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COGNITIVE NEUROSCIENCE

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 polymorphisms in the dopamine D4 receptor (D_4) gene have been implicated in vulnerability to ADHD and the response to MP. Here we examined the contribution of dopamine D4 receptors (D4Rs) to baseline brain glucose metabolism and to the regional metabolic responses to MP. We compared brain glucose metabolism (measured with micro-positron emission tomography and [^{18}F]2-fluoro-2-deoxy-D-glucose) at baseline and after MP (10 mg/kg, i.p.) 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 the 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 the PFC and decreased it in the CBV, whereas in $D_4^{+/+}$ and $D_4^{+/-}$ mice, MP decreased metabolism in the PFC and increased it in the CBV. These findings provide evidence that D4Rs modulate not only the PFC, which may reflect the activation by dopamine of D4Rs located in this region, but also the CBV, which may reflect an indirect modulation as D4Rs are minimally expressed in this region. As 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 patients with ADHD with different D_4 polymorphisms.

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 and impulsivity). MP increases extracellular levels of dopamine (DA) and norepinephrine 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 the effects of MP 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).

Attention deficit hyperactivity disorder has been associated with a polymorphism in the DA D4 receptor (D_4); a seven-repeat variant of the 48 base-pair variable number of tandem repeat 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

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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 the cerebellum (CB) (Monuteaux *et al.*, 2008) and prefrontal cortex (PFC) (Durston *et al.*, 2005).

In the brain, the D_4 is highly expressed in the frontal cortex and hypothalamus (Ariano *et al.*, 1997; Tarazi & Baldessarini, 1999; Oak *et al.*, 2000) with very low expression in the CB (predominantly in the 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.*, 2010).

Here we test the hypothesis that DA D4 receptors (D4Rs) influence baseline activity in the PFC and CB, 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 wild-type ($D_4^{+/+}$) mice. Specifically we hypothesized that, as D4Rs are highly expressed in the PFC, $D_4^{-/-}$ mice would differ in baseline and in MP-induced changes in prefrontal metabolism. Because the 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 Animal Facility. At the time of the experiments, the mice were approximately 3–4 months old and weighed 36.2 ± 2.1 g ($D_4^{+/+}$), 36.5 ± 1.5 g ($D_4^{+/-}$) and 33.7 ± 2.4 g ($D_4^{-/-}$). They were single-housed under a 12 h light/dark cycle in clear acrylic cages with wire-mesh tops and food and water were 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 Brookhaven National Laboratory Institutional Animal Care and Use Committee protocols.

Chemicals

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

In vivo *2-[^{18}F]-fluoro-2-deoxy-d-glucose micro-positron emission tomography*

All animals were scanned with FDG twice and the scans were 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 injection of approximately 0.2 mCi FDG and was immediately placed in its home cage. Each mouse was awake for a period of 40 min 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 at 5–7 min after the administration of anesthesia. The MP scan was the same as baseline except that it was administered 1 week later and each animal was given 10 mg/kg (i.p.) MP at 5 min prior to FDG. After the experiment, mice were killed using CO_2.

Micro-positron emission tomography image acquisition and analysis

An R4 small animal positron emission tomography (micro-PET) scanner (Concorde CTI Siemens, Knoxville, TN, USA) was used for FDG micro-PET imaging. The R4 micro-PET has a transaxial resolution of 2.0 mm full width at half maximum, with a field of view of 11.5 cm. Animals were placed in the center of the field of view and were scanned under a static imaging protocol for 80 min 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 that were previously described (Alexoff *et al.*, 2003; Schiffer *et al.*, 2007).

The micro-PET image analysis was performed as described previously (Pascau *et al.*, 2009) with slight modification for mouse brain. To obtain statistical parametric maps from different animals, these studies must be properly co-registered. For this purpose, all of 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 were also registered against each other. Finally, by combining all of these geometrical transformations, we can automatically detect any incorrect registration, making use of consistency measures. Once the whole dataset was properly registered, an FDG micro-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 intraperitoneal cavity. Images were analyzed using the statistical parametric mapping (SPM2) software package using a method previously described by Thanos *et al.* (2008b). In order to obtain 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 μm isotropic, relaxation time/echo time 250/5 ms) and was subsequently used for micro-PET-MRI co-registration using previously described procedures (Thanos *et al.*, 2008b).

