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ARTICLE

Methylphenidate's effects on thalamic metabolism and functional connectivity in cannabis abusers and healthy controls

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Methylphenidate (MPH) is a first line treatment for ADHD and is also misused as a purported cognitive enhancer, yet its effects on brain function are still poorly understood. Recent functional magnetic resonance imaging (fMRI) studies showed that MPH altered cortico-striatal resting functional connectivity (RFC). Here we investigated the effects of MPH in thalamic connectivity since the thalamus modulates striato-cortical signaling. We hypothesized that MPH would increase thalamic connectivity and metabolism, and that this response would be blunted in cannabis abusers. For this purpose, we measured RFC in seven thalamic nuclei using fMRI and brain glucose metabolism using positron emission tomography (PET) and ^{18}F-fluorodeoxyglucose (FDG) in sixteen healthy controls and thirteen participants with cannabis use disorder (CUD) twice after placebo and after MPH (0.5 mg/kg, iv). MPH significantly increased thalamo-cerebellar connectivity and cerebellar metabolism to the same extent in both groups. Group comparisons revealed that in CUD compared to controls, metabolism in nucleus accumbens was lower for the placebo and MPH measures, that MPH-induced increases in thalamic metabolism were blunted, and that enhanced negative connectivity between thalamus and accumbens in CUD was normalized by MPH (reducing negative connectivity). Our findings identify the thalamus as a target of MPH, which increased its metabolism and connectivity. The reduced metabolism in nucleus accumbens and the disrupted thalamo-accumbens connectivity (enhanced negative connectivity) in CUD is consistent with impaired reactivity of the brain reward's circuit. MPH's normalization of thalamo-accumbens connectivity (reduced negative connectivity) brings forth its potential therapeutic value in CUD, which merits investigation.

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INTRODUCTION

Methylphenidate (MPH) is a psychostimulant medication widely used for therapeutic purposes, but is also globally misused both for its purportedly cognitive enhancing properties [1]. It is accepted that its pharmacological effects are mediated by its dopaminergic and noradrenergic enhancing effects [2, 3], which result from its blockade of dopamine (DA) and norepinephrine (NE) transporters [4, 5].

Positron emission tomography (PET) studies have confirmed that MPH's effects are strongly tied to tonic levels of DA release [6]. MPH might also increase presynaptic striatal DA synthesis as suggested by findings from a PET study using [^{18}F]fluorodopamine ([^{18}F]DOPA) [7]. Additionally functional brain imaging studies have shown that MPH increases brain glucose metabolism (measured with PET and ^{18}F-fluorodeoxyglucose (FDG)) in a diverse set of brain regions including cingulate gyrus, thalamus, and cerebellum during non-stimulation conditions [8–10]. However when given to healthy controls while performing a cognitive (numerical calculation) task, MPH attenuated the task-induced increases in regional brain glucose metabolism, consistent with an enhancement of neuronal efficacy [11]. MPH's effects on brain glucose metabolism are also influenced by prior drug exposure histories. Specifically, in participants with a cannabis use disorder (CUD) the effects of MPH in regional brain glucose metabolism were markedly attenuated [10]; whereas they were enhanced in participants with an alcohol use disorder [8]. Additionally, MPH's behavioral effects in healthy controls [12] and in participants with substance use disorders is state dependent (i.e. influenced by the state of arousal, exposure to cues, task difficulty) [13].

The effects of MPH on brain function have also been assessed with functional magnetic resonance imaging (fMRI) using task-based and resting-state measures in healthy controls [14], and in CUD participants [15]. In healthy controls, MPH reduced BOLD responses to gain and loss trials and to prediction errors [14]; reduced resting functional connectivity (RFC) between anterior cingulate cortex and thalamus when the whole thalamus was selected as seed [16], and reduced RFC between globus pallidus, ventral pallidum, cerebellum and the nucleus accumbens (NAc) [17], which is a ventral striatal region underlying motivation and reward [18].

Though MPH's effects on striato-cortical connectivity are likely to mediate some of its therapeutic and rewarding effects [3], its effects on thalamic activity and connectivity are also likely to be relevant. The thalamus is a relay between midbrain dopamine

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projections into the striatum and the cortex and it's a main target of noradrenergic projections from the locus coeruleus [19], positioning it as a critical node in the networks that mediate MPH's rewarding and therapeutic effects. As it relates to reward, the NAc connects into the thalamus (via its projections to the ventral pallidum), where its primary output is the mediodorsal thalamic nucleus (MD) [20]. Preclinical studies with cocaine, a drug pharmacologically similar to MPH [5] also implicate the posterior paraventricular thalamic nucleus (PVT), which sends glutamatergic projections to NAc and contains D2-like dopamine receptors [21], in its rewarding effects [22]. On the other hand the dorsal lateral geniculate thalamic nucleus has been implicated in MPH's performance enhancement to a visual detection task, presumably via its noradrenergic modulation [23].

