

Cannabis Abusers Show Hypofrontality and Blunted Brain Responses to a Stimulant Challenge in Females but not in Males

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The extent to which cannabis is deleterious to the human brain is not well understood. Here, we test whether cannabis abusers (CA) have impaired frontal function and reactivity to dopaminergic signaling, which are fundamental to relapse in addiction. We measured brain glucose metabolism using PET and [¹⁸F]FDG both at baseline (placebo) and after challenge with methylphenidate (MP), a dopamine-enhancing drug, in 24 active CA (50% female) and 24 controls (HC; 50% female). Results show that (i) CA had lower baseline glucose metabolism than HC in frontal cortex including anterior cingulate, which was associated with negative emotionality. (ii) MP increased whole-brain glucose metabolism in HC but not in CA; and group by challenge effects were most profound in putamen, caudate, midbrain, thalamus, and cerebellum. In CA, MP-induced metabolic increases in putamen correlated negatively with addiction severity. (iii) There were significant gender effects, such that both the group differences at baseline in frontal metabolism and the attenuated regional brain metabolic responses to MP were observed in female CA but not in male CA. As for other drug addictions, reduced baseline frontal metabolism is likely to contribute to relapse in CA. The attenuated responses to MP in midbrain and striatum are consistent with decreased brain reactivity to dopamine stimulation and might contribute to addictive behaviors in CA. The gender differences suggest that females are more sensitive than males to the adverse effects of cannabis in brain.

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INTRODUCTION

Despite the high prevalence of cannabis consumption worldwide, the effects of cannabis abuse in the human brain are not well understood. The expanding legalization of cannabis for medical or recreational purposes in the USA and in other countries poses a sense of urgency towards addressing the limited evidence regarding potential deleterious effects of cannabis to the human brain. Here, we test the hypothesis that chronic cannabis abusers (CA) would show impaired activity of frontal brain regions, which is a robust finding in addiction, and is associated with impaired self-regulation (Goldstein and Volkow, 2011; Volkow and Morales, 2015a). Reduced glucose metabolism (marker of brain function) in frontal brain regions has been shown in CA even after 3–4 months of abstinence (Volkow *et al*, 1992b, 1993), alcoholics (Volkow *et al*, 1992a), and in heavy drinkers (Volkow *et al*, 2015b). Moreover, in a pilot study, we showed decreased frontal (and cerebellar) metabolism in

eight male CA compared with age-matched male controls (Volkow *et al*, 1996).

We recently showed that CA had attenuated behavioral and cardiovascular responses to a methylphenidate challenge (MP), a stimulant drug that increases dopamine (DA), and an attenuated reduction in MP-induced decreases in the distribution volumes of [¹¹C]raclopride, which is a radio-tracer whose binding to DA D2 receptors (D2R) in brain is reduced when DA is increased (Volkow *et al*, 2014). Here we extend these findings to assess whether MP-induced responses on brain glucose metabolism (Sokoloff *et al*, 1977) are also attenuated in CA. We hypothesized that blunted responses to MP in regional brain glucose metabolism would be associated with addiction severity and with attenuated behavioral effects of MP (eg, self-reported measures of control, restlessness, and cannabis craving), as previously found in CA (Volkow *et al*, 1999).

Recent findings suggest higher expression of cannabinoid type-1 (CB1) receptors in the brain of women than men (Normandin *et al*, 2015). There is also evidence that women compared with men are less likely to be chronic cannabis users (Preston, 2006). This led us to additionally investigate whether there were group by gender interactions in baseline and MP-induced changes in brain metabolism.

For these purposes, we measured regional brain glucose metabolism using PET and [¹⁸F]deoxyglucose (FDG) in 24

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active CA (50% females) and 24 healthy control participants (HC) (50% females). Regional brain glucose metabolism was measured twice in each participant in a randomized order: after placebo (which was used as baseline) and after challenge with intravenous MP. MP increases DA by blocking DA transporters (Volkow *et al*, 1998) and was used as challenge to assess the reactivity of the brain to DA stimulation.

We hypothesized that (i) CA compared with HC would show decreased baseline frontal metabolism, (ii) CA compared with HC would show blunted regional brain metabolic responses to MP, and (iii) group differences would be stronger for females than for males.

MATERIALS AND METHODS

Subjects

Twenty-four CA (12 females) and 24 HC (12 females) completed the studies. Participants were recruited from advertisements in local newspapers. At least two clinicians interviewed the patients to ensure that they met DSM-IV diagnostic criteria for cannabis abuse or dependence, with a semi-structured standardized interview. Participants were excluded if they had a history of substance abuse or addiction (other than cannabis abuse/dependence in the cannabis group and nicotine abuse/dependence), a history of psychiatric disease (other than cannabis abuse/dependence), neurological disease, medical conditions that may alter cerebral function (ie, cardiovascular, endocrinological, oncological, or autoimmune diseases), current use of prescribed or over-the-counter medications, and/or head trauma with loss of consciousness of more than 30 min. All subjects had Hamilton's Anxiety and Depression scores < 19 (Hamilton, 1960). Exclusion criteria for HC were the same as for CA other than allowance for regular cannabis use (ie, exclusion for controls was use of cannabis more than 1 day a month). All subjects had a physical, psychiatric, and neurologic examination. Drug screens were performed on the days of the PET studies to exclude the use of psychoactive drugs (other than cannabis in CA). Subjects were free from any over-the-counter medication 2 weeks prior to the PET scan. Food and beverages (except for water) were discontinued at least 4 h prior and cigarettes for at least 2 h prior to the study. This study was approved by the Committee on Research in Human Subjects at Stony Brook University (IRB net number 225114) and written informed consent was obtained from all subjects.

