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

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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 (D₄) gene have been implicated in vulnerability to ADHD and on the response MP. Here we examined the contribution of D₄ receptors (D4Rs) to baseline brain glucose metabolism (BGluM) and to the regional metabolic responses to MP. We compared BGluM (measured with microPET and [¹⁸F]2-fluoro-2-deoxy-D-glucose) at baseline and after MP (10 mg/kg ip) administration in mice with genetic deletion of the D₄. Images were analyzed using a novel automated image registration procedure. Baseline D₄^{-/-} mice had lower metabolism in prefrontal cortex (PFC) and greater metabolism in the cerebellar vermis (CBV) than D₄^{+/+} and D₄^{+/-} mice; when given MP, D₄^{-/-} mice increased metabolism in PFC and decreased it in CBV; whereas in D₄^{+/+} and D₄^{+/-} 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 D₄ 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 (Fergusson, 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 (D₄); a 7-repeat variant of the 48 base-pair (bp) VNTR located in exon 3, which results in decreased D₄ 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 (Durstun *et al.*, 2005).

In the brain, the D₄ 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 D₄ 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 D₄ KO (D₄^{-/-}) with that in heterozygous (D₄^{+/-}) and in wild-type (D₄^{+/+}) mice. Specifically we hypothesized that since D₄Rs are highly expressed in the prefrontal cortex we hypothesized that D₄^{-/-} 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 D₄^{-/-} and wild-type mice.

Materials and Methods

Animals

All mice were produced as previously described (Rubinstein *et al.*, 1997). We studied male D₄ 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 (D₄^{+/+}), 36.5g±1.5

(D₄^{+/-}), and 33.7g±2.4 (D₄^{-/-}). 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-[¹⁸F]-fluoro-2-deoxy-D-glucose (FDG) was synthesized at the BNL Cyclotron.

In-vivo FDG μ PET

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 (μ PET) scanner (Concorde CTI Siemens, Knoxville, TN) was used for FDG μ PET imaging. The R4 μ PET 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 μ PET 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 (50 μ m isotropic, TR/TE 250/5 ms) and was subsequently used for μ PET-MRI coregistration using previously described procedures (Thanos *et al.*, 2008b).

Results

FDG brain μ PET 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 ($\alpha=.05$). 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 D₄ genotypes in baseline and in MP-induced changes in metabolism in PFC and in CBV. D₄ KO mice when studied at baseline showed lower metabolism in PFC regions and greater metabolism in CBV metabolism when compared with D₄^{+/-} and D₄^{-/-} mice. Also MP in D₄ KO mice increased PFC metabolism and decreased metabolism in CBV whereas in D₄^{+/-} and D₄^{-/-} 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 D₄ is associated with ADHD our findings suggest that differences in D₄ 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 D₄ 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 D₄ on basal BGlucose

The lower basal brain glucose metabolism (BGlucose) in the PFC (bilaterally) of D₄ KO compared to wild-type and heterozygote counterparts suggests that D₄ 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. D₄^{-/-} 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 D₄^{+/+} mice (Rubinstein *et al.*, 1997; Rubinstein *et al.*, 2001; Falzone *et al.*, 2002). Furthermore, D₄^{-/-} 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 D₄ 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 D₄ 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, D₄ antagonism (L745,870) has been reported to decrease cognitive-related behavior in rats (Braszkowski, 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 BGlucose

We found a differential effect of MP based on D₄ genotype; MP increased metabolism in PFC in D₄^{-/-} mice but decreased it in D₄^{+/+} and D₄^{+/-} mice. This finding further supports our hypothesis that D4Rs regulate PFC activity. It is also consistent with findings from 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 D₄ KO mice (Wang *et al.*, 2009).

Cerebellum: Effects of D₄ on basal brain metabolism

We found greater basal metabolism in D₄^{-/-} compared to D₄^{+/+} and D₄^{+/-} mice in the CBV, which is a brain region that modulates DA neurotransmission in caudate and accumbens (Nieoullon *et al.*, 1978) via its projections 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, D₄ 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 D₄^{-/-} 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

D₄^{+/+} and D₄^{+/-} mice showed increased metabolic activity in response to MP in the CBV whereas D₄^{-/-} mice showed a decrease. The differential responses to MP in the CBV as a function of D₄ 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]H₂O have 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 D₄^{-/-} mice. A similar but not significant effect ($p=.09$) was observed in D₄^{+/-} mice. This finding suggests a functional interaction between these two brain regions that is mediated by the presence of D₄, 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 D₄ KO mice. This potential interference might be circumvented in future studies through the use of conditional KO, gene-therapy or D₄ 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 μ PET 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 D₄^{-/-} and D₄^{+/-} mice providing evidence of the regulation of the activity of these brain regions by D₄Rs.

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Abbreviations

FDG	2-[¹⁸ F] fluoro-2-deoxy-D-glucose
ADHD	Attention Deficit Hyperactivity Disorder
bp	base-pair
BGlucoseM	Brain glucose metabolism
BNL	Brookhaven National Laboratory
CB	Cerebellum
CBV	Cerebellar Vermis
D₄	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
MTA	Ventral tegmental area

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Figure 1. Small animal positron emission tomography (μ PET) 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 parametric 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