Using graph theoretical approaches [24], MPH was shown to decrease short-range connectivity in putamen and thalamus in controls and cocaine abusers [25]. However, the comparison of MPH's effects on long-range thalamic connectivity between healthy controls and drug-abusers has been minimally investigated. In particular we were interested on assessing its effects in CUD since the thalamus contains high levels of cannabinoid CB1 receptors, which are implicated in its oscillatory activity and in arousal [26]. Moreover, in CUD participants, the thalamus was shown to be involved in reactivity to cannabis cues [27]; to have enhanced local connectivity [28] and to have increased levels of α4β2 nicotinic receptors [29]. The thalamus is structurally and functionally heterogeneous including distinct connectivity patterns [30–32], and metabolic activity for the various thalamic nuclei [33]; and thus in our study we investigated the effects of MPH across thalamic sub-segments and compared its effects in healthy controls to that in CUD participants.

For this purpose we measured brain glucose metabolism, which is a marker of brain activity [34, 35] using PET and FDG, as well as long-range RFC via fMRI seed-based analysis using seven thalamic subregions, referred here as thalamic seeds [31] in healthy controls (HC; $n = 16$, 8 females) and in participants with CUD (CUD; $n = 13$, 7 females). Each of these thalamic seeds dominantly project to motor, sensory, occipital, frontal, premotor, parietal, temporal cortical regions via white matter tracks (Sup. Fig 1, shows the location of the thalamic seeds referred to as TH1 to TH7).

We hypothesized that MPH would increase thalamic metabolism and long-range RFC and that the changes in thalamic metabolism would be associated with changes in thalamic connectivity. We also hypothesized that MPH's effects on thalamic metabolism and connectivity would be blunted in CUD compared to HCs in whom we previously reported reduced behavioral and brain reactivity to MPH [36, 37]. All participants underwent scanning on two separate sessions, after MPH (0.5 mg/kg iv), and after Placebo (saline iv).

MATERIALS AND METHODS

Participants

Thirteen CUD (7 females; age 27.2 ± 7.6) and sixteen HC (8 females; age 28.2 ± 4.9) completed the study (Table 1). Participants were recruited from advertisements in local newspapers, and signed a written informed consent approved by the Committee on Research in Human Subjects at Stony Brook University (IRB net number 225114). See Supplementary Methods for the details of participant selection criteria.

Study design and drug administration

The study was a single blind design: i.e., subjects were blind to the drugs (MPH or Placebo; PL) received. There were two scan days; one for PL administration and one for MPH administration. In each day, either MPH (0.5 mg/kg) or PL (3 cc saline) were injected intravenously 90 min prior to the PET-FDG scan procedure, which was followed by the fMRI scanning session (150–180 min after MPH or PL injection). See Supplementary Methods for the details of PET and fMRI data acquisition.

Seed-based fMRI connectivity analysis

Standard seed-voxel correlation analyses were used to estimate the functional connectivity of the thalamic partitions in the Behrens atlas [31] (Sup. Fig. 1). Pearson correlations were used to compute the strength of the functional connectivity between the average time-varying signals within each of the thalamic partitions (i.e. seeds, bilateral) and the rest of the brain voxels. See Supplementary Methods for the details of RFC analysis.

Thalamic seed metabolic analyses

Average absolute metabolic values were extracted for whole thalamus and each of the seven thalamic seeds (bilateral) for each participant and condition from the PET-FDG images and were used to compare GROUP, DRUG, and SEED effects.

Metabolic changes and correlations across thalamic seeds and target ROIs

After finding resting state seed-based connectivity clusters as defined above, brain metabolism was further evaluated with region-of-interest (ROI) analysis to obtain signal values in the selected target volumes (Supplementary Methods).

We focused on the ROIs showing significant drug effects in RFC (MPH > PL or MPH < PL). We first calculated and compared changes in glucose metabolism in these ROIs. Then, in the final step, metabolic changes in these ROIs were compared to the metabolic changes in the thalamic seeds -which project onto those ROIs- via Pearson correlation (r) analyses; to assess whether metabolic changes in thalamic seeds correlated with the metabolic changes in these target brain regions.