Behavioral Measures

Personality measures. Participants completed the Multi-dimensional Personality Questionnaire (MPQ), which provides rating for three main factors: positive emotional temperament (PEM), negative emotional temperament (NEM), and constraint (Tellegen and Waller, 2008). PEM is a combination of scores for well-being (reward sensitivity), social potency, achievement (motivation), and social closeness; high PEM reflects behavior and temperamental characteristics conducive to joy, and to active and rewarding engagement with social and work environments, whereas low PEM reflects the tendency to experience joylessness, loss

of interest, and fatigue, reflecting non-pleasurable and possibly depressive disengagement. NEM is a combination of scores for stress reaction, alienation, and aggression; high NEM reflects proneness to experience anxiety, anger, and related emotional and behavioral negative engagement, whereas low NEM reflects a disposition to calm, relaxation, and other non-pleasurable states of disengagement. Constraint is a combination of scores for self-control, harm avoidance, and traditionalism; high Constraint reflects a tendency to inhibit and restrain impulse expression, unconventional behavior, and risk-taking, whereas a person with low Constraint is inclined to act on impulse, take risks, and ignore conventional restrictions. We also particularly looked at the NEM subscale Alienation; with high scores reflecting people who believe that others wish them harm; being victims of false and nasty rumors; feeling pushed around; feeling used by friends; having been betrayed and deceived; and generally having had a lot of bad luck (Tellegen and Waller, 2008).

Marijuana dependency questionnaire. The Marijuana Dependency Questionnaire (MDQ) scores 7 symptoms of dependence as per DSM IV, each on a range from 0 to 3. The MPQ and MDQ were obtained on the day of screening.

We also assessed subjective ratings and cardiovascular measures 60 min after placebo and MP injection, which are reported in the Supplementary Material.

PET Scanning

PET studies were performed with a Siemens HR+ tomograph. Images were reconstructed using filtered back projection (Hann filter with a 4.9 mm Kernel FWHM). Each subject underwent two PET FDG scans on two separate days. On a given day, each subject was injected with placebo (3 cc saline iv) and then 90 min later with FDG, and on another they were first injected with MP (0.5 mg/kg iv) followed 90 min later with FDG. The order of administration was randomized across group and gender. The study was a single blind design: ie, subjects were blind to the drugs received. Imaging procedure consisted of an emission scan obtained for 20 min and started 35 min after injection of 4–6 mCi of FDG using previously described procedures (Wang *et al*, 1993). Blood sampling was obtained from a catheter placed in the radial artery, which was used to measure the concentration of radiotracer in plasma. During the uptake period of FDG, subjects remained in a supine position with their eyes open in a darkly lit room and noise was kept to a minimum except for the periodic assessment of drug effects. Metabolic rates were computed using an extension of Sokoloff's model (Phelps *et al*, 1979). To reduce effects of menstrual cycle, females were scanned during their mid-follicular phase.

The data were normalized to the FDG template in MNI space in SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/>). Whole-brain and SPM analyses were performed on the absolute metabolic images. For SPM, the metabolic images were spatially smoothed using an 8-mm Gaussian kernel to account for the variability of the brain anatomy across subjects.

Table 1 Demographics, Clinical Characteristics, and Personality Scores (MPQ) in Male and Female Cannabis Abusers and Healthy Controls

Characteristic	Cannabis abusers		Healthy controls		P-value		
	Male N = 12	Female N = 12	Male N = 12	Female N = 12	Group	Gender	Group × Gender
Age, years	29.0 ± 8.8	24.6 ± 4.3	30.3 ± 7.0	26.2 ± 26.2	NS	0.032	NS
Years of education	12.9 ± 1.3	13.5 ± 1.5	13.6 ± 1.3	14.2 ± 1.8	NS	NS	NS
BMI	25.7 ± 3.1	22.6 ± 3.5	24.9 ± 3.1	23.7 ± 2.2	NS	0.017	NS
Cigarette smokers	7 active 1 former	3 active 1 former	3 active 1 former	1 active 0 former	NS	NS	NS
Cannabis age initiation	14.8 ± 3.0	15.2 ± 2.4	—	—		NS	
Days/week	6.7 ± .7	6.8 ± .5	—	—		NS	
Joints/day	4.9 ± 3.8	4.8 ± 2.9	—	—		NS	
Years abuse	12.9 ± 9.1	9.0 ± 4.7	—	—		NS	
MDQ sum	6.5 ± 2.7	4.3 ± 2.6	—	—		NS	
BDI	8.6 ± 4.1	9.0 ± 8.2	6.1 ± 9.5	5.4 ± 5.6	0.06	NS	NS
MPQ PEM	51.3 ± 10.4	43.2 ± 9.6	50.8 ± 5.6	51.7 ± 10.1	0.05	NS	NS
MPQ NEM	22.8 ± 7.7	21.9 ± 10.0	16.0 ± 10.4	11.4 ± 6.0	0.001	NS	NS
MPQ Constraint	45.6 ± 7.4	49.8 ± 8.1	48.6 ± 10.9	54.0 ± 7.9	NS	NS	NS

Abbreviations: BDI, Beck's Depression Index; BMI, body mass index; MDQ, Marijuana Dependence Questionnaire; MPQ, Multidimensional Personality Questionnaire; NEM, negative emotionality; NS, non-significant = $p \geq 0.1$; PEM, positive emotionality.

Characteristics are listed with mean ± SD. Mann–Whitney *U* test. Bold values indicate *P*-values < 0.1.

