Dopamine D4 Receptors Modulate Brain Metabolic Activity in the Prefrontal Cortex and Cerebellum at Rest and in Response to Methylphenidate

Michael Michaelides[2,3], **Javier Pascau**[4], **Juan-Domingo Gispert**[5], **Foteini Delis**[1], **David K. Grandy**[6], **Gene-Jack Wang**[1], **Manuel Desco**[4], **Marcelo Rubinstein**[7], **Nora D. Volkow**[1], and **Panayotis K. Thanos**[1,2,3]

[1] Laboratory of Neuroimaging, NIAAA, NIH, Bethesda, MD

[2] Behavior Neuropharmacology and Neuroimaging Lab., Brookhaven National Lab, Upton, NY

[3] Dept. of Psychology, Stony Brook University, Stony Brook, NY

[4] Hosp. Gen. Universitario Gregorio Marañón, Madrid, Spain

[5] Inst. d'Alta Tecnología, Barcelona, Spain

[6] Oregon Health Sciences University, Portland, OR

[7] Department of Biology, University of Buenos Aires, Buenos Aires, Argentina

Abstract

Methylphenidate (MP) is widely used to treat Attention-Deficit Hyperactivity Disorder (ADHD). Variable number of tandem repeats (VNTR) polymorphisms in the dopamine D4 receptor (D_4) gene have been implicated in vulnerability to ADHD and on the response MP. Here we examined the contribution of D_4 receptors (D4Rs) to baseline brain glucose metabolism (BGluM) and to the regional metabolic responses to MP. We compared BGluM (measured with microPET and [^{18}F]2-fluoro-2-deoxy-D-glucose) at baseline and after MP (10 mg/kg ip) administration in mice with genetic deletion of the D_4. Images were analyzed using a novel automated image registration procedure. Baseline $D_4^{-/-}$ mice had lower metabolism in prefrontal cortex (PFC) and greater metabolism in the cerebellar vermis (CBV) than $D_4^{+/+}$ and $D_4^{+/-}$ mice; when given MP, $D_4^{-/-}$ mice increased metabolism in PFC and decreased it in CBV; whereas in $D_4^{+/+}$ and $D_4^{+/-}$ mice MP decreased metabolism in PFC and increased it in CBV. These findings provide evidence that D4Rs modulate not only the PFC, which may reflect activation by dopamine of D4Rs located in this region, but also the CBV, which may reflect an indirect modulation since D4Rs are minimally expressed in this region. Since individuals with ADHD show structural and/or functional abnormalities in these brain regions the association of ADHD with D4Rs may reflect its modulation of these brain regions. The differential response to MP as a function of genotype could explain differences in brain functional responses to MP between patients with ADHD and healthy controls and between ADHD patients with different D_4 polymorphisms.

Keywords

mice; microPET; positron emission tomography; FDG; ADHD

Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is the most frequent psychiatric disorder of childhood and it is increasingly being recognized in adults (Fergason, 2000; Volkow *et al.*, 2001), (Pary *et al.*, 2002). The stimulant methylphenidate (MP) is widely used to treat symptoms of ADHD (inattention, hyperactivity, impulsivity). MP increases extracellular levels of dopamine (DA) and norepinephrine (NE) by blocking their respective transporters (Kuczenski & Segal, 1997). Imaging studies have shown that MP increases cerebellar glucose metabolism in normal individuals (Volkow *et al.*, 1997a), and that it attenuates task-induced increases in brain glucose metabolic activity during exposure to a cognitive task in proportion to the baseline metabolic measures (Volkow *et al.*, 2008). These findings suggest that MP's effects on brain metabolic activity depend on the context (task performed) as well as the individual (baseline brain metabolism) and that the therapeutic effects of MP may reside in part on its ability to increase the efficiency of the brain when performing a cognitive task (Volkow *et al.*, 2008).

ADHD has been associated with a polymorphism in the dopamine D4 receptor (D_4); a 7-repeat variant of the 48 base-pair (bp) VNTR located in exon 3, which results in decreased D_4 efficacy (Terwilliger & Ott, 1992; LaHoste *et al.*, 1996; Swanson *et al.*, 1998; Himelstein *et al.*, 2000; Sunohara *et al.*, 2000; Tahir *et al.*, 2000; Cheon *et al.*, 2007). This polymorphism has also been associated with poor response to MP treatment in ADHD (Seeger *et al.*, 2001; Hamarman *et al.*, 2004; Cheon *et al.*, 2007) and with volumetric changes in cerebellum (Monuteaux *et al.*, 2008) and prefrontal cortex (Durston *et al.*, 2005).

In the brain, the D_4 is highly expressed in the frontal cortex and in the hypothalamus (Ariano *et al.*, 1997; Tarazi & Baldessarini, 1999; Oak *et al.*, 2000) and with very low expression in cerebellum (CB) (predominantly in white matter) (Barili *et al.*, 2000). Deletion of D_4 in knockout (KO) mice results in lower basal extracellular DA levels in the striatum as well as decreased KCl-evoked overflow of DA in the striatum and nucleus accumbens core (Thomas *et al.*, 2007). These mice also show reduced exploration of novel stimuli, decreased spontaneous locomotor activity (Rubinstein *et al.*, 1997; Dulawa *et al.*, 1999) and abnormal behavioral responses (locomotor activity and conditioned place preference) to stimulant drugs (amphetamine, MP and cocaine) (Thanos *et al.*, 2009).

Here we test the hypothesis that dopamine D4 receptors (D4Rs) influence baseline activity in prefrontal cortex and cerebellum, which are brain regions implicated in ADHD, and that D4Rs also modulate the response to stimulant medication in these brain regions. For this purpose we compared regional brain glucose metabolism (marker of brain activity) at baseline and in response to MP in D_4 KO ($D_4^{-/-}$) with that in heterozygous ($D_4^{+/-}$) and in wild-type ($D_4^{+/+}$) mice. Specifically we hypothesized that since D4Rs are highly expressed in the prefrontal cortex we hypothesized that $D_4-/-$ mice would differ in baseline and in MP-induced changes in prefrontal metabolism. Because MP-induced increases seen in earlier human studies have been postulated to reflect prefrontal regulation of cerebellar activity we also hypothesized differences in baseline and in MP-induced changes in cerebellar metabolism between $D_4-/-$ and wild-type mice.

Materials and Methods

Animals

All mice were produced as previously described (Rubinstein *et al.*, 1997). We studied male D_4 wild-type (n=8), heterozygous (n=8) and KO mice (n=8) that had been bred at the Brookhaven National Laboratory (BNL) Animal Facility. At the time of the experiments, the mice were approximately 3–4 months old and weighed 36.2g±2.1 ($D_4^{+/+}$), 36.5g±1.5

($D_4^{+/-}$), and 33.7g±2.4 ($D_4^{-/-}$). They were single-housed under a 12 hour light/dark cycle in clear acrylic cages with wire-mesh tops and food and water was available ad-libitum. All experiments were conducted in conformity with the National Academy of Sciences Guide for the Care and Use of Laboratory Animals (NAS & NRC, 1996) and BNL Institutional Animal Care and Use Committee protocols.

Chemicals

MP hydrochloride (Sigma, St. Louis, MO) was dissolved in saline to produce a concentration of 10 mg/kg. 2-[^{18}F]-flouro-2-deoxy-D-glucose (FDG) was synthesized at the BNL Cyclotron.