Statistical approach

For voxel-wise analysis of RFC data to assess thalamic connectivity we used a flexible factorial general lineal model with DRUG (within-subject) and GROUP (between-subject) (2 × 2) factors in SPM12. Follow-up post-hoc analyses were conducted with paired (for DRUG contrasts; MPH > PL, and PL > MPH) or unpaired (for GROUP contrasts; HC > CUD, and CUD > HC) F-tests in SPM12. We first used a standard double threshold method; first chose a cluster forming voxel threshold of $p < 0.005$ with $k > 50$ (minimum of 50 neighboring voxels), and then applied a threshold of $p < 0.05$ to correct for family-wise error (FWE) across the p-values of the surviving clusters as described elsewhere [38]. Following this standard SPM approach, we additionally used Bonferroni to correct for multiple observations planned across thalamic seeds (i.e., for seven thalamic seeds cluster FWER p-threshold was set to 0.05/7 (0.0072), see Table 2). The surviving clusters were then used to form ROIs around the voxel with peak intensity in that cluster for further comparisons.

For the brain metabolic images (PET-FDG) we performed a 3-way ANOVA using GROUP, DRUG and SEED (2 × 2 × 7) to assess the effects of MPH across the thalamic seeds.

For the metabolic analysis of the target ROIs, we first picked ROIs showing significant effect of RFC for DRUG (MPH > PL, shaded rows in Table 2; note that there were no significant RFC effects for the contrast PL > MPH that survived correction for multiple comparisons). We then ran a 2-way ANOVA using GROUP and DRUG (2 × 2) for the metabolic changes in each of these regions. Finally, we measured the metabolic changes (MPH-PL) in the thalamic seeds as well as their target ROIs to assess the correlation between MPH-induced metabolic changes in these connected regions using pearson product moment correlation analyses. The p-values were adjusted for multiple comparisons via Bonferroni correction on all of these analyses. ANOVA and correlation analyses on the extracted values from the thalamic

Table 1. Participant demographics

	CUD		HC		*p*-values		
	Female ($N = 7$)	Male ($N = 6$)	Female ($N = 8$)	Male ($N = 8$)	Group	Gender	Group × gender
Age, years	23.23 (2.33)	31.78 (9.64)	27.63 (5.65)	28.78 (4.49)	NS	0.052	NS
Years of education	12.83 (1.33)	12.42 (1.52)	13.21 (1.64)	12.98 (1.14)	NS	NS	NS
BMI	22 (3.28)	26.84 (2.58)	24.12 (2.05)	24.37 (2.86)	NS	0.032	0.033
Cigarette smokers	2 active	2 active	1 active	1 active	NS	NS	NS
	0 former	0 former	1 former	1 former	NS	NS	NS
Cannabis age initiation	15.29 (2.81	16 (3.1)	–	–	–	NS	–
Days/week	6.86 (0.38)	6.5 (0.84)	–	–	–	NS	–
Joints/day	5.43 (3.6)	4.5 (3.27)	–	–	–	NS	–
Years abuse	7 (3.74)	14.33 (10.76)	–	–	–	NS	–
MDQ Sum	4.86 (2.54)	4.83 (2.14)	–	–	–	NS	–
MPQ PEM	41.43 (12.07)	51.67 (13.03)	54 (4.93)	52.88 (3.76)	0.038	NS	NS
MPQ NEM	19.86 (10.09)	19.5 (6.38)	10.12 (6.24)	17 (9.06)	0.055	NS	NS
MPQ Constraint	49.29 (7.18)	47.67 (8.41)	53.38 (7.42)	49.62 (11.29)	NS	NS	NS

CUD cannabis use disorder, *HC* healthy control, *MDQ* Mood Disorder Questionnaire, *MPQ* Multidimensional Personality Questionnaire, *PEM* positive emotionality, *NEM* negative emotionality

seeds and target ROIs were conducted using R software version 3.4.3 [39].

RESULTS

Head motion

The average frame displacement (FD) did not differ between HC (0.228 ± 0.144 mm and 0.208 ± 0.076 mm for PL and MPH), and CUD (0.225 ± 0.089 mm and 0.185 ± 0.053 mm for PL and MPH). There was no effect of drug on FD nor was there an interaction between drug × group ($F < 1$).