Statistical Analyses

To calculate the main effects of group, gender, and drug challenge on behavioral, cardiovascular, and whole-brain glucose metabolism and its interaction (group × gender × drug-challenge), we used mixed ANOVAs in SPSS 22 (IBM, Armonk, NY). *Post hoc t*-tests were performed to determine the direction of findings. There was a main effect of gender on body-mass index ($F = 6.2$, $p = 0.017$) and age ($F = 4.9$, $p = 0.032$), with females having lower body-mass indexes and being younger than males. We therefore used body-mass index and age as covariates in gender interactions, for we had previously shown that regional brain glucose metabolism correlated negatively with age (De Santi *et al*, 1995; Volkow *et al*, 2000b) and with body-mass index in healthy volunteers (Volkow *et al*, 2009).

Second-level voxel-wise analyses were performed in SPM8 using a mixed design ANOVA model with group (CA and HC) as between-subject factor and challenge (MP and Placebo) as within subject factor. We also investigated group × gender interactions for baseline and MP-induced differences in metabolism. The statistical significance for the *a priori* hypotheses: (i) that baseline frontal and cerebellar metabolism would be lower in CA than HC, we set significance at $p < 0.005$ uncorrected, $k \geq 10$; (ii) that metabolic responses to MP would be attenuated in DA regions and their projections targets (caudate, putamen, nucleus accumbens, midbrain) in CA compared with HC was tested with small volume correction (SVC) for caudate, putamen, nucleus accumbens, and midbrain using the WFU Pickatlas (Maldjian *et al*, 2003) with a significance threshold of $p < 0.05$ family-wise error corrected (FWE); and for the whole brain at $p < 0.001$ uncorrected, $k \geq 10$; (iii) that group differences would be stronger in females than males, we set significance at $p < 0.001$ uncorrected, $k \geq 10$. We computed

average metabolic values within clusters that showed significant difference in CA < HC using SPM8 and tested their associations with severity of dependence (MDQ) using Pearson's correlations in SPSS 22. We tested normality for demographics and physiological measures using the Kolmogorov-Smirnov (K-S) test for each group separately. Demographic variables were normally distributed on the K-S test (all $p > 0.05$), except education years for HC ($D = 0.2$, $p = 0.007$) and CA ($D = 0.3$, $p < 0.001$), BDI for HC ($D = 0.2$, $p = 0.006$), and cannabis age initiation ($D = 0.2$, $p = 0.04$), cannabis days/week ($D = 0.45$, $p < 0.001$) joints/day ($D = 0.3$, $p < 0.001$), and years of abuse ($D = 0.2$, $p = 0.007$). Cardiovascular measures and whole-brain measures of glucose metabolism (PL and MP) were distributed normally in both groups (K-S test: all $p > 0.05$). For variables that were not normally distributed, we used the Mann–Whitney *U* test for group comparisons and Spearman's rank-order rho for correlation analyses.

RESULTS

Group Characteristics

There were no differences in demographics between the groups (Table 1). Groups differed significantly in personality measures on the MPQ; CA had significantly lower scores in Positive Emotionality (PEM) ($t = 2.4$, $p = 0.02$) and higher scores in Negative Emotionality (NEM) ($t = 3.5$, $p = 0.001$) than HC (Table 1). There were no gender or gender by group interactions for the personality measures.

PET Measures

Baseline brain glucose metabolism in CA vs HC. Whole-brain measures of glucose metabolism at baseline did not differ between groups ($F = 0.05$, $p = 0.82$).

Table 2 Baseline Regional Brain Glucose Metabolism in Cannabis Abusers vs Controls

Brain region	BA	Hemi	K	MNI (x y z)	T-value
<i>CA < HC</i>					
Fusiform gyrus	37	R	20	42 -43 -17	3.87
Inferior parietal cortex	40	R	21	57 -43 28	3.68
Middle frontal gyrus	8	L	22	-54 8 46	3.60
Inferior frontal gyrus	44	L	11	-48 5 25	3.43
Anterior cingulate	24	R	12	9 8 22	3.34
Anterior cingulate	24, 32	L	41	-9 14 22	3.28

HC < CA

No significant voxels

Abbreviations: BA, Brodmann area; CA, cannabis abusers; HC, healthy controls; Hemi, hemisphere; K, cluster size; L, left; MNI (x y z), Coordinates in Montreal Neurological Institute space (x y z); R, right.
 $p < 0.005$ uncorrected, $k \geq 10$.

SPM analysis for the *a priori* analysis (threshold $p < 0.005$ uncorrected, $k \geq 10$) revealed that CA had lower regional metabolic measures in frontal regions, including anterior cingulate cortex (BA 24, BA 32, BA 31), medial and inferior frontal gyrus compared with HC (see Table 2; Figure 1). There were no significant voxels for the reverse contrast of $CA > HC$. The SPM also revealed differences in fusiform gyrus and inferior parietal cortex, which were regions that we had not identified *a priori* and thus we are not considering them as significant.

Correlation with MPQ. In both groups pooled together, whole-brain glucose metabolism correlated significantly with NEM ($r = -0.314$, $p = 0.028$), particularly with the alienation subscale ($r = -0.421$, $p = 0.003$). This was due to a negative correlation in HC (NEM: $r = -0.535$, $p = 0.007$, AL: $r = -0.656$, $p = 0.001$), but not in CA (ns). This pattern was also shown for brain areas that showed lower uptake in CA than HC: alienation correlated with all brain areas in HC (fusiform gyrus $r = -0.424$; inferior parietal cortex $r = -0.439$, medial frontal gyrus $r = -0.50$, and inferior frontal gyrus $r = -0.443$, anterior cingulate cortex $r = -0.375$; all $p < 0.05$), but only with inferior frontal gyrus in CA ($r = -0.367$, $p < 0.05$) (Figure 2).