In-vivo FDG μPET

All animals were scanned with FDG twice and each scan was performed 1 week apart. Animals were fasted overnight prior to the scan. The *Baseline-Scan* was conducted first and used as a control scan during which each animal received an intraperitoneal (IP) injection of approximately 0.2 mCi FDG and was immediately placed in its home cage. Each mouse was awake for a period of 40 minutes during the FDG uptake. Mice were then anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) and placed in a stereotaxic head holder (David Kopf Instruments; Tujunga, CA, USA) in a prone position on the bed of the scanner 5–7 minutes after the anesthesia administration. The *MP-scan* was the same as *Baseline* except that it was administered 1 week later and each animal was given 10 mg/kg (IP) MP 5 min prior to FDG.

MicroPET Image Acquisition & Analysis

An R4 small animal positron emission tomography (μPET) scanner (Concorde CTI Siemens, Knoxville, TN) was used for FDG μPET imaging. The R4 μPET has a transaxial resolution of 2.0 mm full width at half maximum, with a field of view (FOV) of 11.5 cm. Animals were placed in the center of the FOV and were scanned under a static imaging protocol for 80 minutes using a ramp filter with cutoff at Nyquist frequency. After scanning, all images were corrected for photon scatter and reconstructed using the ordered subset expectation maximization algorithm provided by Concorde CTI. Attenuation correction was not carried out due to factors previously described (Alexoff *et al.*, 2003; Schiffer *et al.*, 2007).

MicroPET image analysis was performed as described previously (Pascau *et al.*, 2009) with slight modification especially for mouse brain. To obtain statistical parametric maps from different animals, these studies must be properly co-registered. For this purpose, all the images in the dataset were co-registered to a reference image (manually selected by the user). The registration algorithm makes use of normalized mutual information to find the rigid transformation that co-registers both images, and works in two multiresolution steps: At the first step (lower resolution), the whole reference image is used, whereas at the second step (higher resolution) the reference image is masked in such a way that only those pixels inside the brain are used to compute the cost function (which is used as a quantitative measure of the quality of the alignment). To minimize registration errors, this process was repeated three times, selecting different reference images in every repetition. These three reference images are also registered against each other. Finally, by combining all these geometrical transformations, we can automatically detect any incorrect registration making use of consistency measures. Once the whole dataset is properly registered, an FDG μPET template image was created by averaging all co-registered images. In order to circumvent potential confounds associated with differences in metabolism that could underlie glucose uptake values, we assessed the regional metabolic change of each animal relative to its global activity (whole-brain). This prevented the characterization of metabolic changes being attributed to differences in animal metabolism. It also prevented misleading glucose

uptake effects due to injected dose, weight differences between animals and variable absorption from the IP cavity. Images were analyzed using the statistical parametric mapping (SPM2) software package using a method previously described (Thanos *et al.*, 2008b). In order to get a more accurate anatomical representation of potential areas of activation, a high-resolution magnetic resonance imaging (MRI) brain scan of an age and body weight matched C57/BL6 wild-type mouse was acquired on a 21.1 Tesla magnet (50μm isotropic, TR/TE 250/5 ms) and was subsequently used for μPET-MRI coregistration using previously described procedures (Thanos *et al.*, 2008b).

Results

FDG brain μPET image analysis

An analysis of variance (ANOVA) model was used that defined six different groups that corresponded to $D_4^{+/+}$, $D_4^{+/-}$ and $D_4^{-/-}$ mice with and without MP. Images were subtracted after intensity normalization to 100 by the proportional scaling method. After estimation of the statistical model, an F contrast was applied to reveal the effects of interest. These effects were overlaid on the previously generated high-resolution MRI template to get a more accurate representation of the areas of activation as previously described (Thanos *et al.*, 2008a). An uncorrected P-value of 0.001 was used as threshold to determine statistical significance for the model.

The original F contrasts for the comparisons between $D_4^{+/+}$ vs. $D_4^{+/-}$, $D_4^{+/+}$ vs. $D_4^{-/-}$ and $D_4^{+/-}$ vs. $D_4^{-/-}$ did not reveal any significant cluster differences at baseline (α=.05). In contrast the F contrast that assessed the differences between the combined scans of $D_4^{+/+}$ and $D_4^{+/-}$ vs. those of $D_4^{-/-}$ mice yielded three significant clusters: right prefrontal cortex (PFC) (K_E=13; F=24.5; Z_{score}=4.14; p<0.001), left PFC (K_E=6; F=26.8; Z_{score}=4.3; p<0.001), cerebellar vermis (CBV) (K_E=23; F=26.3; Z_{score}=4.26; p<0.001) (Figure 1 and Table 1).

Differences in metabolism prior to MP between $D_4^{+/+}$, $D_4^{+/-}$ $D_4^{-/-}$ mice

The SPM analysis yielded three significant interaction effects (Table 1). In the first interaction, and most significant, the $D_4^{+/+}$ and $D_4^{+/-}$ mice showed greater relative baseline metabolism in two regions located in the right (K_E=13; F=24.5; Z_{score}=4.14; p<0.001) and left PFC (K_E=6; F=26.8; Z_{score}=4.3; p<0.001), compared to $D_4^{-/-}$ mice whereas the $D_4^{-/-}$ mice had greater relative baseline metabolism in a region located in the CBV (K_E=23; F=26.3; Z_{score}=4.26; p<0.001) than $D_4^{+/-}$ and $D_4^{+/+}$ mice (Figure 2).

Differences in metabolism induced by MP between $D_4^{+/+}$, $D_4^{+/-}$ $D_4^{-/-}$ mice

MP significantly increased metabolic activity in the CBV in $D_4^{+/+}$ and $D_4^{+/-}$ mice but decreased it in $D_4^{-/-}$ mice (K_E=23; F=26.3; Z_{score}=4.26; p<0.001) (Table 1; Figure 3) and it decreased metabolism in left (K_E=6; F=26.8; Z_{score}=4.3; p<0.001), and right PFC (K_E=13; F=24.5; Z_{score}=4.14; p<0.001) in $D_4^{+/+}$ and $D_4^{+/-}$ mice but increased it in $D_4^{-/-}$ mice (Table 1 and Figure 3).

Linear Regression Analysis

The linear regression analysis performed between MP-induced changes in metabolism in the left and right PFC and those in the CBV showed that this was significant for a negative correlation between the left PFC and CBV in $D_4^{-/-}$ mice but not in $D_4^{+/+}$ and $D_4^{+/-}$ mice (r=0.88; p=0.007; Figure 4).

Discussion

In this study, we document significant differences between D_4 genotypes in baseline and in MP-induced changes in metabolism in PFC and in CBV. D_4 KO mice when studied at baseline showed lower metabolism in PFC regions and greater metabolism in CBV metabolism when compared with $D_4^{+/-}$ and $D_4^{-/-}$ mice. Also MP in D_4 KO mice increased PFC metabolism and decreased metabolism in CBV whereas in $D_4^{+/-}$ and $D_4^{-/-}$ mice MP elicited the opposite pattern of metabolic changes in these brain regions. These findings support our hypothesis that D4Rs modulate baseline activity in prefrontal regions and in CBV. Since studies have shown both volumetric and functional differences in the PFC and CB of ADHD patients (for review see (Giedd *et al.*, 2001; Brennan & Arnsten, 2008)) and the D_4 is associated with ADHD our findings suggest that differences in D_4 expression or function may underlie the reported dysfunction of these brain regions in subjects with ADHD. Studies comparing prefrontal and CBV activity between ADHD patients with the D_4 VNTR 7 repeat polymorphism and those without it are necessary to test this hypothesis.