Thalamic seed functional connectivity

We examined long-range connectivity of the thalamus using seven bilateral seed regions that project to motor, sensory, occipital, frontal, premotor, parietal, temporal cortical regions via white matter tracks (referred to as TH1 to TH7, respectively).

We found that only five of these seeds, namely motor (TH1), sensory (TH2), premotor (TH5), parietal (TH6), and temporal (TH7) seeds formed surviving resting state connectivity clusters, resulting in a total of nine thalamo-cortical or thalamo-cerebellar connectivity pairs throughout the brain. This was because three seeds, namely premotor, sensory, and motor seeds, formed more than one surviving connectivity cluster, while two of the thalamic seeds, namely parietal and temporal seeds, formed only one surviving connectivity cluster. The occipital (TH3) and frontal (TH4) seeds did not form any surviving connectivity cluster (Table 2). Thus, five of the seven seeds are reported in the paper.

Drug effects. Thalamic seed 1 (*motor*) showed increased RFC with right cerebellum for MPH than for PL ($p < 0.001$) (Fig. 1a) . Thalamic seed 2 (*sensory*) showed increased RFC with right cerebellum for MPH than for PL ($p < 0.006$) (Fig. 1b).

Group effects. Thalamic seed 1 (*motor*) showed stronger RFC with left pars triangularis ($p < 0.006$) and left supramarginal gyrus ($p < 0.001$) (Fig. 2a, c), and weaker RFC with visual areas for CUD than HC ($p < 0.005$) (Fig. 2e). Thalamic seed 2 (*sensory*) showed stronger RFC with right parietal cortex, for CUD than HC ($p < 0.005$) (Fig. 2b). Thalamic seed 6 (*parietal*) showed stronger RFC with left parietal cortex, for HC than CUD ($p < 0.005$) (Fig. 2f). Thalamic seed 7 (*temporal*) showed stronger RFC with right temporo-parietal cortex for CUD than HC ($p < 0.001$) (Fig. 2d).

Group × drug interaction. Thalamic seed 5 (*premotor*) showed a GROUP and a DRUG × GROUP interaction effect with nucleus accumbens (NAc) cluster (left hemisphere dominant, henceforth named as left NAc) (Fig. 1c). The CUD group showed negative RFC with left NAc for PL that was significantly larger than for HC ($p < 0.005$). The DRUG × GROUP effect ($p < 0.005$) (Fig. 1c) was due to MPH's reducing negative RFC in CUD ($p < 0.05$) whereas it increased negative RFC in HC ($p < 0.01$).

Thalamic metabolism: effects of GROUP and MPH

Absolute metabolic values (mean and SD; expressed as micromol/100 g/min) for the whole thalamus in HC and CUD for placebo and MPH were as follows: for HC: PL condition, 26.66 (6.11), MPH condition, 29.56 (7.05); for CUD: PL condition, 26.76 (4.87), MPH condition, 27.35 (4.90). We computed the mean metabolic value per thalamic seed, per subject per condition, and ran a 3-way ANOVAs (2 × 2 × 7; GROUP × DRUG × SEED). Comparisons showed a trend effect of GROUP, where HC showed higher values than CUD, $F(1,28) = 3.77$, $p = 0.052$. MPH significantly increased thalamic metabolism compared to PL, $F(1,28) = 11.88$, $p < 0.001$. There was also a significant DRUG and GROUP interaction effect, $F(1,28) = 4.54$, $p < 0.05$; driven by a significant increase in thalamic metabolism with MPH in HC, $F(1,28) = 10.87$, $p < 0.01$, but not in CUD ($F < 1$). There was also a main effect of SEED, $F(6,34) = 14.67$, $p < 001$, where some thalamic seeds (i.e., TH4, projecting to frontal regions) had highest baseline metabolism compared to the others (Sup. Fig. 2).

Metabolic changes on target ROIs due to GROUP and MPH administration

We contrasted the metabolic changes in the three target ROIs that were found to be significantly affected by MPH in the RFC analyses, namely (i) right cerebellum ROI connected to motor seed (TH1), (ii) right cerebellum ROI connected to sensory seed (TH2) (these two ROIs showed a main effect of MPH on RFC), and (iii) NAc ROI connected to pre-motor seed (TH5) (which showed an interaction between GROUP and DRUG in RFC). For this purpose we compared the metabolic values with ANOVA to assess GROUP and DRUG effects and their interactions in these ROIs (Fig. 3).

