontal function through alpha2-adrenergic and dopamine D1 receptors. J. Am. Acad. Child Adolesc. Psychiatry 49, 1011–1023. https://doi.org/10.1016/j.jaac.2010.06.015.

Gray, J.D., Punsoni, M., Tabori, N.E., Melton, J.T., Fanslow, V., Ward, M.J., Zupan, B., Menzer, D., Rice, J., Drake, C.T., Romeo, R.D., Brake, W.G., Torres-Reveron, A., Milner, T.A., 2007. Methylphenidate administration to juvenile rats alters brain areas involved in cognition, motivated behaviors, appetite, and stress. J. Neurosci. 27, 7196–7207 27/27/7196 [pii].

Gronier, B., Aston, J., Liauzun, C., Zetterstrom, T., 2010. Age-dependent effects of methylphenidate in the prefrontal cortex: evidence from electrophysiological and Arc gene expression measurements. J. Psychopharmacol. 24, 1819–1827. https://doi.org/10.1177/0269881109359100.

Gutierrez, R., Lobo, M.K., Zhang, F., de Lecea, L., 2011. Neural integration of reward, arousal, and feeding: recruitment of VTA, lateral hypothalamus, and ventral striatal neurons. IUBMB Life 63, 824–830. https://doi.org/10.1002/iub.539.

Hazell, P., 2011. The challenges to demonstrating long-term effects of psychostimulant treatment for attention-deficit/hyperactivity disorder. Curr. Opin. Psychiatr. 24, 286–290. https://doi.org/10.1097/YCO.0b013e32834742db.

Heilman, K.M., Bowers, D., Valenstein, E., Watson, R.T., 1986. The right hemisphere: neuropsychological functions. J. Neurosurg. 64, 693–704. https://doi.org/10.3171/jns.1986.64.5.0693.

Heilman, K.M., Van Den Abell, T., 1980. Right hemisphere dominance for attention: the mechanism underlying hemispheric asymmetries of inattention (neglect). Neurology 30, 327–330.

Hesse, S., Ballaschke, O., Barthel, H., Sabri, O., 2009. Dopamine transporter imaging in adult patients with attention-deficit/hyperactivity disorder. Psychiatr. Res. 171, 120–128. https://doi.org/10.1016/j.pscychresns.2008.01.002.

James, S.N., Cheung, C.H.M., Rijsdijk, F., Asherson, P., Kuntsi, J., 2016. Modifiable arousal in attention-deficit/hyperactivity disorder and its etiological association with fluctuating reaction times. Biol. Psychiatry. Cogn. Neurosci. Neuroimaging 1, 539–547 S2451-9022(16)30068-4 [pii].

Jucaite, A., Fernell, E., Halldin, C., Forssberg, H., Farde, L., 2005. Reduced midbrain dopamine transporter binding in male adolescents with attention-deficit/hyperactivity disorder: association between striatal dopamine markers and motor hyperactivity. Biol. Psychiatry 57, 229–238 S0006-3223(04)01167-9 [pii].

Koda, K., Ago, Y., Cong, Y., Kita, Y., Takuma, K., Matsuda, T., 2010. Effects of acute and chronic administration of atomoxetine and methylphenidate on extracellular levels of noradrenaline, dopamine and serotonin in the prefrontal cortex and striatum of mice. J. Neurochem. 114, 259–270. https://doi.org/10.1111/j.1471-4159.2010.06750.x.

Kuczenski, R., Segal, D.S., 2005. Stimulant actions in rodents: implications for attention-deficit/hyperactivity disorder treatment and potential substance abuse. Biol. Psychiatry 57, 1391–1396 S0006-3223(04)01379-4 [pii].

Kuczenski, R., Segal, D.S., 2002. Exposure of adolescent rats to oral methylphenidate: preferential effects on extracellular norepinephrine and absence of sensitization and cross-sensitization to methamphetamine. J. Neurosci. 22, 7264–7271 20026690 [doi].