Results

2-[^{18}F]-fluoro-2-deoxy-d-glucose brain micro-positron emission tomography image analysis

An 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 obtain 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 ($\alpha = 0.05$). 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 PFC ($K_E = 13$; $F = 24.5$; $Z_{score} = 4.14$; $P < 0.001$), left PFC ($K_E = 6$; $F = 26.8$; $Z_{score} = 4.3$; $P < 0.001$) and cerebellar vermis (CBV) ($K_E = 23$; $F = 26.3$; $Z_{score} = 4.26$; $P < 0.001$) (Fig. 1 and Table 1).

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

The statistical parametric mapping analysis yielded three significant interaction effects (Table 1). In the first, and most significant, interaction 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 ($K_E = 6$; $F = 26.8$; $Z_{score} = 4.3$; $P < 0.001$) PFC compared with $D_4^{-/-}$ 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 (Fig. 2).

Differences in metabolism induced by methylphenidate between $D_4^{+/+}$, $D_4^{+/-}$ and $D_4^{-/-}$ 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 and Fig. 3); it decreased metabolism in the left ($K_E = 6$; $F = 26.8$; $Z_{score} = 4.3$; $P < 0.001$) and right ($K_E = 13$; $F = 24.5$; $Z_{score} = 4.14$; $P < 0.001$) PFC in $D_4^{+/+}$ and $D_4^{+/-}$ mice but increased it in $D_4^{-/-}$ mice (Table 1 and Fig. 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 ($r = 0.88$; $P = 0.007$; Fig. 4).

Discussion

In this study, we document significant differences between D_4 genotypes in baseline and MP-induced changes in metabolism in the PFC and CBV. D_4 KO mice, when studied at baseline, showed lower metabolism in PFC regions and greater metabolism in CBV

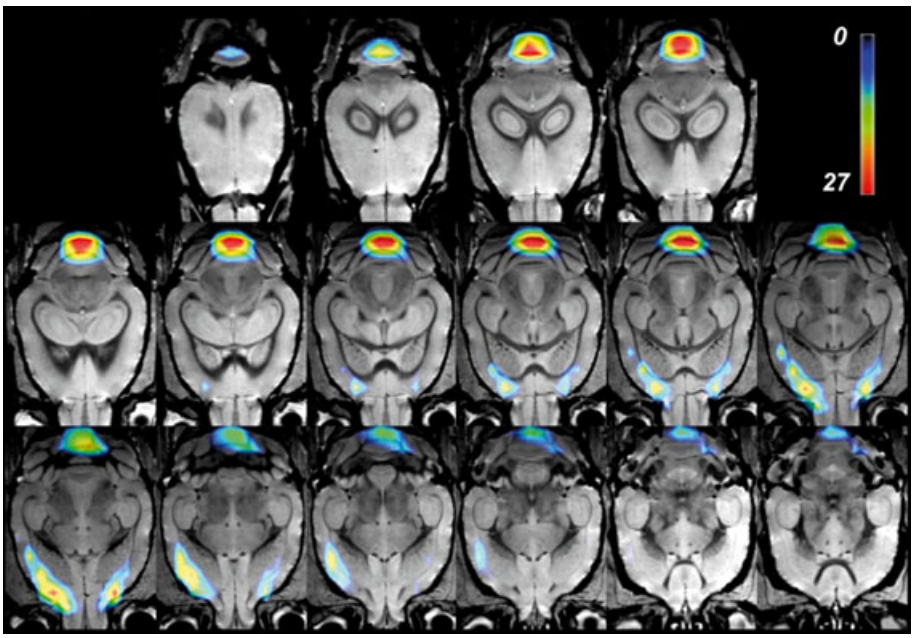

FIG. 1. Micro-PET and statistical parametric mapping (SPM2) results co-registered to an MRI template. A multivariate statistical model with factors Genotype ($D_4^{+/+}$ and $D_4^{+/-}$ vs. $D_4^{-/-}$) and Treatment (Baseline vs. MP) was used. After model estimation, an *F* contrast ($P = 0.001$) was 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 co-registered 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 Fig. 2.