Right cerebellum ROIs. MPH increased glucose metabolism in the right cerebellar ROIs compared to PL both for the ROI formed by the motor seed ($F(1,28) = 22.64$, Fig. 3ai) and the ROI formed by

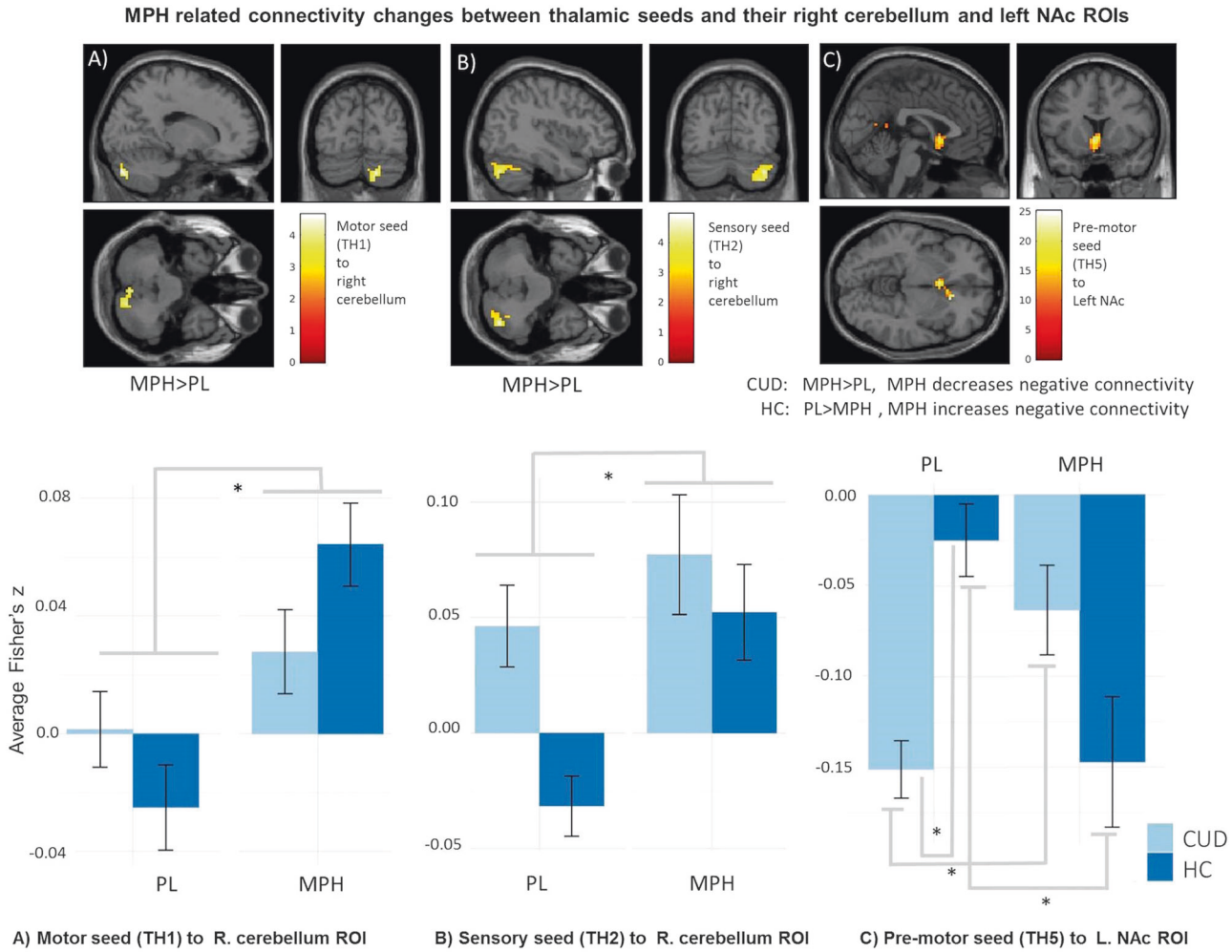

Fig. 1 MPH-induced changes in thalamic connectivity in cerebellum and left NAc. Top row: brain regions with strong seed-based connectivity modulation with **a** motor, **b** sensory, and **c** pre-motor thalamic seeds are shown. Thalamic seed names are indicated near the color scale along with their target clusters shown in the figures. In **a** and **b**, the scale shows *t*-values contrasting MPH > PL. In **c**, scale shows F-value (contrasting interaction between DRUG and GROUP). MPH impacts connectivity on thalamo-cerebellar networks in both groups, that was strongest for the right cerebellum with the sensory and motor thalamic seeds. MPH's effects on pre-motor thalamic seed connectivity with NAc differed between groups; in CUD it decreased negative connectivity whereas in HC it increased negative connectivity. Initial clustering threshold was chosen as $p = 0.005$, with $k > 50$; final pFWE < 0.0072. Bottom row: drug effects on connectivity across subjects are shown in barplots. Effect of MPH as compared to PL in HC and CUD in resting connectivity ROIs formed by motor, sensory and pre-motor thalamic seeds. Mean and SD of z-scores of the correlations are plotted across subjects. **a** Motor thalamic seed connectivity with right cerebellum was increased by MPH. **b** Sensory thalamic seed connectivity with right cerebellum was increased by MPH. **c** Pre-motor thalamic seed connectivity with NAc showed that for PL CUD showed greater negative connectivity than for HC and MPH reduced the negative connectivity in CUD whereas in HC it had the opposite effect enhancing it

the sensory seed ($F(1,28) = 9.55$, $p < 0.01$), Fig. 3aii), consistent with the increases in RFC.

Left NAc ROI. In the NAc ROI, MPH did not significantly affect metabolism, instead HC showed higher glucose metabolism than CUD ($F(1,28) = 31.74$, $p < 0.001$; Fig. 3aiii) both for the placebo and the MPH conditions.

Correlations between metabolic changes (MPH-PL) in thalamic seeds and their target ROIs

Since MPH affected metabolism on both target ROIs and the thalamic seeds, we calculated the correlations between the metabolic changes induced by MPH (MPH-PL) between each of the three thalamic seeds and their corresponding ROIs across subjects (i.e., motor, sensory and pre-motor thalamic seeds and their corresponding target ROIs, Fig. 3b).