Labbe, A., Liu, A., Atherton, J., Gizenko, N., Fortier, M.E., Sengupta, S.M., Ridha, J., 2012. Refining psychiatric phenotypes for response to treatment: contribution of LPHN3 in ADHD. Am. J. Med. Genet. B. Neuropsychiatr. Genet. 159B, 776–785. https://doi.org/10.1002/ajmg.b.32083.

Liu, L., Sun, L., Li, Z.H., Li, H.M., Wei, L.P., Wang, Y.F., Qian, Q.J., 2013. BAIAP2 exhibits association to childhood ADHD especially predominantly inattentive subtype in Chinese Han subjects. Behav. Brain Funct. 9https://doi.org/10.1186/1744-9081-9-48. 48-9081-9-48.

Mehta, M.A., Owen, A.M., Sahakian, B.J., Mavaddat, N., Pickard, J.D., Robbins, T.W., 2000. Methylphenidate enhances working memory by modulating discrete frontal and parietal lobe regions in the human brain. J. Neurosci. 20, RC65.

Napolitano, F., Bonito-Oliva, A., Federici, M., Carta, M., Errico, F., Magara, S., Martella, G., Nistico, R., Centonze, D., Pisani, A., Gu, H.H., Mercuri, N.B., Usiello, A., 2010. Role of aberrant striatal dopamine D1 receptor/cAMP/protein kinase A/DARPP32 signaling in the paradoxical calming effect of amphetamine. J. Neurosci. 30, 11043–11056. https://doi.org/10.1523/JNEUROSCI.1682-10.2010.

Narendran, R., Jedema, H.P., Lopresti, B.J., Mason, N.S., Himes, M.L., Bradberry, C.W., 2015. Decreased vesicular monoamine transporter type 2 availability in the striatum following chronic cocaine self-administration in nonhuman primates. Biol. Psychiatry 77, 488–492. https://doi.org/10.1016/j.biopsych.2014.06.012.

Pardey, M.C., Kumar, N.N., Goodchild, A.K., Clemens, K.J., Homewood, J., Cornish, J.L., 2012. Long-term effects of chronic oral Ritalin administration on cognitive and neural development in adolescent wistar kyoto rats. Brain Sci. 2, 375–404. https://doi.org/10.3390/brainsci2030375.

Quansah, E., Ruiz-Rodado, V., Grootveld, M., Probert, F., Zetterstrom, T.S.C., 2017a. (1)H NMR-based metabolomics reveals neurochemical alterations in the brain of adolescent rats following acute methylphenidate administration. Neurochem. Int. 108, 109–120 S0197-0186(16)30506-X [pii].

Quansah, E., Sgamma, T., Jaddoa, E., Zetterstrom, T.S.C., 2017b. Chronic methylphenidate regulates genes and proteins mediating neuroplasticity in the juvenile rat brain. Neurosci. Lett. 654, 93–98 S0304-3940(17)30492-5 [pii].

Ribases, M., Bosch, R., Hervas, A., Ramos-Quiroga, J.A., Sanchez-Mora, C., Bielsa, A., Gastaminza, X., Guijarro-Domingo, S., Nogueira, M., Gomez-Barros, N., Kreiker, S., Gross-Lesch, S., Jacob, C.P., Lesch, K.P., Reif, A., Johansson, S., Plessen, K.J., Knappskog, P.M., Haavik, J., Estivill, X., Casas, M., Bayes, M., Cormand, B., 2009. Case-control study of six genes asymmetrically expressed in the two cerebral hemispheres: association of BAIAP2 with attention-deficit/hyperactivity disorder. Biol. Psychiatry 66, 926–934. https://doi.org/10.1016/j.biopsych.2009.06.024.

Roessner, V., Sagvolden, T., Dasbanerjee, T., Middleton, F.A., Faraone, S.V., Walaas, S.I., Becker, A., Rothenberger, A., Bock, N., 2010. Methylphenidate normalizes elevated dopamine transporter densities in an animal model of the attention-deficit/hyperactivity disorder combined type, but not to the same extent in one of the attention-deficit/hyperactivity disorder inattentive type. Neuroscience 167, 1183–1191. https://doi.org/10.1016/j.neuroscience.2010.02.073. ([doi]).