Effects of MP on brain metabolism in CA and HC. For whole-brain glucose metabolism, there was a main effect of challenge ($F = 6.94$, $p = 0.012$) and an interaction of group \times gender ($F = 4.14$, $p = 0.048$). *Post hoc* paired *t*-tests showed that MP increased whole-brain metabolism in HC (Placebo: 32.8 ± 5.3 SD vs MP: 36.5 ± 6.5 $\mu\text{mol/g/min}$; $t = 3.0$, $p = 0.006$), but in CA, the effects were not significant (Placebo: 32.5 ± 4.8 vs MP: 33.9 ± 5.6 $\mu\text{mol/g/min}$, $t = 1.1$, $p = 0.27$).

SPM showed that the largest MP-induced increase in regional brain glucose metabolism occurred in hippocampus, bilateral thalamus, bilateral occipital cortex (BA 17), insula, and inferior temporal gyrus (all $p < 0.001$ uncorrected, $k \geq 10$; see Table 3).

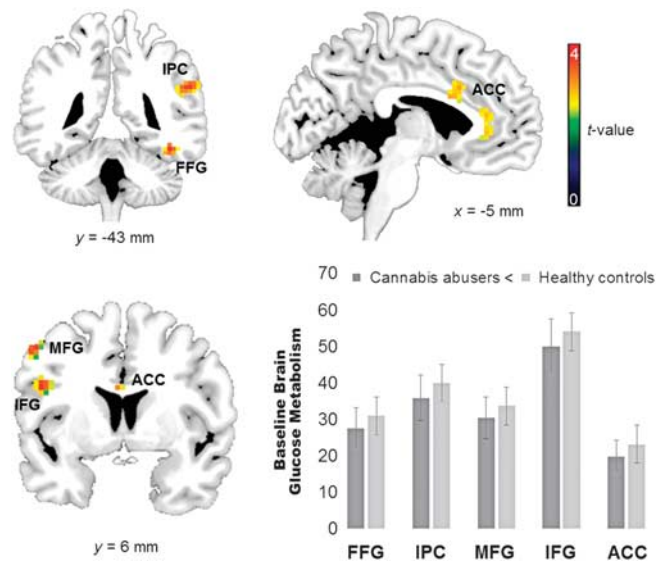


Figure 1 SPM results for the comparison of baseline regional brain glucose metabolism between cannabis abusers and healthy controls. CA had lower metabolism in the fusiform gyrus (FFG), inferior parietal gyrus (IPG), inferior frontal gyrus (IFG), anterior cingulate cortex (ACC), and middle frontal gyrus (MFG) ($p < 0.005$ uncorrected). For visualization purposes, activations were plotted at $p < 0.01$ uncorrected.

For the *a priori* hypothesis that CA would have blunted response to MP in DA projection regions, we showed that the right putamen, left caudate, and midbrain (all $p < 0.05$ FWE-corrected SVC) but not nucleus accumbens ($p > 0.05$ FWE-corrected) had lower MP-induced activation in CA vs HC (see Table 3). The exploratory whole-brain analysis showed that increases in metabolism were stronger in HC than CA not just in midbrain, putamen, and caudate (*a priori* regions), but also in cerebellum and thalamus (all $p < 0.001$ uncorrected) (see Figure 3; Table 3).

Correlations with addiction severity. Metabolic values in the regions that showed a main MP-induced difference of $CA < HC$ (ie, putamen, caudate, midbrain, cerebellum, thalamus) were extracted using SPM8.

In CA, the measures of addiction severity (MDQ scores) correlated with MP-induced changes in metabolism in putamen ($r = -0.37$, $p = 0.04$, one-tailed; see Figure 4). Please see Supplementary Material for exploratory correlations between MP-induced changes in behavioral ratings with MP-induced changes in regional brain glucose metabolism.

Group by gender interactions on baseline and MP-induced changes in regional brain glucose metabolism. At baseline, there was no group by gender interaction effect for whole-brain glucose metabolism ($F = 0.51$, $p = 0.48$), nor an effect of gender ($F = 2.43$, $p = 0.13$). For the regional brain metabolic measures at baseline, SPM analyses showed a significant group \times gender interaction in bilateral medial frontal gyrus, right superior frontal gyrus, and right occipital cortex, all $p < 0.001$ uncorrected, $k \geq 10$ (Table 4). *Post hoc* contrasts showed no group differences in male $CA < HC$ (all $p > 0.005$ uncorrected), but female CA had significant lower metabolism in left superior frontal gyrus, right occipital