In the wildtype and heterozygous mice acute treatment with MP evoked a relative increase in metabolism in CBV, which is consistent with results from imaging studies reporting MP-induced increases in cerebellar metabolism, which is most accentuated in the CBV (Volkow *et al.*, 1997b). Moreover studies measuring cerebral blood flow or BOLD responses with functional MRI (fMRI) have also consistently identified the cerebellum and the prefrontal cortex as targets for MP effects (Giedd *et al.*, 2001; Schweitzer *et al.*, 2003; Volkow *et al.*, 2005).

Prefrontal Cortex: Effects of D_4 on basal BGluM

The lower basal brain glucose metabolism (BGluM) in the PFC (bilaterally) of D_4 KO compared to wild-type and heterozygote counterparts suggests that D_4 modulate activity in the PFC and thus are likely to influence PFC-related behaviors. Studies in humans have shown that the PFC is involved in cognitive inhibition, impulse control, organizational planning, working memory, sensory gating and attention (Godefroy *et al.*, 1996; Goldman-Rakic, 1996; Itami & Uno, 2002; Aron *et al.*, 2004). Similarly, animal lesion studies have shown that the PFC is associated with attentional impairment in primates and rodents (Bartus & Levere, 1977; Birrell & Brown, 2000). Interestingly, individuals with ADHD show deficits in the above PFC-related behaviors as well as decreased PFC volume (Castellanos *et al.*, 1996; Casey *et al.*, 1997; Bush *et al.*, 1999; Rubia *et al.*, 1999; Sowell *et al.*, 2003) and activity (Zametkin *et al.*, 1990; Vaidya *et al.*, 1998; Bush *et al.*, 1999; Rubia *et al.*, 1999; Schweitzer *et al.*, 2000; Schweitzer *et al.*, 2003).

D4Rs are expressed preferentially in the PFC (Tarazi & Baldessarini, 1999) and are localized on glutamatergic and GABAergic neurons (Mrzljak *et al.*, 1996; Wedzony *et al.*, 2000) where they have been shown to regulate cognitive functions (Fuster, 2001). Similarly mice lacking D4Rs show impaired inhibitory and/or excitatory activation in the prefrontal cortex (Rubinstein *et al.*, 2001), a trait that may underlie their behavioral phenotype. $D_4^{-/-}$ mice show less locomotor activity in novel and familiar environments, greater vigilance in approach avoidance paradigms and increased excitability in prefrontal cortical neurons when compared to $D_4^{+/+}$ mice (Rubinstein *et al.*, 1997; Rubinstein *et al.*, 2001; Falzone *et al.*, 2002). Furthermore, $D_4^{-/-}$ mice show reduced exploration of novel stimuli (Dulawa *et al.*, 1999; Tan *et al.*, 2003). Evidence for the role for prefrontal D4Rs in attention is given by a recent study showing that the Spontaneously-Hyperactive Rat, a rat which exhibits increased hyperactivity and impaired attentional, show lower D_4 levels in PFC when compared with control strains that don't show attentional deficits (Li *et al.*, 2007).

Pharmacological studies also support the role of D4Rs in cognitive tasks linked with prefrontal cortex (working memory and attention). Specifically, the selective D_4 agonist A-412997 was reported to improve performance in the novel object recognition task and to increase extracellular DA concentration in the PFC in rats (Woolley *et al.*, 2009). On the other hand, D_4 antagonism (L745,870) has been reported to decrease cognitive-related behavior in rats (Braszko, 2009a; b) including decreases in working-memory performance in rats with good baseline performance but increases in rats with poor baseline performance (Zhang *et al.*, 2004).

Prefrontal Cortex: Effects of MP on BGluM

We found a differential effect of MP based on D_4 genotype; MP increased metabolism in PFC in $D_4^{-/-}$ mice but decreased it in $D_4^{+/+}$ and $D_4^{+/-}$ mice. This finding further supports our hypothesis that D4Rs regulate PFC activity. It is also consistent with findings form imaging studies in non-ADHD humans that showed MP-induced decreases in PFC cerebral blood flow (CBF) in controls (Mehta *et al.*, 2000), and with CBF increases in PFC in children with ADHD (Teicher *et al.*, 1996; Vaidya *et al.*, 1998). PET imaging studies using FDG observed both increases and decreases in metabolism in frontal brain regions after intravenous MP in normal adults (Volkow *et al.*, 1997a) but no changes after oral MP in adults with ADHD tested under no stimulation conditions (Matochik *et al.*, 1993; Matochik *et al.*, 1994). The PFC effects of MP are likely to reflect in part its DAergic effects in the PFC since MP increases synaptic DA in the PFC of human subjects (Montgomery *et al.*, 2007).

Thus, our results support the hypothesis that increased PFC activation in ADHD patients (in response to MP as observed in fMRI studies), may reflect dysfunctional D4Rs on GABA and glutamate neurons in PFC, a hypothesis that would have to be tested by further investigation. Though some caution the interpretation of behavioral, physiological and biochemical studies of the PFC in D_4 KO mice (Wang *et al.*, 2009).

Cerebellum: Effects of D_4 on basal brain metabolism

We found greater basal metabolism in $D_4^{-/-}$ compared to $D_4^{+/+}$ and $D_4^{+/-}$ mice in the CBV, which is a brain region that modulates DA neurotransmission in caudate and accumbens (Nieoullon *et al.*, 1978) via itsprojections to the ventral tegmental area (Snider *et al.*, 1976), and has furthermore been implicated both in the pathophysiology of ADHD and on the therapeutic effects of MP (Anderson *et al.*, 2002). Thus, D_4 regulation of CBV activity may also underlie the association between D4Rs and ADHD. The opposite pattern of relative metabolism between the CBV and the PFC across genotype suggests that these two brain regions may work in concert in $D_4^{-/-}$ mice. Indeed, projections in the form of parallel, closed-loop circuits, from the PFC to the striatum, CB and back, have been previously described (for review see (Brennan & Arnsten, 2008). Finally, the CB and striatum, where MP has significant effects on DA release, are connected via a polysynaptic pathway involving the intralaminar nuclei of the thalamus (Ichinohe *et al.*, 2000; Hoshi *et al.*, 2005). Based on these findings, it has been suggested that such circuits may be involved in impaired regulation of higher-order cognitive functions as well as impaired motor control (Brennan & Arnsten, 2008), both of which are observed in ADHD patients.

Cerebellum: Effects of MP on Metabolism

$D_4^{+/+}$ and $D_4^{+/-}$ mice showed increased metabolic activity in response to MP in the CBV whereas $D_4^{-/-}$ mice showed a decrease. The differential responses to MP in the CBV as a function of D_4 are also consistent with the differential response of the CBV to MP as a function of the ADHD phenotype; specifically MP increased T(2) relaxation time in the ADHD children with high score on hyperactivity whereas it reduced it in ADHD children

without symptoms of hyperactivity (Anderson *et al.*, 2002). The results in the CBV are opposite to the differential activation observed in the PFC with MP as described above. Apart from the traditionally defined role of the CB in motor control and coordination, findings have shown that the CB plays a role in cognitive and emotional processes including memory, learning, and attention processes (Leiner *et al.*, 1989; Barkley *et al.*, 1992; Andreasen *et al.*, 1995; Desmond *et al.*, 1998; Rapoport *et al.*, 2000; Schmahmann, 2004) that are disrupted in ADHD (Barkley *et al.*, 1992) and have been shown to be improved by MP (Wilens & Biederman, 1992). The involvement of the CB in ADHD has further been suggested by structural imaging studies that showed smaller CBV in children with ADHD than in controls (Berquin *et al.*, 1998; Mostofsky *et al.*, 1998; Castellanos *et al.*, 2001). fMRI studies have also showed a specific activation profile of the CB in response to MP in normal subjects (Anderson *et al.*, 2006). PET imaging studies with FDG and [^{15}O]H_2Ohave also shown MP-specific increases in cerebellar energy metabolism and CBF respectively (Matochik *et al.*, 1993; Volkow *et al.*, 1997a; Schweitzer *et al.*, 2003; Udo de Haes *et al.*, 2007).