TABLE 1. Greatest regional brain metabolic activation: Interaction Effects of Genotype & MP

Brain structure	Cluster level (K_E)	*F* value	*Z* score	*P* level	Stereotaxic location *x, y, z* (mm)
Right PFC	13	24.5	4.14	< 0.001	−3, −5, 10
Left PFC	6	26.8	4.3	< 0.001	1, −5, 10
CBV	23	26.3	4.26	< 0.001	0, −8, 19

when compared with $D_4^{+/-}$ and $D_4^{-/-}$ mice. Also MP in D_4 KO mice increased PFC metabolism and decreased metabolism in the CBV, whereas MP in $D_4^{+/-}$ and $D_4^{-/-}$ mice 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 the CBV. As studies have shown both volumetric and functional differences in the PFC and CB of patients with ADHD (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 patients with ADHD with the D_4 variable number of tandem repeat seven-repeat polymorphism and those without it are necessary to test this hypothesis.

In the wild-type and heterozygous mice, acute treatment with MP evoked a relative increase in metabolism in the 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 blood oxygen level-dependent responses with functional MRI have also consistently identified the CB and PFC as targets for MP effects (Giedd *et al.*, 2001; Schweitzer *et al.*, 2003; Volkow *et al.*, 2005).

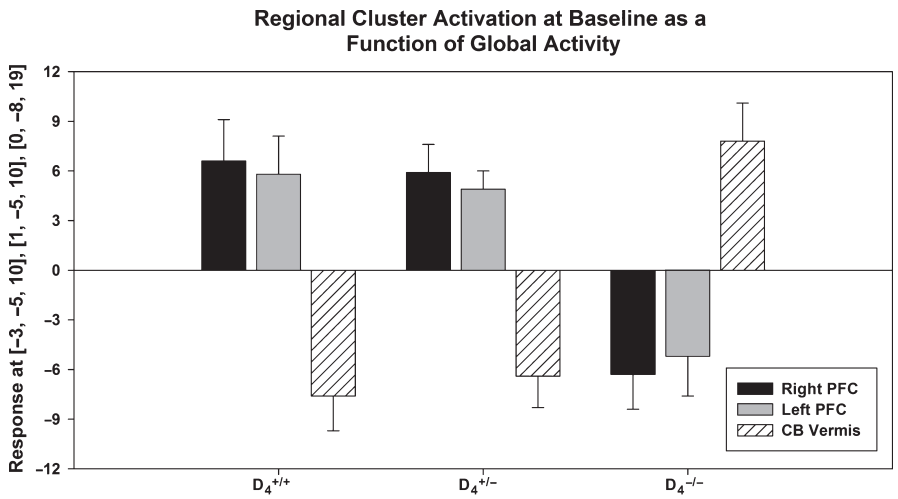

FIG. 2. Mean (+SEM) contrast responses in the three surviving clusters, left PFC, right PFC and CBV ($P = 0.001$), as obtained from statistical parametric mapping (SPM2) analysis.

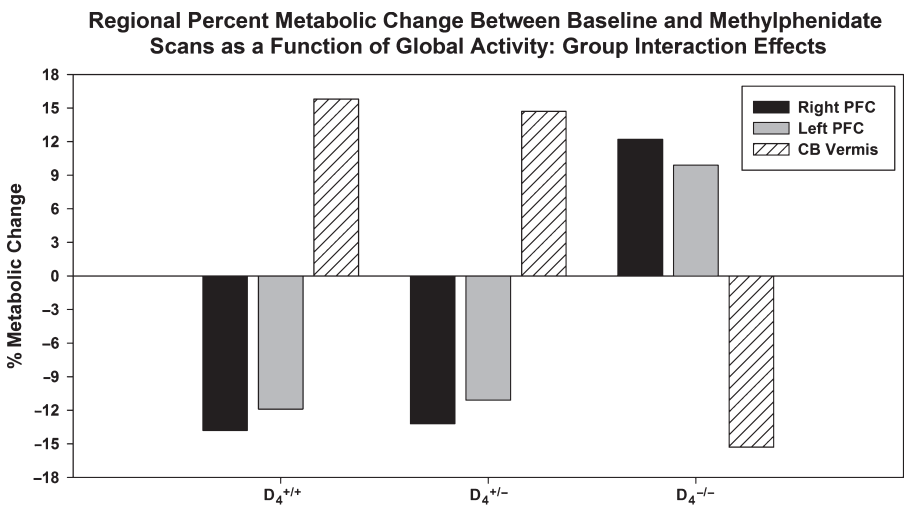

FIG. 3. Percent change in contrast response between baseline and MP scans.