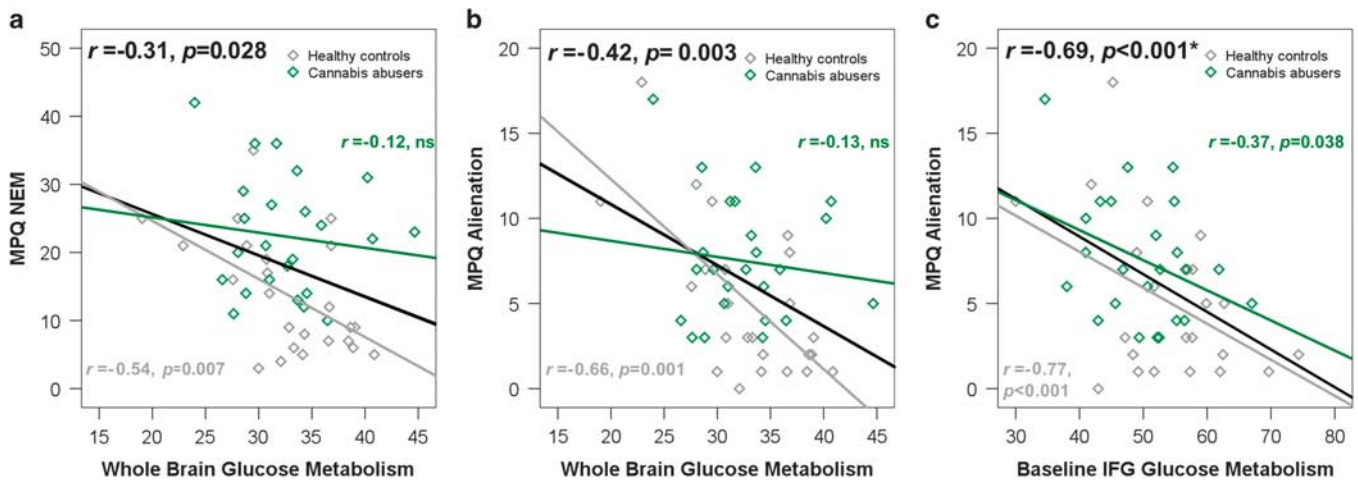


Figure 2 Whole-brain glucose metabolism correlated with negative emotionality (NEM) (a) particularly with the alienation subscale (b). Regional glucose metabolism in frontal areas that showed cannabis abusers < healthy controls (CA < HC) group differences correlated with alienation in all brain areas in HC, and only with IFG in CA (c). * Note that this correlation may be artificially high owing to drug group differences in both IFG glucose metabolism and MPQ alienation. Regressions for groups pooled together are depicted in black, for HC in gray and for CA in green. MPQ, Multidimensional Personality Questionnaire.

Table 3 Effects of MP on Regional Brain Glucose Metabolism: Main Effects and Interaction with Group (Cannabis Abusers vs Controls)

Brain region	BA	Hemi	K	MNI (x y z)	T-value
<i>PL < MP</i>					
Cerebellum/Midbrain		R/L	4598	6 -70 -32	7.87 ^a
Hippocampus	17	R	30	30 -28 -8	4.50
Thalamus		L	33	-21 -22 7	4.14
Thalamus		R	35	9 -13 7	4.06
Occipital cortex		R	56	12 -76 10	4.05
Mid temp gyrus/insula	21	L	24	-39 2 -32	4.01
Inferior temporal gyrus	20	R	57	78 -25 -17	3.98
Occipital cortex	17	L	13	-9 -88 7	3.51
<i>Interaction PL < MP, CA < HC</i>					
Cerebellum		R	38	21 -49 -23	4.20
Putamen		R	10	24 -1 13	3.80 ^b
Caudate		L	5	-21 11 22	3.60 ^b
Midbrain		L	4	15 -22 -17	3.63 ^b
Thalamus		R	10	9 -13 10	3.39

Abbreviations: BA, Brodmann area; CA, cannabis abusers; FWE, family-wise error corrected; HC, healthy controls; Hemi, hemisphere; K, cluster size; L, left; MNI (x y z), Coordinates in Montreal Neurological Institute space (x y z); MP, methylphenidate; PL, placebo; R, right; SVC, small-volume corrected. $p < 0.001$ uncorrected, $k \geq 10$.

^a $p < 0.05$ FWE.

^b $p < 0.05$ FWE, SVC.

cortex, and right anterior cingulate cortex than female HC (Table 4; Figure 5).

There was an interaction of group \times challenge \times gender at trend level for whole-brain metabolism ($F = 3.93$, $p = 0.054$): Increases in whole-brain metabolism with MP were observed in females in both groups pooled together ($9.8 \pm 16.4\%$ increase; $t = 2.9$, $p = 0.007$), but not in males ($5.4 \pm 20.2\%$ increase, $t = 1.3$, $p = 0.21$). However, increases in females were driven by HC who were the only ones showing

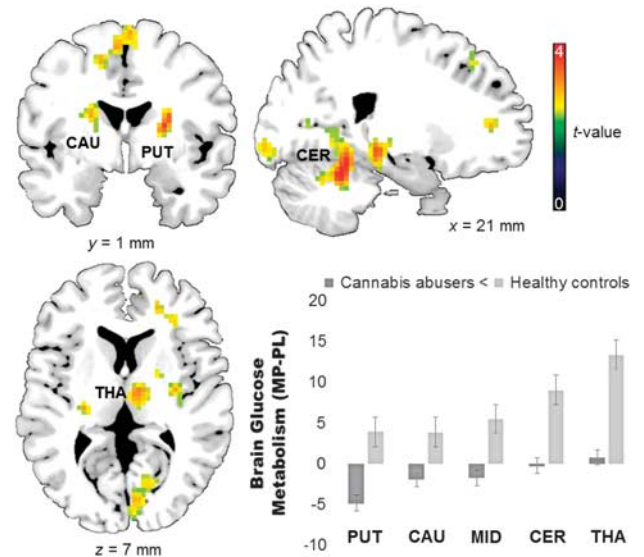


Figure 3 Group comparison cannabis abusers vs healthy controls (CA vs HC) of MP effects (MP-PL) on regional brain glucose metabolism. Cannabis abusers showed lower MP-PL activation in putamen (PUT), caudate (CAU), midbrain (MID), cerebellum (CER), and thalamus (THA) ($p < 0.001$ uncorrected). There were no regions where MP induced stronger metabolic increases in CA vs HC. For visualization purposes, activations were plotted at $p < 0.01$ uncorrected.

significant increases with MP (16.9% increase, $t = 3.9$, $p = 0.002$), whereas neither female/male CA nor male HC showed significant effects (all $p > 0.36$; Figure 5).

For regional effects, the exploratory whole-brain SPM analyses also showed a significant gender by group interaction effect. Female CA showed blunted MP-induced responses in cerebellum, medial frontal gyrus, pons, and in a cluster that encompassed hippocampus, thalamus, and midbrain, whereas there were no differences in males (Table 4; Figure 5).