In addition to the NE transporter (NET) (Kung *et al.*, 2004), the DA transporter (DAT) is also expressed in the CBV (albeit at low levels) (Melchitzky & Lewis, 2000). Thus MP effects in the CBV are likely to reflect both its blockade of the NET as well as that of DAT (Patrick *et al.*, 1987; Volkow *et al.*, 1992; Houk & Wise, 1995; Pontieri *et al.*, 1995; Gatley *et al.*, 1996; Strazielle *et al.*, 1999; Middleton & Strick, 2001; Glaser *et al.*, 2006). However, we contend that the increases in cerebellar metabolism with MP are also likely to reflect striatal cerebellar networks as evidenced by imaging studies that showed that striatal D2 receptor levels predicted MP-induced increases in cerebellar metabolism in healthy controls (Volkow *et al.*, 1997a).

Prefrontal Cortex-Cerebellum Interactions

We found a significant negative correlation between MP-induced metabolic changes in the left PFC and those in the CBV in $D_4^{-/-}$ mice. A similar but not significant effect (p=.09) was observed in $D_4^{+/-}$ mice. This finding suggests a functional interaction between these two brain regions that is mediated by the presence of D_4, a conjecture that should be further investigated.

Limitations

Our interpretation of the present FDG findings is limited by the fact that we cannot account for compensatory changes in D_4 KO mice. This potential interference might be circumvented in future studies through the use of conditional KO, gene-therapy or D_4 vectors. Another limitation is that the baseline scan always preceded the MP scan and thus we cannot rule out the possibility that differences in metabolism observed with MP may be confounded by an order effect and/or a differential effect of the anesthetic used in the first measurement. Nevertheless, this possibility seems unlikely, given the excellent within-subject reproducibility reported in previous μPET studies (Alexoff *et al.*, 2003; Marsteller *et al.*, 2006). Finally, this is the first study to report the use of SPM in mice and thus further work will be required to determine the sensitivity of this analytical method, especially as it relates to our use of global normalization (Borghammer *et al.*, 2009).

In summary this study identifies the PFC and the CBV as regions that distinguish $D_4^{-/-}$ and $D_4^{+/+}$ mice providing evidence of the regulation of the activity of these brain regions by D4Rs.

Acknowledgments

This work was supported by the NIAAA (AA 11034 & AA07574, AA07611).

Abbreviations

FDG	2-[^{18}F] fluoro-2-deoxy-D-glucose
ADHD	Attention Deficit Hyperactivity Disorder
bp	base-pair
BGluM	Brain glucose metabolism
BNL	Brookhaven National Laboratory
CB	Cerebellum
CBV	Cerebellar Vermis
D_4	Dopamine D4 receptor
D4Rs	Dopamine D4 receptors
DA	Dopamine
DAT	Dopamine transporter
FOV	Field of view
fMRI	Functional magnetic resonance imaging
IP	Intraperitoneal
MRI	Magnetic Resonance Imaging
MP	Methylphenidate
NE	Norepinephrine
NET	Norepinephrine transporter
PET	positron emission tomography
PFC	Prefrontal Cortex
rCBF	Regional cerebral blood flow
SPM	statistical parametric mapping
VNTR	Variable number tandem repeat
VTA	Ventral tegmental area

References

Alexoff DL, Vaska P, Marsteller D, Gerasimov T, Li J, Logan J, Fowler JS, Taintor NB, Thanos PK, Volkow ND. Reproducibility of ^{11}C-raclopride binding in the rat brain measured with the microPET R4: effects of scatter correction and tracer specific activity. J Nucl Med. 2003; 44:815–822. [PubMed: 12732684]

Anderson CM, Lowen SB, Renshaw PF. Emotional task-dependent low-frequency fluctuations and methylphenidate: Wavelet scaling analysis of 1/f-type fluctuations in fMRI of the cerebellar vermis. J Neurosci Methods. 2006; 151:52–61. [PubMed: 16427128]

Anderson CM, Polcari A, Lowen SB, Renshaw PF, Teicher MH. Effects of methylphenidate on functional magnetic resonance relaxometry of the cerebellar vermis in boys with ADHD. Am J Psychiatry. 2002; 159:1322–1328. [PubMed: 12153824]

Andreasen NC, O'Leary DS, Arndt S, Cizadlo T, Hurtig R, Rezai K, Watkins GL, Ponto LL, Hichwa RD. Short-term and long-term verbal memory: a positron emission tomography study. Proc Natl Acad Sci U S A. 1995; 92:5111–5115. [PubMed: 7761457]

Ariano MA, Wang J, Noblett KL, Larson ER, Sibley DR. Cellular distribution of the rat D4 dopamine receptor protein in the CNS using anti-receptor antisera. Brain Res. 1997; 752:26–34. [PubMed: 9106437]

Aron AR, Robbins TW, Poldrack RA. Inhibition and the right inferior frontal cortex. Trends Cogn Sci. 2004; 8:170–177. [PubMed: 15050513]

Barili P, Bronzetti E, Ricci A, Zaccheo D, Amenta F. Microanatomical localization of dopamine receptor protein immunoreactivity in the rat cerebellar cortex. Brain Res. 2000; 854:130–138. [PubMed: 10784114]

Barkley RA, Grodzinsky G, DuPaul GJ. Frontal lobe functions in attention deficit disorder with and without hyperactivity: a review and research report. J Abnorm Child Psychol. 1992; 20:163–188. [PubMed: 1593025]

Bartus RT, Levere TE. Frontal decortication in rhesus monkeys: a test of the interference hypothesis. Brain Res. 1977; 119:233–248. [PubMed: 401465]

Berquin PC, Giedd JN, Jacobsen LK, Hamburger SD, Krain AL, Rapoport JL, Castellanos FX. Cerebellum in attention-deficit hyperactivity disorder: a morphometric MRI study. Neurology. 1998; 50:1087–1093. [PubMed: 9566399]

Birrell JM, Brown VJ. Medial frontal cortex mediates perceptual attentional set shifting in the rat. J Neurosci. 2000; 20:4320–4324. [PubMed: 10818167]

Borghammer P, Cumming P, Aanerud J, Forster S, Gjedde A. Subcortical elevation of metabolism in Parkinson's disease--a critical reappraisal in the context of global mean normalization. Neuroimage. 2009; 47:1514–1521. [PubMed: 19465133]

Braszko JJ. Dopamine D4 receptor antagonist L745,870 abolishes cognitive effects of intracerebroventricular angiotensin IV and des-Phe(6)-Ang IV in rats. Eur Neuropsychopharmacol. 2009a; 19:85–91. [PubMed: 18835699]

Braszko JJ. Participation of D(1–4) dopamine receptors in the pro-cognitive effects of angiotensin IV and des-Phe(6) angiotensin IV. Neurosci Biobehav Rev. 2009b

Brennan AR, Arnsten AF. Neuronal mechanisms underlying attention deficit hyperactivity disorder: the influence of arousal on prefrontal cortical function. Ann N Y Acad Sci. 2008; 1129:236–245. [PubMed: 18591484]

Bush G, Frazier JA, Rauch SL, Seidman LJ, Whalen PJ, Jenike MA, Rosen BR, Biederman J. Anterior cingulate cortex dysfunction in attention-deficit/hyperactivity disorder revealed by fMRI and the Counting Stroop. Biol Psychiatry. 1999; 45:1542–1552. [PubMed: 10376114]