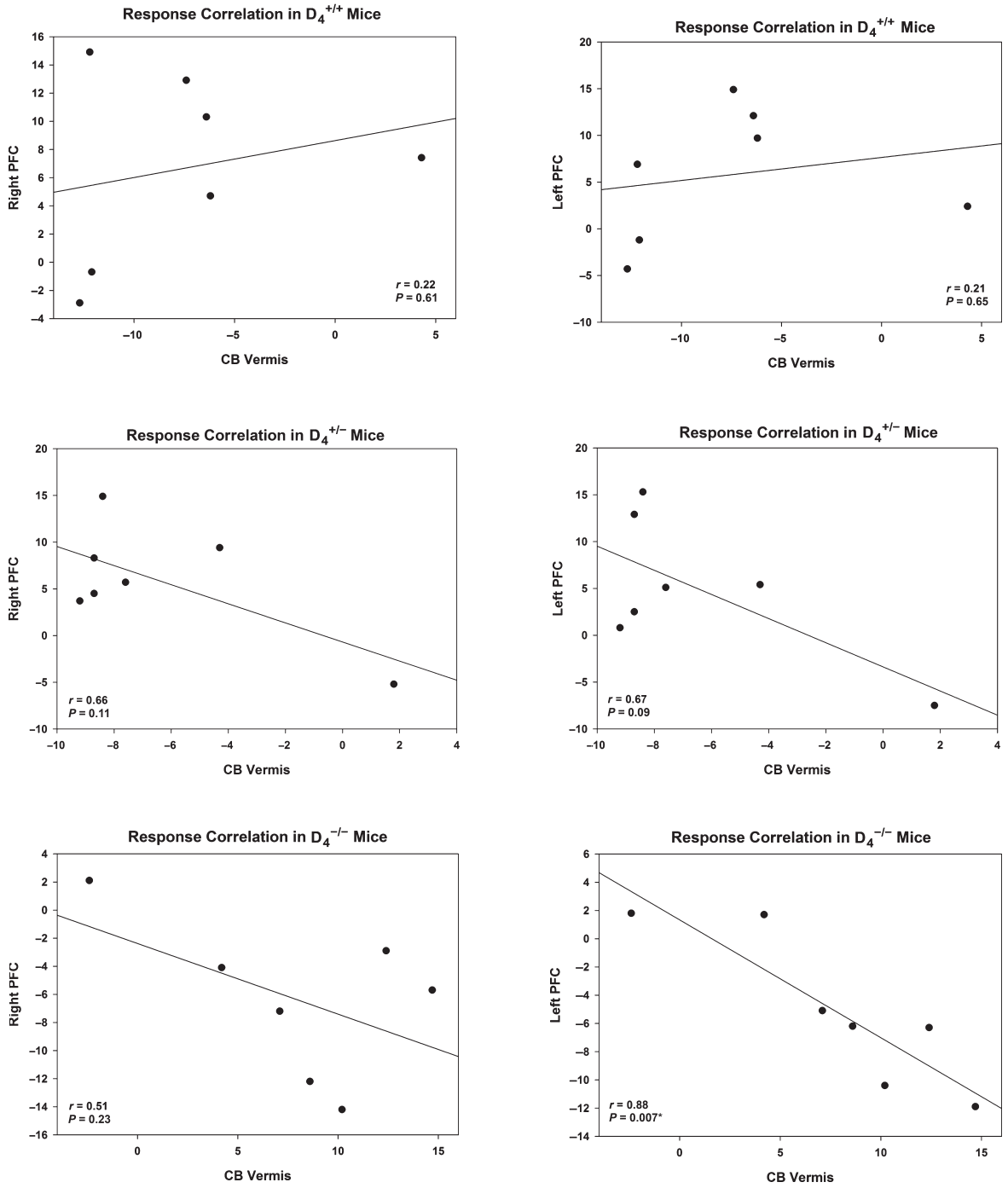

FIG. 4. Contrast response correlations between PFC and CBV by genotype.