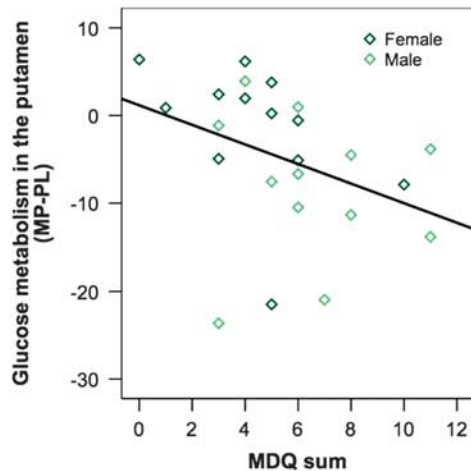


Figure 4 Measures of addiction severity (MDQ scores) correlated with MP-induced metabolic changes in putamen ($r = -0.37$, $p < 0.05$, one-tailed). There were no effects of gender.

DISCUSSION

Baseline Brain Glucose Metabolism in CA vs HC

Here, we show, first, reduced baseline metabolism in frontal brain regions in CA compared with controls, which is consistent with prior findings of impaired frontal baseline metabolism in other drug addictions (Volkow and Fowler, 2000a; Goldstein and Volkow, 2011), including CA (Volkow *et al*, 1996; Jacobus *et al*, 2012). Decreased baseline frontal metabolic activity has been associated with low DA D2 receptors in striatum (Volkow *et al*, 1993, 2001, 2013) and the associated reduced dopaminergic tonic stimulation (Volkow and Morales, 2015a), and clinically, it is associated with reductions in self-regulation and higher rates of relapse (Goldstein and Volkow, 2011; Volkow and Morales, 2015a). Moreover, enhancing DA signaling with oral MP has been shown to normalize frontal activation and to improve cognitive task performance in cocaine addiction (Goldstein *et al*, 2010; Moeller *et al*, 2014).

In the current study, decreased whole-brain and frontal metabolism correlated with negative emotionality (NEM) scores on the MPQ, particularly the subscale of alienation for which CA showed significantly higher scores than HC. These findings are consistent with a study that showed decreased frontal BOLD activation on an emotion-arousal task in CA that mediated the relationship between cannabis abuse and negative emotionality (Heitzeg *et al*, 2015). They are also in line with previous findings that in CA, reduced brain DA signaling was associated with negative emotionality (Volkow *et al*, 2014) and negative symptoms and inattention (van de Giessen *et al*, 2016), and reduced DA synthesis with subjective apathy (Bloomfield *et al*, 2014b). Further, DA synthesis capacity was negatively associated with higher levels of cannabis use and positively associated with age of onset of cannabis use (Bloomfield *et al*, 2014a), and stress-induced DA release was shown to be associated with duration of cannabis use in chronic CA (Mizrahi *et al*, 2013). This suggests that cannabis use leads to impairment in

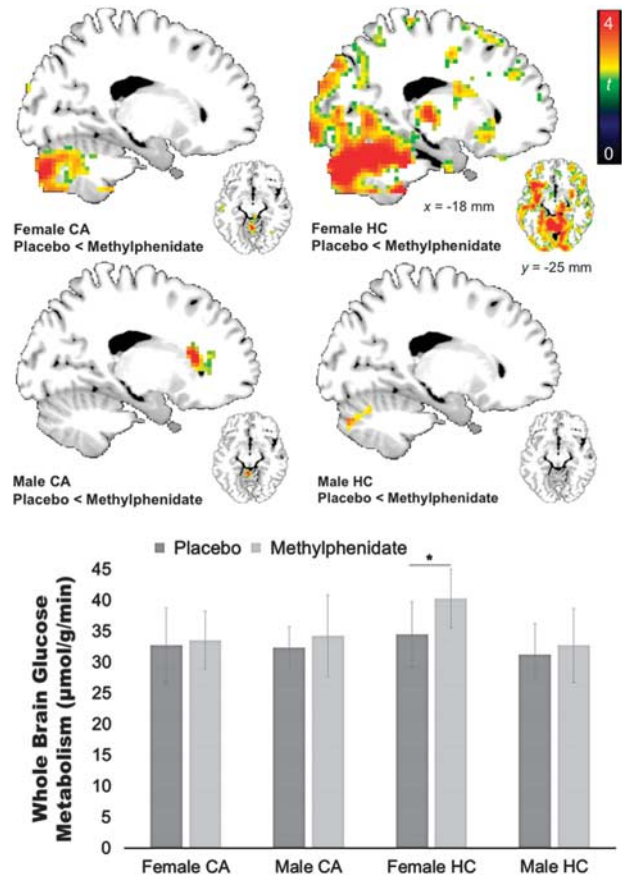


Figure 5 MP increased whole-brain metabolism in controls (HC) ($p < 0.01$), but the effects in cannabis abusers (CA) were not significant. In HC, the increases in whole-brain metabolism with MP were driven by gender because increases with MP were observed in females ($p < 0.01$) but not in males. Only female HC significantly increased whole-brain metabolism ($p < 0.01$), but not female CA, male CA, or male HC. SPM analyses showed that female CA compared with female HC had lower MP-induced increases in regional metabolism in cerebellum, medial frontal gyrus, pons, hippocampus, and thalamus ($p < 0.001$ uncorrected). For visualization purposes, activations were plotted at $p < 0.01$ uncorrected, cluster size 50 voxels. There were no differences in MP reactivity in male CA vs HC.

frontal brain function and in DA neurotransmission, which contribute to negative emotional states in CA.