Casey BJ, Castellanos FX, Giedd JN, Marsh WL, Hamburger SD, Schubert AB, Vauss YC, Vaituzis AC, Dickstein DP, Sarfatti SE, Rapoport JL. Implication of right frontostriatal circuitry in response inhibition and attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry. 1997; 36:374–383. [PubMed: 9055518]

Castellanos FX, Giedd JN, Berquin PC, Walter JM, Sharp W, Tran T, Vaituzis AC, Blumenthal JD, Nelson J, Bastain TM, Zijdenbos A, Evans AC, Rapoport JL. Quantitative brain magnetic resonance imaging in girls with attention-deficit/hyperactivity disorder. Arch Gen Psychiatry. 2001; 58:289–295. [PubMed: 11231836]

Castellanos FX, Giedd JN, Marsh WL, Hamburger SD, Vaituzis AC, Dickstein DP, Sarfatti SE, Vauss YC, Snell JW, Lange N, Kaysen D, Krain AL, Ritchie GF, Rajapakse JC, Rapoport JL. Quantitative brain magnetic resonance imaging in attention-deficit hyperactivity disorder. Arch Gen Psychiatry. 1996; 53:607–616. [PubMed: 8660127]

Cheon KA, Kim BN, Cho SC. Association of 4-repeat allele of the dopamine D4 receptor gene exon III polymorphism and response to methylphenidate treatment in Korean ADHD children. Neuropsychopharmacology. 2007; 32:1377–1383. [PubMed: 17077808]

Desmond JE, Gabrieli JD, Glover GH. Dissociation of frontal and cerebellar activity in a cognitive task: evidence for a distinction between selection and search. Neuroimage. 1998; 7:368–376. [PubMed: 9626676]

Dulawa SC, Grandy DK, Low MJ, Paulus MP, Geyer MA. Dopamine D4 receptor-knock-out mice exhibit reduced exploration of novel stimuli. J Neurosci. 1999; 19:9550–9556. [PubMed: 10531457]

Durston S, Fossella JA, Casey BJ, Hulshoff Pol HE, Galvan A, Schnack HG, Steenhuis MP, Minderaa RB, Buitelaar JK, Kahn RS, van Engeland H. Differential effects of DRD4 and DAT1 genotype on fronto-striatal gray matter volumes in a sample of subjects with attention deficit hyperactivity disorder, their unaffected siblings, and controls. Mol Psychiatry. 2005; 10:678–685. [PubMed: 15724142]

Falzone TL, Gelman DM, Young JI, Grandy DK, Low MJ, Rubinstein M. Absence of dopamine D4 receptors results in enhanced reactivity to unconditioned, but not conditioned, fear. Eur J Neurosci. 2002; 15:158–164. [PubMed: 11860516]

Fergason JH. National Institutes of Health Consensus Development Conference Statement: diagnosis and treatment of attention-deficit/hyperactivity disorder (ADHD). J Am Acad Child Adolesc Psychiatry. 2000; 39:182–193. [PubMed: 10673829]

Fuster JM. The prefrontal cortex--an update: time is of the essence. Neuron. 2001; 30:319–333. [PubMed: 11394996]

Gatley SJ, Pan D, Chen R, Chaturvedi G, Ding YS. Affinities of methylphenidate derivatives for dopamine, norepinephrine and serotonin transporters. Life Sci. 1996; 58:231–239. [PubMed: 8786705]

Giedd JN, Blumenthal J, Molloy E, Castellanos FX. Brain imaging of attention deficit/hyperactivity disorder. Ann N Y Acad Sci. 2001; 931:33–49. [PubMed: 11462751]

Glaser PE, Surgener SP, Grondin R, Gash CR, Palmer M, Castellanos FX, Gerhardt GA. Cerebellar neurotransmission in attention-deficit/hyperactivity disorder: does dopamine neurotransmission occur in the cerebellar vermis? J Neurosci Methods. 2006; 151:62–67. [PubMed: 16451810]

Godefroy O, Lhullier C, Rousseaux M. Non-spatial attention disorders in patients with frontal or posterior brain damage. Brain. 1996; 119 (Pt 1):191–202. [PubMed: 8624681]

Goldman-Rakic PS. The prefrontal landscape: implications of functional architecture for understanding human mentation and the central executive. Philos Trans R Soc Lond B Biol Sci. 1996; 351:1445–1453. [PubMed: 8941956]

Hamarman S, Fossella J, Ulger C, Brimacombe M, Dermody J. Dopamine receptor 4 (DRD4) 7-repeat allele predicts methylphenidate dose response in children with attention deficit hyperactivity disorder: a pharmacogenetic study. J Child Adolesc Psychopharmacol. 2004; 14:564–574. [PubMed: 15662148]

Himelstein J, Newcorn JH, Halperin JM. The neurobiology of attention-deficit hyperactivity disorder. Front Biosci. 2000; 5:D461–478. [PubMed: 10762596]

Hoshi E, Tremblay L, Feger J, Carras PL, Strick PL. The cerebellum communicates with the basal ganglia. Nat Neurosci. 2005; 8:1491–1493. [PubMed: 16205719]

Houk JC, Wise SP. Distributed modular architectures linking basal ganglia, cerebellum, and cerebral cortex: their role in planning and controlling action. Cereb Cortex. 1995; 5:95–110. [PubMed: 7620294]

Ichinohe N, Mori F, Shoumura K. A di-synaptic projection from the lateral cerebellar nucleus to the laterodorsal part of the striatum via the central lateral nucleus of the thalamus in the rat. Brain Res. 2000; 880:191–197. [PubMed: 11033006]

Itami S, Uno H. Orbitofrontal cortex dysfunction in attention-deficit hyperactivity disorder revealed by reversal and extinction tasks. Neuroreport. 2002; 13:2453–2457. [PubMed: 12499848]

Kuczenski R, Segal DS. Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: comparison with amphetamine. J Neurochem. 1997; 68:2032–2037. [PubMed: 9109529]

Kung MP, Choi SR, Hou C, Zhuang ZP, Foulon C, Kung HF. Selective binding of 2-[125I]iodo-nisoxetine to norepinephrine transporters in the brain. Nucl Med Biol. 2004; 31:533–541. [PubMed: 15219270]

LaHoste GJ, Swanson JM, Wigal SB, Glabe C, Wigal T, King N, Kennedy JL. Dopamine D4 receptor gene polymorphism is associated with attention deficit hyperactivity disorder. Mol Psychiatry. 1996; 1:121–124. [PubMed: 9118321]

Leiner HC, Leiner AL, Dow RS. Reappraising the cerebellum: what does the hindbrain contribute to the forebrain? Behav Neurosci. 1989; 103:998–1008. [PubMed: 2679667]

Li Q, Lu G, Antonio GE, Mak YT, Rudd JA, Fan M, Yew DT. The usefulness of the spontaneously hypertensive rat to model attention-deficit/hyperactivity disorder (ADHD) may be explained by the differential expression of dopamine-related genes in the brain. Neurochem Int. 2007; 50:848–857. [PubMed: 17395336]

Marsteller DA, Barbarich-Marsteller NC, Fowler JS, Schiffer WK, Alexoff DL, Rubins DJ, Dewey SL. Reproducibility of intraperitoneal 2-deoxy-2-[^{18}F]-fluoro-D-glucose cerebral uptake in rodents through time. Nucl Med Biol. 2006; 33:71–79. [PubMed: 16459261]