Right cerebellum ROIs. The metabolic changes in the motor thalamic seed and its corresponding right cerebellar target ROI were correlated across all participants ($r = 0.69$, $p < 0.001$) and separate analyses by group showed they were correlated in HC ($r = 0.803$, $p < 0.001$), but not in CUD ($r = 0.470$, $p > 0.1$) (Fig. 3bi). Similarly the correlations between metabolic changes in the sensory thalamic seed region and its right cerebellar target ROI were significant across all participants ($r = 0.709$, $p < 0.001$), and separate analyses by group showed they were significant in HC ($r = 0.872$, $p < 0.001$), but not in CUD ($r = 0.457$, $p > 0.1$) (Fig. 3bii).

Left NAc ROI. The correlations between metabolic changes in the pre-motor thalamic seed and its left NAc target ROI were significant across all participants ($r = 0.671$, $p < 0.001$) and separate analyses by group showed they were significant both in HC ($r = 0.695$, $p < 0.01$) and CUD ($r = 0.713$, $p < 0.01$) (Fig. 3biii).

Table 2. fMRI resting state SPM clusters formed by thalamic seeds

Thalamus seed label	Target cortical area from the thalamic seed (Behrens atlas)	ROI target MNI space max intensity voxel (*x*, *y*, *z*)	Cluster location	Cluster level pFWER value			*k*	Post hoc tests
				Drug	Group	Drug × Group		
TH1	Motor	54, −28, 40	Right cerebellum	0.0051			98	MPH > PL
		−36, 41, 4	Left supramarginal		0.0001		117	CUD > HC
		−15, −49, −8	Left broca		0.0052		97	CUD > HC
		18, −82, −38	Fusiform/lingual		0.0034		106	HC > CUD
TH2	Sensory	51, −40, 43	Right cerebellum	0.0001			150	MPH > PL
		39, −70, −38	Right parietal		0.0022		108	CUD > HC
TH5	Premotor	−3, 11, −5	Left nucleus Accumbens			0.002	92	For CUD: MPH > PL, $p < 0.01$ For HC: MPH < PL, $p < 0.05$ For PL: CUD < HC, $p < 0.05$
TH6	Parietal	−42, −82, 31	Left parietal		0.0043		102	HC > CUD
TH7	Temporal	54, −49, 34	Right temporal-parietal		0.0001		147	CUD > HC

CUD cannabis use disorder, *PL* placebo, *HC* healthy control, *MPH* methylphenidate
Cortical areas with the highest probabilistic white matter connectivity from the thalamic seed (Behrens atlas) are given in the second column. Five out of seven seed-cluster pairs were selected on the basis of statistical criteria applied to the resting functional connectivity data. First, voxel-wise threshold was selected for $p < 0.005$ with minimum cluster size $k > 50$ voxels, and then correction with family-wise error was applied where seeds with $p = 0.0072$ (0.05/7) were selected for further analyses. Drug effects were observed only in three clusters (shaded). Post hoc pairwise comparisons were made if the interaction reached significance (e.g., TH5, left nucleus accumbens cluster)