Effects of MP on Brain Metabolism in CA and HC

Second, we show attenuated responses to MP in CA vs HC in the hypothesized striatal DA projections, though not in prefrontal cortex or NAc, which is consistent with reduced reactivity to dopaminergic stimulation previously found in CA (Volkow *et al*, 2014). MP-induced increases in brain metabolism in the putamen of CA were negatively correlated with Marijuana Dependency Questionnaire scores, suggesting that blunted striatal DA reactivity to MP is associated with addiction severity. The mechanism responsible for reduced striatal reactivity to MP may reflect downregulation of DA transporters, because lower than normal DA transporter has been reported in CA (Leroy *et al*, 2012). Similarly, we reported an attenuation of MP-induced reduction in the distribution volume of [^{11}C]raclopride, which is an indication of DA release in CA (Volkow *et al*,

Table 4 Gender Effects in Baseline and MP-induced Brain Glucose Metabolism

Brain region	BA	Hemi	K	MNI (x y z)			T-value
Baseline, interaction female < male, CA < HC							
Middle frontal gyrus	8	R	24	39	14	49	4.33
Superior frontal gyrus	9	R	17	0	11	67	3.97
Occipital cortex	17	R	12	54	-85	-2	3.64
Baseline, female CA < HC							
Superior frontal gyrus	9	L	13	-12	17	55	3.79
Occipital cortex	17	R	10	45	-61	-14	3.68
Anterior cingulate	24	L	10	-9	23	31	3.54
Baseline, male CA < HC							
No significant voxels							
PL < MP, female CA < HC							
Cerebellum		R	19	6	-61	-14	4.10
Medial frontal gyrus		R	50	0	-22	55	4.08
Cerebellum		R	10	18	-46	-17	3.98
Pons		L	25	-18	-28	-50	3.90
Hippocampus/thalamus/midbrain		R	22	18	-25	-11	3.84
PL < MP, male CA < HC							
No significant voxels							

Abbreviations: BA, Brodmann area; CA, cannabis abusers; HC, healthy controls; Hemi, hemisphere; K, cluster size; L, left; MNI (x y z), Coordinates in Montreal Neurological Institute space (x y z); MP, methylphenidate; PL, placebo; R, right. $p < 0.001$ uncorrected, $k \geq 10$.

2014), consistent with prior findings in CA with psychosis of reduced DA release when challenged with a stimulant drug or when exposed to a stressor (Thompson *et al*, 2013), though others have not shown reduced DA release in CA (Urban *et al*, 2012). In controls, we previously showed that MP-induced increases in brain glucose metabolism in cortical regions were modulated by striatal D2R availability, such that MP increased brain metabolism in subjects with high striatal D2R, whereas it decreased metabolism in subjects with low D2R (Volkow *et al*, 1997). Thus, down-regulation of D2R could also result in an attenuated brain metabolic response to MP. This is, however, unlikely to account for our results because striatal D2R availability was not reduced in CA (Volkow *et al*, 2014). Finally, it is also possible that the attenuated responses reflect downstream modulation from D2R or other DA receptors (D1, D3, D4, or D5). In this respect, our findings are opposite to what we observed in alcoholics in whom changes in regional brain metabolism induced by MP were enhanced compared with controls even though DA release to MP was attenuated (Volkow *et al*, 2013).

The exploratory analyses also revealed group differences in MP-induced metabolic changes in the cerebellum and thalamus, which are regions that receive dense noradrenergic innervation (Olson and Fuxe, 1971; Sara, 2009). Moreover, the cerebellum has minimal DA innervation, which suggests that reduced metabolic increases to MP in CA could also reflect attenuated reactivity to MP-induced noradrenergic stimulation (Tilley and Gu, 2008). The cannabinoid system is

an important regulator of noradrenergic systems in part via CB1 receptors (Reyes *et al*, 2009; Carvalho and Van Bockstaele, 2012; Cathel *et al*, 2014) and downregulation of CB1 receptors in CA (Hirvonen *et al*, 2012) could underlie the attenuated responses to MP-induced changes in metabolism observed in these brain regions. A prior study in CA showed that co-administration of Δ -9-tetrahydrocannabinol (THC) with ecstasy, which like MP is a monoamine transporter blocker, enhanced its cardiovascular effects and the increases in epinephrine and norepinephrine in plasma (Dumont *et al*, 2009). This is indicative of enhanced effects to ecstasy when CB1 receptors are co-stimulated by THC. Thus, downregulation of CB1 receptors in CA could contribute to their attenuated responses to MP.

Group by Gender Interactions on Baseline and MP-Induced Changes in Regional Brain Glucose Metabolism

A third important finding was the interaction between gender and drug effects as well as an interaction between gender and group on both baseline and MP-induced changes in regional brain metabolism. Female CA showed lower baseline metabolism in frontal and occipital cortices than HC, whereas these baseline differences were not significant for males. Females also had significantly higher MP-induced increases in brain glucose metabolism than males, which was an effect driven by the HC. Specifically, MP produced greater metabolic increases in female HC than in female CA or in male HC or CA. In turn, this drove the significant gender by group drug interaction because only in females was the difference between the groups significant. Female CA showed decreased responses to MP in the cerebellum, medial frontal gyrus, pons, hippocampus, and thalamus, compared with female HC. In contrast, there were no group differences in male participants on MP-induced metabolism. Preclinical studies have shown higher sensitivity of females than males to the rewarding effects of cannabinoids and a greater vulnerability to reinstatement during abstinence (Fattore *et al*, 2008). However, adolescent male rats were recently shown to be more sensitive to the anxiolytic and antidepressant effects of THC, as measured by the elevated plus maze and forced swim test (Silva *et al*, 2016). Clinical studies have shown that male CA are more likely than female CA to be chronic cannabis users (eg, Preston, 2006), experience more withdrawal symptoms (Crowley *et al*, 1998), and suffer a higher prevalence of panic attacks and personality disorders (Hasin *et al*, 2008). Gender differences have also been reported in subjective responses to oral THC administration: while female CA reported greater responses to a 5 mg THC dose than male CA, male CA were more sensitive to a higher (ie, 15 mg) THC dose than female CA (Fogel *et al*, 2016). Epidemiologic studies have shown important gender differences in clinical characteristics and psychiatric comorbidities in CA. That is, smoking cannabis was associated with decreased quality of life, particularly in female CA (Lev-Ran *et al*, 2012). Further, male CA were more likely diagnosed with any psychiatric disorder, any substance use disorder and antisocial personality disorder compared with female CA, whereas female CA had more mood, anxiety, and externalizing disorders (Khan *et al*, 2013). Male CA also had longer episodes of abuse, smoked more joints, and were