Matochik JA, Liebenauer LL, King AC, Szymanski HV, Cohen RM, Zametkin AJ. Cerebral glucose metabolism in adults with attention deficit hyperactivity disorder after chronic stimulant treatment. Am J Psychiatry. 1994; 151:658–664. [PubMed: 8166305]

Matochik JA, Nordahl TE, Gross M, Semple WE, King AC, Cohen RM, Zametkin AJ. Effects of acute stimulant medication on cerebral metabolism in adults with hyperactivity. Neuropsychopharmacology. 1993; 8:377–386. [PubMed: 8512624]

Mehta MA, Owen AM, Sahakian BJ, Mavaddat N, Pickard JD, Robbins TW. Methylphenidate enhances working memory by modulating discrete frontal and parietal lobe regions in the human brain. J Neurosci. 2000; 20:RC65. [PubMed: 10704519]

Melchitzky DS, Lewis DA. Tyrosine hydroxylase- and dopamine transporter-immunoreactive axons in the primate cerebellum. Evidence for a lobular- and laminar-specific dopamine innervation. Neuropsychopharmacology. 2000; 22:466–472. [PubMed: 10731621]

Middleton FA, Strick PL. Cerebellar projections to the prefrontal cortex of the primate. J Neurosci. 2001; 21:700–712. [PubMed: 11160449]

Montgomery AJ, Asselin MC, Farde L, Grasby PM. Measurement of methylphenidate-induced change in extrastriatal dopamine concentration using [^{11}C]FLB 457 PET. J Cereb Blood Flow Metab. 2007; 27:369–377. [PubMed: 16685253]

Monuteaux MC, Seidman LJ, Faraone SV, Makris N, Spencer T, Valera E, Brown A, Bush G, Doyle AE, Hughes S, Helliesen M, Mick E, Biederman J. A preliminary study of dopamine D4 receptor genotype and structural brain alterations in adults with ADHD. Am J Med Genet B Neuropsychiatr Genet. 2008; 147B:1436–1441. [PubMed: 18951431]

Mostofsky SH, Reiss AL, Lockhart P, Denckla MB. Evaluation of cerebellar size in attention-deficit hyperactivity disorder. J Child Neurol. 1998; 13:434–439. [PubMed: 9733289]

Mrzljak L, Bergson C, Pappy M, Huff R, Levenson R, Goldman-Rakic PS. Localization of dopamine D4 receptors in GABAergic neurons of the primate brain. Nature. 1996; 381:245–248. [PubMed: 8622768]

NAS & NRC. Guide for the Care and Use of Laboratory Animals. National Academy Press; Washington D.C: 1996.

Nieoullon A, Cheramy A, Glowinski J. Release of dopamine in both caudate nuclei and both substantia nigrae in response to unilateral stimulation of cerebellar nuclei in the cat. Brain Res. 1978; 148:143–152. [PubMed: 656921]

Oak JN, Oldenhof J, Van Tol HHM. The dopamine D_4 receptor: one decade of research. European Journal of Pharmacology. 2000; 405:303–327. [PubMed: 11033337]

Pary R, Lewis S, Matuschka PR, Rudzinskiy P, Safi M, Lippmann S. Attention deficit disorder in adults. Ann Clin Psychiatry. 2002; 14:105–111. [PubMed: 12238735]

Pascau J, Gispert JD, Michaelides M, Thanos PK, Volkow ND, Vaquero JJ, Soto-Montenegro ML, Desco M. Automated method for small-animal PET image registration with intrinsic validation. Mol Imaging Biol. 2009; 11:107–113. [PubMed: 18670824]

Patrick KS, Caldwell RW, Ferris RM, Breese GR. Pharmacology of the enantiomers of threo-methylphenidate. J Pharmacol Exp Ther. 1987; 241:152–158. [PubMed: 3572779]

Pontieri FE, Mainero C, La Riccia M, Passarelli F, Orzi F. Functional correlates of repeated administration of cocaine and apomorphine in the rat. Eur J Pharmacol. 1995; 284:205–209. [PubMed: 8549628]

Rapoport M, van Reekum R, Mayberg H. The role of the cerebellum in cognition and behavior: a selective review. J Neuropsychiatry Clin Neurosci. 2000; 12:193–198. [PubMed: 11001597]

Rubia K, Overmeyer S, Taylor E, Brammer M, Williams SC, Simmons A, Bullmore ET. Hypofrontality in attention deficit hyperactivity disorder during higher-order motor control: a study with functional MRI. Am J Psychiatry. 1999; 156:891–896. [PubMed: 10360128]

Rubinstein M, Cepeda C, Hurst RS, Flores-Hernandez J, Ariano MA, Falzone TL, Kozell LB, Meshul CK, Bunzow JR, Low MJ, Levine MS, Grandy DK. Dopamine D4 receptor-deficient mice display cortical hyperexcitability. J Neurosci. 2001; 21:3756–3763. [PubMed: 11356863]

Rubinstein M, Phillips TJ, Bunzow JR, Falzone TL, Dziewczapolski G, Zhang G, Fang Y, Larson JL, McDougall JA, Chester JA, Saez C, Pugsley TA, Gershanik O, Low MJ, Grandy DK. Mice lacking dopamine D4 receptors are supersensitive to ethanol, cocaine, and methamphetamine. Cell. 1997:991–1001. [PubMed: 9323127]

Schiffer WK, Mirrione MM, Dewey SL. Optimizing experimental protocols for quantitative behavioral imaging with 18F-FDG in rodents. J Nucl Med. 2007; 48:277–287. [PubMed: 17268026]

Schmahmann JD. Disorders of the cerebellum: ataxia, dysmetria of thought, and the cerebellar cognitive affective syndrome. J Neuropsychiatry Clin Neurosci. 2004; 16:367–378. [PubMed: 15377747]

Schweitzer JB, Faber TL, Grafton ST, Tune LE, Hoffman JM, Kilts CD. Alterations in the functional anatomy of working memory in adult attention deficit hyperactivity disorder. Am J Psychiatry. 2000; 157:278–280. [PubMed: 10671402]

Schweitzer JB, Lee DO, Hanford RB, Tagamets MA, Hoffman JM, Grafton ST, Kilts CD. A positron emission tomography study of methylphenidate in adults with ADHD: alterations in resting blood flow and predicting treatment response. Neuropsychopharmacology. 2003; 28:967–973. [PubMed: 12700698]

Seeger G, Schloss P, Schmidt MH. Functional polymorphism within the promotor of the serotonin transporter gene is associated with severe hyperkinetic disorders. Mol Psychiatry. 2001; 6:235–238. [PubMed: 11317229]

Snider RS, Maiti A, Snider SR. Cerebellar pathways to ventral midbrain and nigra. Exp Neurol. 1976; 53:714–728. [PubMed: 1001395]

Sowell ER, Thompson PM, Welcome SE, Henkenius AL, Toga AW, Peterson BS. Cortical abnormalities in children and adolescents with attention-deficit hyperactivity disorder. Lancet. 2003; 362:1699–1707. [PubMed: 14643117]

Strazielle C, Lalonde R, Hebert C, Reader TA. Regional brain distribution of noradrenaline uptake sites, and of alpha1-alpha2- and beta-adrenergic receptors in PCD mutant mice: a quantitative autoradiographic study. Neuroscience. 1999; 94:287–304. [PubMed: 10613519]

Sunohara GA, Roberts W, Malone M, Schachar RJ, Tannock R, Basile VS, Wigal T, Wigal SB, Schuck S, Moriarty J, Swanson JM, Kennedy JL, Barr CL. Linkage of the dopamine D4 receptor gene and attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry. 2000; 39:1537–1542. [PubMed: 11128331]