DISCUSSION

Here, we examined the effects of MPH in seed-based connectivity and brain glucose metabolism in HC and CUD. MPH modulated thalamic functional connectivity in a set of brain regions including NAc. Independent of MPH, thalamic connectivity with NAc and various cortical regions differed between HC and CUD, presumably due to the effects of long-term CUD on brain function or due to pre-existing differences that might have increased vulnerability for CUD. On the other hand, MPH impacted connectivity in interesting ways; for CUD MPH normalized the negative connectivity between NAc and thalamus by bringing connectivity close to the pattern recorded in HC for the PL condition, whereas in HC, MPH increased negative connectivity bringing connectivity close to the pattern recorded in CUD for the PL condition. In addition, MPH increased thalamo-cerebellar connectivity for both HC and CUD.

While MPH significantly increased metabolism in the cerebellar target ROIs, concomitant changes in metabolism between thalamic seeds and cerebellar ROIs were significantly correlated only in HC, and not in CUD. The group differences in the correlations was likely driven by the group differences in metabolic effects of MPH on the thalamus, where metabolism was increased in HC—but minimally in CUD. In contrast, in the NAc, a brain region that plays a critical role in drug reward and addiction [18], MPH-induced metabolic changes were correlated with the metabolic changes in the thalamic seed for both in HC and CUD even though the main effect of MPH on NAc metabolism was not significant, highlighting the inter-subject variability for MPH effects in NAc metabolism.

RFC changes

MPH effects. MPH increased connectivity between sensory and motor thalamic seeds and right cerebellum (for both HC and CUD). On the other hand, MPH produced a contrasting connectivity pattern between premotor thalamic seed region and NAc, where MPH effects differed between groups. Specifically, MPH decreased the negative connectivity between pre-motor thalamic seed region and NAc in CUD, while it increased negative connectivity in HC. This finding is in line with previous reports on connectivity changes in NAc [17] and of abnormal dopaminergic responses to MPH challenge in CUD versus controls [10, 40]. The disrupted thalamo-accumbens connectivity in CUD adds to the growing evidence of the importance of thalamic inputs to NAc in drug abuse and addiction [41].

Group effects. We observed group differences in the functional connectivity of a diverse set of regions connecting to thalamic seeds. CUD showed a main effect of hyperconnectivity in motor, sensory and temporal thalamic seeds with many cortical clusters across the brain, including Broca's region and parietal and temporo-parietal regions. Given the widespread distribution of cannabinoid receptors in brain [42], and the complex nature of the interactions between cannabis and other types of drugs with which it is frequently consumed [43], it is not unexpected to find effects of chronic cannabis use on long-range thalamic connectivity. Our findings are consistent with prior imaging studies that identified increases in functional connectivity in subcortical regions (short-range connectivity) in CUD compared to HC [28].

Metabolic changes in the thalamus

Recent findings showed that glucose metabolism differed across thalamic nuclei, which was tied to cell density differences; for instance medial dorsal regions of thalamus projecting dominantly to prefrontal and temporal regions show higher glucose metabolism, and also higher cell density [33]. Confirming this finding, we showed that thalamic seeds projecting heavily to the frontal cortical regions showed the highest metabolic values. We also showed that MPH significantly increased thalamic glucose