older at remission than female CA (Khan *et al*, 2013). Nevertheless, it has to be noted that some of these gender differences might reflect social and cultural factors influencing exposure and responses to cannabis rather than biological differences in the sensitivity to cannabis.

A recent brain imaging study documented higher CB1 receptor levels in the brains of women compared with men (Neumeister *et al*, 2013; Normandin *et al*, 2015), and a preclinical study showed that THC altered CB1 receptor expression and function in females but not in males (Silva *et al*, 2016). This could provide a neurobiological basis for differences in sensitivity to the effects of cannabis between the genders. Sex differences in cannabinoid action have been ascribed in part to menstrual phase and fluctuating hormonal levels (Fattore and Fratta, 2010; Riebe *et al*, 2010). In our study, we scanned women during the mid-follicular phase when levels of estrogen, FSH, LH, and progesterone are low, to minimize the effects of menstrual cycle. The study reporting higher brain CB1 receptors in females than males was also performed during the follicular phase. Preclinical studies also support sex effects on cannabinoid-induced changes in regional brain glucose in responses to a stimulant drug. Interestingly, however, the findings were opposite to our results; in male rats but not in females, cannabinoid exposure decreased the metabolic responses to cocaine (Higuera-Matas *et al*, 2011).

Gender differences in regional brain metabolic response to drug cues have also been reported: female but not male cocaine abusers show significant metabolic decreases in cognitive control networks (ie, fronto-parietal and cingulo-opercular network) (Volkow *et al*, 2011). In the current study, we also found preliminary evidence for a gender by group effect in subjective ratings of *control*: MP significantly decreased *control* in female HC and in male CA, but not in female CA and male HC (see Supplementary Material). In this respect, female CA reacted similarly to male HC for MP-induced changes in self-reports of *control* as well as *restlessness*, *desire for MP* and *cannabis craving*. Although the particulars of the sex and gender differences in responses to acute and chronic cannabis are far from elucidated, it is clear that there are important differences between males and females that might reflect in part the interaction of gonadal hormones with the endocannabinoid system (Gorzalka and Dang, 2012). Future studies investigating the influence of gender on the effects of cannabis in brain are needed to elucidate the nature of the differences and the mechanisms underlying them.

Study Limitations

Study limitations include that we did not quantify hormonal measures from blood of participants. This would have given us the opportunity to assess whether there was a relationship between estrogen and progesterone levels and MP-induced changes in brain glucose metabolism and to assess whether these differed between controls and CA. Further, despite a lack of group differences in tobacco smoking between CA and HC ($\chi^2 = 4.48$, $p = 0.11$, ns; see Table 1) and most CA expressed smoking joints with cannabis only, we did not systematically assess whether CA smoked cannabis together with tobacco on a regular basis. As such, brain metabolism differences between CA and HC reported in this study may,

partly, be due to tobacco use. In our study, the administration of saline and MP was single rather than double-blind, which may have influenced our measures. However, we chose a single-blind design to ensure that medical and nursing support was present for potential adverse reactions to MP, which can result in tachycardia and in blood pressure increases. Finally, though we interpret our findings of reduced brain metabolic changes in response to MP challenge to reflect blunting of dopaminergic and/or noradrenergic signaling because these neurotransmitters are increased by MP (Berridge and Arnsten, 2013), we cannot rule out the possibility that the blunting reflects a downstream effect because CB1 receptors, which are the targets for cannabis, modulate multiple signaling pathways (Puighermanal *et al*, 2012).

MP was given 90 min prior to FDG. Because the initial brain uptake of iv MP is associated with its rewarding effects, which return to baseline 20–30 min after its injection even when MP brain levels are still high (Volkow *et al*, 1995), the metabolic changes have to be interpreted as reflecting changes in activity that follow the fast DA increases induced by MP. We chose this design because we wanted to assess MP's effects when the levels in brain had stabilized (Volkow *et al*, 1995). The pharmacological effects of MP persist for at least 2–3 h (Patrick and Markowitz, 1997), which is within the time window of the FDG measures. Interestingly, when we measured the effects of MP 1 min after its intravenous administration, we observed a similar pattern of changes in regional brain glucose metabolism to those reported in this study (Volkow *et al*, 2003).

CONCLUSION

In summary, we show evidence of decreased baseline activity in frontal brain regions in CA, which has been implicated in other types of addiction. We also report attenuated regional brain metabolic responses to MP challenge, which are consistent not only with reduced neuronal reactivity to DA but also because of noradrenergic stimulation in CA. Finally, consistent with greater sensitivity of the female brain to the effects of repeated cannabis exposure, we showed that the reduced baseline frontal activity and the blunted MP-induced changes in brain metabolism in CA were predominantly driven by differences in females but not male participants. This suggests that women are more sensitive to the adverse effects of cannabis on the brain than men.

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The authors declare no conflict of interest.

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