Swanson JM, Sunohara GA, Kennedy JL, Regino R, Fineberg E, Wigal T, Lerner M, Williams L, LaHoste GJ, Wigal S. Association of the dopamine receptor D4 (DRD4) gene with a refined phenotype of attention deficit hyperactivity disorder (ADHD): a family-based approach. Mol Psychiatry. 1998; 3:38–41. [PubMed: 9491811]

Tahir E, Yazgan Y, Cirakoglu B, Ozbay F, Waldman I, Asherson PJ. Association and linkage of DRD4 and DRD5 with attention deficit hyperactivity disorder (ADHD) in a sample of Turkish children. Mol Psychiatry. 2000; 5:396–404. [PubMed: 10889550]

Tan S, Hermann B, Borrelli E. Dopaminergic mouse mutants: investigating the roles of the different dopamine receptor subtypes and the dopamine transporter. Int Rev Neurobiol. 2003; 54:145–197. [PubMed: 12785287]

Tarazi FI, Baldessarini RJ. Dopamine D4 receptors: significance for molecular psychiatry at the millennium. Mol Psychiatry. 1999; 4:529–538. [PubMed: 10578234]

Teicher MH, Ito Y, Glod CA, Barber NI. Objective measurement of hyperactivity and attentional problems in ADHD. J Am Acad Child Adolesc Psychiatry. 1996; 35:334–342. [PubMed: 8714322]

Terwilliger JD, Ott J. A haplotype-based ‘haplotype relative risk’ approach to detecting allelic associations. Hum Hered. 1992; 42:337–346. [PubMed: 1493912]

Thanos P, Bermeo C, Rubinstein M, Suchland K, Wang G, Grandy D, Volkow N. Conditioned place preference and locomotor activity in response to methylphenidate, amphetamine and cocaine in mice lacking dopamine D4 receptors. J Psychopharm. 2009 Apr 7. [Epub ahead of print].

Thanos PK, Michaelides M, Benveniste H, Wang GJ, Volkow ND. The effects of cocaine on regional brain glucose metabolism is attenuated in dopamine transporter knockout mice. Synapse. 2008a; 62:319–324. [PubMed: 18286542]

Thanos PK, Michaelides M, Gispert JD, Pascau J, Soto-Montenegro ML, Desco M, Wang R, Wang GJ, Volkow ND. Differences in response to food stimuli in a rat model of obesity: in-vivo assessment of brain glucose metabolism. International Journal of Obesity. 2008b; 32:1171–1179. [PubMed: 18475275]

Thomas TC, Kruzich PJ, Joyce BM, Gash CR, Suchland K, Surgener SP, Rutherford EC, Grandy DK, Gerhardt GA, Glaser PE. Dopamine D4 receptor knockout mice exhibit neurochemical changes consistent with decreased dopamine release. J Neurosci Methods. 2007; 166:306–314. [PubMed: 17449106]

Udo de Haes JI, Maguire RP, Jager PL, Paans AM, den Boer JA. Methylphenidate-induced activation of the anterior cingulate but not the striatum: a [^{15}O]H2O PET study in healthy volunteers. Hum Brain Mapp. 2007; 28:625–635. [PubMed: 17080442]

Vaidya CJ, Austin G, Kirkorian G, Ridlehuber HW, Desmond JE, Glover GH, Gabrieli JD. Selective effects of methylphenidate in attention deficit hyperactivity disorder: a functional magnetic resonance study. Proc Natl Acad Sci U S A. 1998; 95:14494–14499. [PubMed: 9826728]

Volkow ND, Fowler JS, Wang GJ, Telang F, Logan J, Wong C, Ma J, Pradhan K, Benveniste H, Swanson JM. Methylphenidate decreased the amount of glucose needed by the brain to perform a cognitive task. PLoS ONE. 2008; 3:e2017. [PubMed: 18414677]

Volkow ND, Levy A, Brodie JD, Wolf AP, Cancro R, Van Gelder P, Henn F. Low cerebellar metabolism in medicated patients with chronic schizophrenia. Am J Psychiatry. 1992; 149:686–688. [PubMed: 1575261]

Volkow ND, Wang GJ, Fowler JS, Logan J, Angrist B, Hitzemann R, Lieberman J, Pappas N. Effects of Methylphenidate on Regional Brain Glucose Metabolism in Humans: Relationship to Dopamine D2 Receptors. Am J Psychiatry. 1997a; 154:50–55. [PubMed: 8988958]

Volkow ND, Wang G, Fowler JS, Logan J, Gerasimov M, Maynard L, Ding Y, Gatley SJ, Gifford A, Franceschi D. Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. J Neurosci. 2001; 21:RC121. [PubMed: 11160455]

Volkow ND, Wang GJ, Fowler JS, Ding YS. Imaging the effects of methylphenidate on brain dopamine: new model on its therapeutic actions for attention-deficit/hyperactivity disorder. Biol Psychiatry. 2005; 57:1410–1415. [PubMed: 15950015]

Volkow ND, Wang GJ, Fowler JS, Logan J, Angrist B, Hitzemann R, Lieberman J, Pappas N. Effects of methylphenidate on regional brain glucose metabolism in humans: relationship to dopamine D2 receptors. Am J Psychiatry. 1997b; 154:50–55. [PubMed: 8988958]

Wang HD, Stanwood GD, Grandy DK, Deutch AY. Dystrophic dendrites in prefrontal cortical pyramidal cells of dopamine D1 and D2 but not D4 receptor knockout mice. Brain Res. 2009; 1300:58–64. [PubMed: 19747903]

Wedzony K, Chocyk A, Mackowiak MKF, Czyrak A. Cortical Localization of Dopamine D4 Receptors in the Rat Brain - Immunocytochemical Study. Journal of Physiology and Pharmacology. 2000; 51:205–221. [PubMed: 10898094]

Wilens TE, Biederman J. The stimulants. Psychiatr Clin North Am. 1992; 15:191–222. [PubMed: 1347939]

Woolley ML, Waters KA, Gartlon JE, Lacroix LP, Jennings C, Shaughnessy F, Ong A, Pemberton DJ, Harries MH, Southam E, Jones DN, Dawson LA. Evaluation of the pro-cognitive effects of the AMPA receptor positive modulator, 5-(1-piperidinylcarbonyl)-2,1,3-benzoxadiazole (CX691), in the rat. Psychopharmacology (Berl). 2009; 202:343–354. [PubMed: 18795266]

Zametkin AJ, Nordahl TE, Gross M, King AC, Semple WE, Rumsey J, Hamburger S, Cohen RM. Cerebral glucose metabolism in adults with hyperactivity of childhood onset. N Engl J Med. 1990; 323:1361–1366. [PubMed: 2233902]

Zhang K, Grady CJ, Tsapakis EM, Andersen SL, Tarazi FI, Baldessarini RJ. Regulation of working memory by dopamine D4 receptor in rats. Neuropsychopharmacology. 2004; 29:1648–1655. [PubMed: 15138447]