Rosa-Neto, P., Lou, H.C., Cumming, P., Pryds, O., Karrebaek, H., Lunding, J., Gjedde, A., 2005. Methylphenidate-evoked changes in striatal dopamine correlate with inattention and impulsivity in adolescents with attention deficit hyperactivity disorder. Neuroimage 25, 868–876 S1053-8119(04)00714-1 [pii].

Rubia, K., Overmeyer, S., Taylor, E., Brammer, M., Williams, S.C., Simmons, A., Bullmore, E.T., 1999. Hypofrontality in attention deficit hyperactivity disorder during higher-order motor control: a study with functional MRI. Am. J. Psychiatry 156, 891–896. https://doi.org/10.1176/ajp.156.6.891.

Sagvolden, T., Johansen, E.B., 2012. Rat models of ADHD. Curr. Top. Behav. Neurosci. 9, 301–315. https://doi.org/10.1007/7854_2011_126.

Sagvolden, T., Johansen, E.B., Woien, G., Walaas, S.I., Storm-Mathisen, J., Bergersen, L.H., Hvalby, O., Jensen, V., Aase, H., Russell, V.A., Killeen, P.R., Dasbanerjee, T., Middleton, F.A., Faraone, S.V., 2009. The spontaneously hypertensive rat model of ADHD–the importance of selecting the appropriate reference strain. Neuropharmacology 57, 619–626. https://doi.org/10.1016/j.neuropharm.2009.08.004.

Sandoval, V., Riddle, E.L., Hanson, G.R., Fleckenstein, A.E., 2002. Methylphenidate redistributes vesicular monoamine transporter-2: role of dopamine receptors. J. Neurosci. 22, 8705–8710 22/19/8705 [pii].

Schiffer, W.K., Volkow, N.D., Fowler, J.S., Alexoff, D.L., Logan, J., Dewey, S.L., 2006. Therapeutic doses of amphetamine or methylphenidate differentially increase synaptic and extracellular dopamine. Synapse 59, 243–251. https://doi.org/10.1002/syn.20235.

Schmitt, K.C., Rothman, R.B., Reith, M.E., 2013. Nonclassical pharmacology of the dopamine transporter: atypical inhibitors, allosteric modulators, and partial substrates. J. Pharmacol. Exp. Therapeut. 346, 2–10. https://doi.org/10.1124/jpet.111.191056.

Schrantee, A., Tamminga, H.G., Bouziane, C., Bottelier, M.A., Bron, E.E., Mutsaerts, H.J., Zwinderman, A.H., Groote, I.R., Rombouts, S.A., Lindauer, R.J., Klein, S., Niessen, W.J., Opmeer, B.C., Boer, F., Lucassen, P.J., Andersen, S.L., Geurts, H.M., Reneman, L., 2016. Age-dependent effects of methylphenidate on the human dopaminergic system in young vs adult patients with attention-deficit/hyperactivity disorder: a randomized clinical trial. JAMA Psychiatry 73, 955–962. https://doi.org/10.1001/jamapsychiatry.2016.1572.

Self, D.W., 2014. Diminished role for dopamine D1 receptors in cocaine addiction? Biol. Psychiatry 76, 2–3. https://doi.org/10.1016/j.biopsych.2014.04.006.

Semrud-Clikeman, M., Steingard, R.J., Filipek, P., Biederman, J., Bekken, K., Renshaw, P.F., 2000. Using MRI to examine brain-behavior relationships in males with attention deficit disorder with hyperactivity. J. Am. Acad. Child Adolesc. Psychiatry 39, 477–484 S0890-8567(09)66191-8 [pii].

Shastry, B.S., 2004. Molecular genetics of attention-deficit hyperactivity disorder (ADHD): an update. Neurochem. Int. 44, 469–474.

Simchon, Y., Weizman, A., Rehavi, M., 2010. The effect of chronic methylphenidate administration on presynaptic dopaminergic parameters in a rat model for ADHD. Eur. Neuropsychopharmacol. 20, 714–720. https://doi.org/10.1016/j.euroneuro.2010.04.007.