Prefrontal cortex: effects of D_4 on basal brain glucose metabolism

The lower basal brain glucose metabolism in the PFC (bilaterally) of D_4 KO compared with 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, 2003).

The 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 PFC (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 with $D_4^{+/+}$ mice (Rubinstein *et al.*, 1997, 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 of prefrontal D4Rs in attention is given by a recent study showing that the Spontaneously-Hyperactive Rat, a rat that exhibits increased hyperactivity and impaired attention, shows lower D_4 levels in the PFC when compared with control strains that do not show attentional deficits (Li *et al.*, 2007).

Pharmacological studies also support the role of D4Rs in cognitive tasks linked with the PFC (working memory and attention). Specifically, the selective D_4 agonist A-412 997 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). However, D_4 antagonism (L745,870) has been reported to decrease cognitive-related behavior in rats (Braszko, 2009, 2010), 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 methylphenidate on brain glucose metabolism

We found a differential effect of MP based on D_4 genotype; MP increased metabolism in the 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 from imaging studies in non-ADHD humans that showed MP-induced decreases in PFC cerebral blood flow in controls (Mehta *et al.*, 2000) and with cerebral blood flow increases in the PFC in children with ADHD (Teicher *et al.*, 1996; Vaidya *et al.*, 1998). Positron emission tomography 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, 1994). The PFC effects of MP are likely to reflect in part its dopaminergic effects in the PFC as 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 patients with ADHD (in response to MP as observed in functional MRI studies) may reflect dysfunctional D4Rs on GABA and glutamate neurons in the PFC, a hypothesis that would have to be tested by further investigation. Some caution is required in 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 with $D_4^{+/+}$ and $D_4^{+/-}$ mice in the CBV, which is a brain region that modulates DA neurotransmission in the caudate and accumbens (Nieoullon *et al.*, 1978) via its projections to the ventral tegmental area (Snider *et al.*, 1976), and has furthermore been implicated in both the pathophysiology of ADHD and 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 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 patients with ADHD.

Cerebellum: effects of methylphenidate on metabolism

The $D_4^{+/+}$ 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 the T(2) relaxation time in the children with ADHD with a high score on hyperactivity, whereas it reduced it in children with ADHD 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 a smaller CBV in children with ADHD than in controls (Berquin *et al.*, 1998; Mostofsky *et al.*, 1998; Castellanos *et al.*, 2001). Functional MRI studies have also shown a specific activation profile of the CB in response to MP in normal subjects (Anderson *et al.*, 2006). Positron emission tomography imaging studies with FDG and $[^{15}O]H_2O$ have also shown MP-specific increases in cerebellar energy metabolism and cerebral blood flow, respectively (Matochik *et al.*, 1993; Volkow *et al.*, 1997a; Schweitzer *et al.*, 2003; Udo de Haes *et al.*, 2007).

In addition to the norepinephrine transporter (Kung *et al.*, 2004), the DA transporter is also expressed in the CBV (albeit at low levels) (Melchitzky & Lewis, 2000). Thus, MP effects in the CBV are likely to reflect its blockade of both the norepinephrine transporter and DA transporter (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 demonstrated 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 ($P = 0.09$) 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 micro-PET studies (Alexoff *et al.*, 2003; Marsteller *et al.*, 2006). Finally, this is the first study to report the use of statistical parametric mapping 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 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.

Acknowledgements

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Abbreviations

ADHD, attention deficit hyperactivity disorder; CB, cerebellum; CBV, cerebellar vermis; D_4, dopamine D4 receptor; D4R, dopamine D4 receptor; DA, dopamine; FDG, 2-[^{18}F]-fluoro-2-deoxy-D-glucose; KO, knockout; micro-PET, small animal positron emission tomography; MP, methylphenidate; MRI, magnetic resonance imaging; PFC, prefrontal cortex.

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