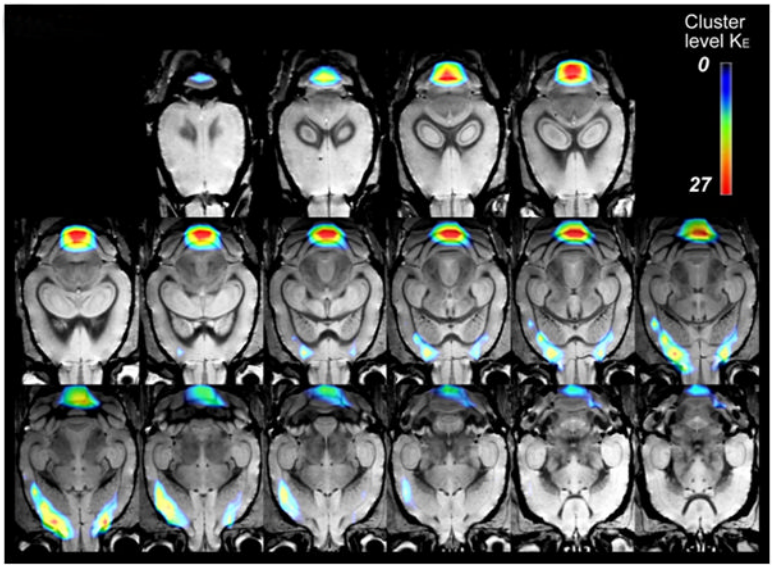

Figure 1. Small animal positron emission tomography (μPET) and statistical parametric mapping (SPM2) results coregistered to a magnetic resonance imaging (MRI) template

A multivariate statistical model with factors *Genotype* ($D_4^{+/+}$ & $D_4^{+/-}$ vs. $D_4^{-/-}$) and *Treatment* (Baseline vs. Methylphenidate (MP)) was used. After model estimation an F contrast (p=.001) was applied to all scans to identify voxels with significant response deviation. The three clusters that are shown survived the analysis threshold for the specific contrast, and represent the regional brain areas of significant change within the given statistical comparison across all groups. In order to identify the anatomical brain regions corresponding to each cluster, the SPM2 image for this contrast was coregistered to an MRI template of an age-matched C57/BL6 mouse using the PMOD v2.8 Image Fusion module (PMOD Technologies, Zurich, Switzerland). Individual responses from scans that corresponded to each of the six groups previously defined were independently examined per cluster and reported in Figure 2.

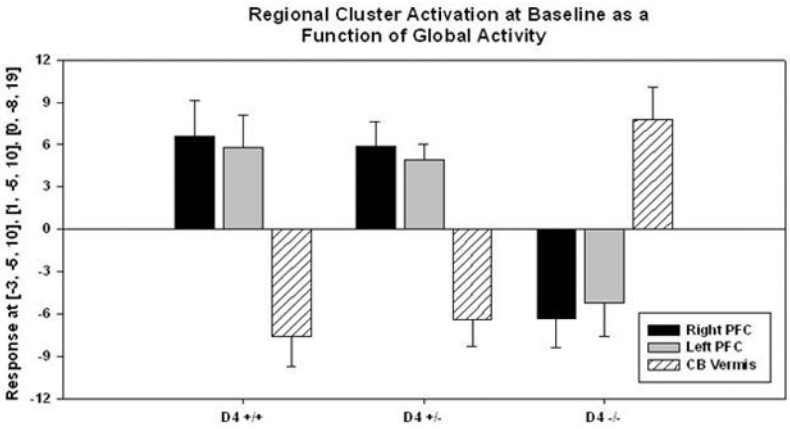

Figure 2.

Mean (+SEM) contrast responses in the three surviving clusters: left prefrontal cortex (PFC), right PFC, and cerebellar vermis (CBV) (p=.001) as obtained from statistical parametric mapping (SPM2) analysis.

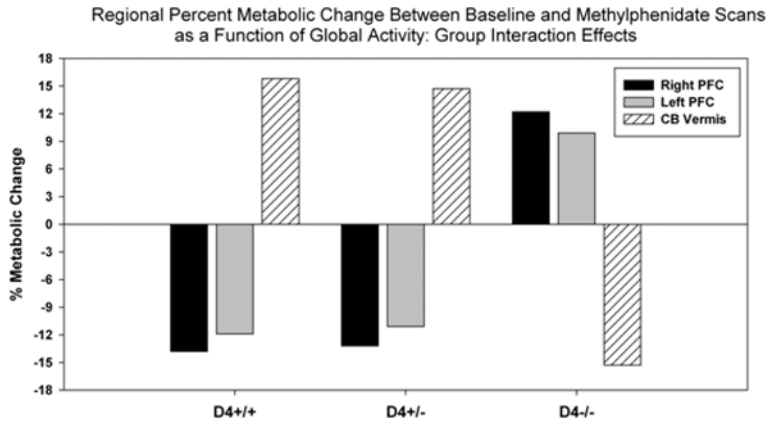

Figure 3.
Percent change in contrast response between Baseline and Methylphenidate (MP) scans.

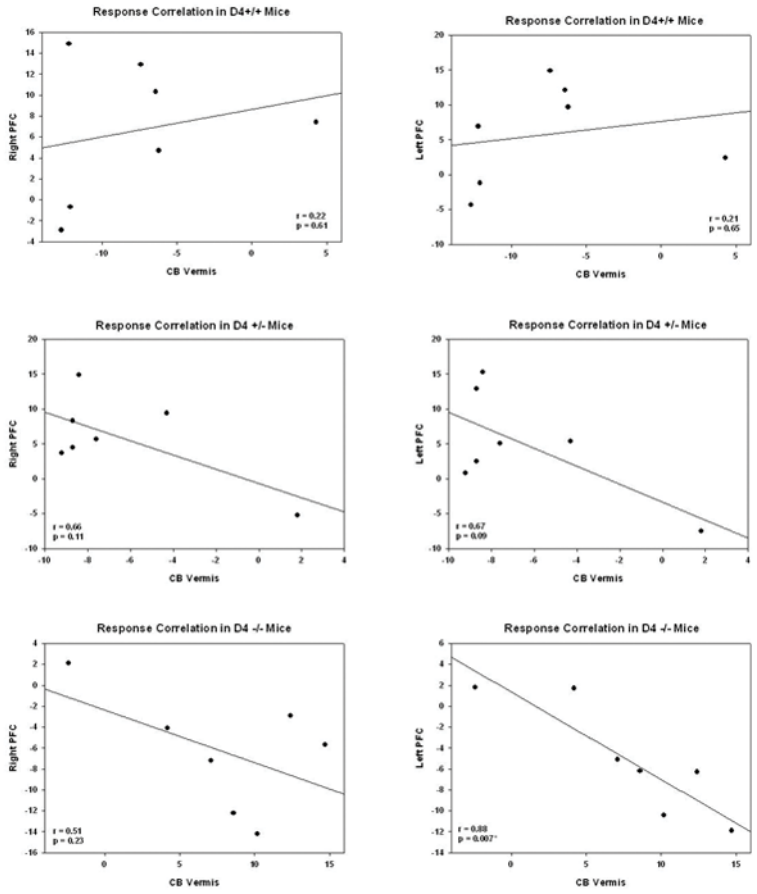

Figure 4.
Contrast response correlations between prefrontal cortex (PFC) and cerebellar vermis (CBV) by genotype.

Table 1

Greatest regional brain metabolic activation: Interaction Effects of Genotype & MP

Statistical paramtetric mapping (SPM2) results comparing interaction effects of *Genotype* ($D_4^{+/+}$ & $D_4^{+/-}$ vs. $D_4^{-/-}$) and *Treatment* (Baseline vs. Methylphenidate (MP)).

Brain Structure	Cluster Level (K_E)	F value	Z score	P level	Stereotaxic location x, y, z (mm)
Right Prefrontal Cortex	13	24.5	4.14	<0.001	−3 −5 10
Left Prefrontal Cortex	6	26.8	4.3	<0.001	1 −5 10
Cerebellum Vermis	23	26.3	4.26	<0.001	0 −8 19