Somkuwar, S.S., Darna, M., Kantak, K.M., Dwoskin, L.P., 2013. Adolescence methylphenidate treatment in a rodent model of attention deficit/hyperactivity disorder: dopamine transporter function and cellular distribution in adulthood. Biochem. Pharmacol. 86, 309–316. https://doi.org/10.1016/j.bcp.2013.04.013.

Valera, E.M., Faraone, S.V., Murray, K.E., Seidman, L.J., 2007. Meta-analysis of structural imaging findings in attention-deficit/hyperactivity disorder. Biol. Psychiatry 61, 1361–1369 S0006-3223(06)00803-1 [pii].

van Dyck, C.H., Quinlan, D.M., Cretella, L.M., Staley, J.K., Malison, R.T., Baldwin, R.M., Seibyl, J.P., Innis, R.B., 2002. Unaltered dopamine transporter availability in adult attention deficit hyperactivity disorder. Am. J. Psychiatry 159, 309–312. https://doi.org/10.1176/appi.ajp.159.2.309.

Vance, A., Silk, T.J., Casey, M., Rinehart, N.J., Bradshaw, J.L., Bellgrove, M.A., Cunnington, R., 2007. Right parietal dysfunction in children with attention deficit hyperactivity disorder, combined type: a functional MRI study. Mol. Psychiatr. 12, 826–832 793 doi: 4001999 [pii].

Vles, J.S., Feron, F.J., Hendriksen, J.G., Jolles, J., van Kroonenburgh, M.J., Weber, W.E., 2003. Methylphenidate down-regulates the dopamine receptor and transporter system in children with attention deficit hyperkinetic disorder (ADHD). Neuropediatrics 34, 77–80. https://doi.org/10.1055/s-2003-39602.

Volkow, N.D., Wang, G.J., Kollins, S.H., Wigal, T.L., Newcorn, J.H., Telang, F., Fowler, J.S., Zhu, W., Logan, J., Ma, Y., Pradhan, K., Wong, C., Swanson, J.M., 2009. Evaluating dopamine reward pathway in ADHD: clinical implications. J. Am. Med. Assoc. 302, 1084–1091. https://doi.org/10.1001/jama.2009.1308.

Volkow, N.D., Wang, G.J., Newcorn, J., Telang, F., Solanto, M.V., Fowler, J.S., Logan, J., Ma, Y., Schulz, K., Pradhan, K., Wong, C., Swanson, J.M., 2007. Depressed dopamine activity in caudate and preliminary evidence of limbic involvement in adults with attention-deficit/hyperactivity disorder. Arch. Gen. Psychiatr. 64, 932–940 64/8/932 [pii].

Volkow, N.D., Wang, G., Fowler, J.S., Logan, J., Gerasimov, M., Maynard, L., Ding, Y., Gatley, S.J., Gifford, A., Franceschi, D., 2001. Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. J. Neurosci. 21, RC121 20014896 [pii].

Wagner, A.K., Drewencki, L.L., Chen, X., Santos, F.R., Khan, A.S., Harun, R., Torres, G.E., Michael, A.C., Dixon, C.E., 2009. Chronic methylphenidate treatment enhances striatal dopamine neurotransmission after experimental traumatic brain injury. J. Neurochem. 108, 986–997. https://doi.org/10.1111/j.1471-4159.2008.05840.x.

Wang, G.J., Volkow, N.D., Wigal, T., Kollins, S.H., Newcorn, J.H., Telang, F., Logan, J.,

Jayne, M., Wong, C.T., Han, H., Fowler, J.S., Zhu, W., Swanson, J.M., 2013. Long-term stimulant treatment affects brain dopamine transporter level in patients with attention deficit hyperactive disorder. PLoS One 8, e63023. https://doi.org/10.1371/journal.pone.0063023.

Wu, J., Xiao, H., Sun, H., Zou, L., Zhu, L.Q., 2012. Role of dopamine receptors in ADHD: a systematic meta-analysis. Mol. Neurobiol. 45, 605–620. https://doi.org/10.1007/s12035-012-8278-5.

Zahniser, N.R., Sorkin, A., 2004. Rapid regulation of the dopamine transporter: role in stimulant addiction? Neuropharmacology 47 (Suppl 1), 80–91 S0028390804002023 [pii].