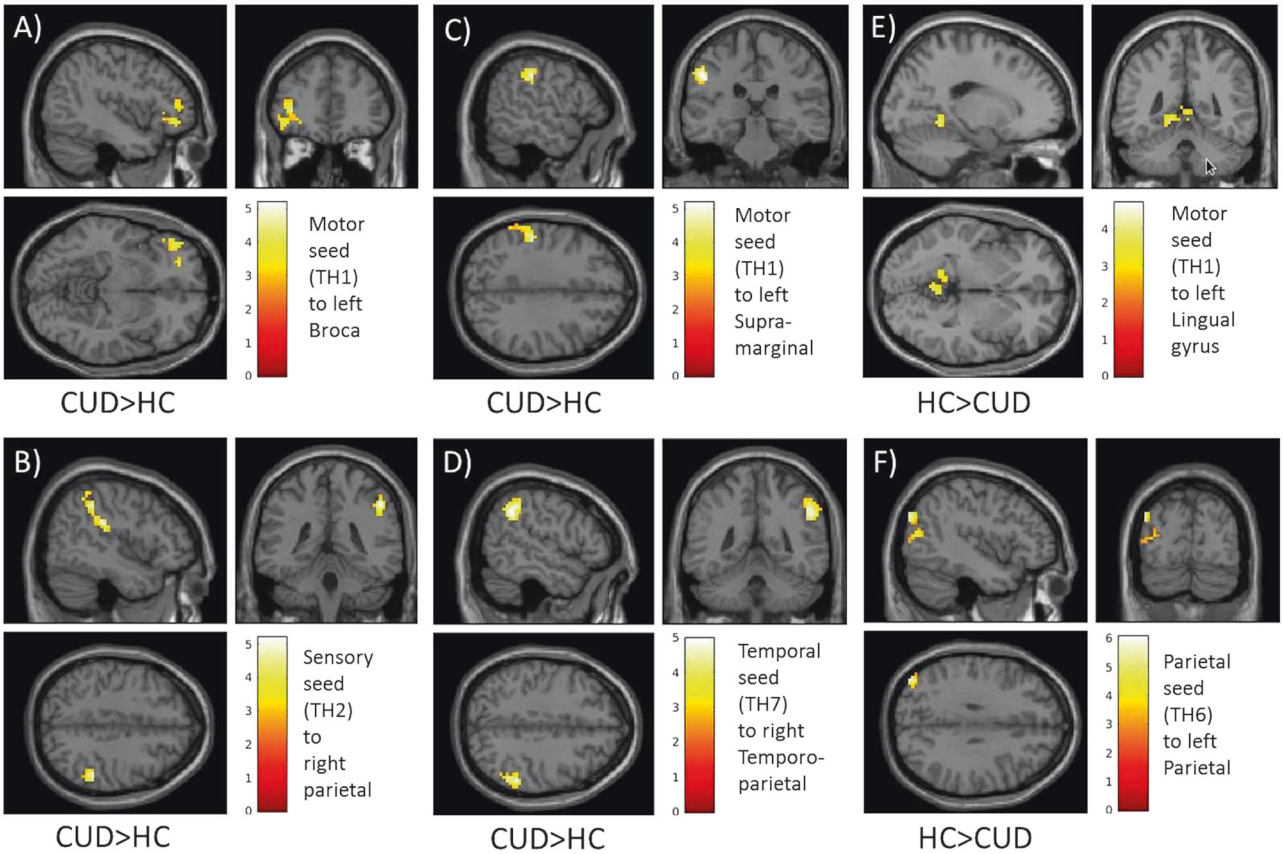

Fig. 2 Group differences in thalamo-cortical connectivity. Brain regions with significant seed-based connectivity differences between groups. Thalamic seed region names are indicated near the color scale along with their target clusters. In **a**–**d**, CUD > HC, for **e**, and **f**, HC > CUD. In **a**, **c** and **e**, the motor thalamic seed (TH1) showed group differences in connectivity with left Broca, left supramarginal gyrus, and left lingual regions respectively; in **b**, the sensory seed (TH2) shows group differences in the right parietal region; in **d**, the temporal seed (TH7) shows group differences in the temporal-parietal region; in **f**, the parietal seed (TH6) showed group differences in the left parietal region. All tests are *t*-tests with either HC > CUD or CUD > HC contrasts. Initial clustering threshold was chosen as $p = 0.005$, with $k > 50$; final pFWE < 0.0072.

metabolism across all seed regions and this effect was mainly driven by HC since CUD had a blunted metabolic response. However we did not observe differences in sensitivity to MPH among sub-sections of the thalamus, revealing the broad effect of MPH over thalamus.

Metabolic changes in target ROIs

Cerebellar ROIs. The right cerebellar regions showed increases in glucose metabolism (in parallel to increases in RFC) with MPH and these metabolic changes did not differ between HC and CUD. We recently reported that MPH increased metabolism in whole brain and in whole cerebellar gray matter predominantly in HC, and that MPH-induced metabolic changes were blunted in CUD in a larger sample that included the participants from the current study [10]. The absence of Group and Drug interaction effects on the cerebellar metabolic findings in the current study could be due to the pre-selection of ROIs (determined by RFC analysis), which confined them into smaller cerebellar subregions. Regional specificity of the cerebellum has been documented for diverse cognitive and emotional functions [44]; for sleep [45] and in neurodegenerative diseases [46].

NAc ROI. In NAc we showed a significant Group effect in metabolism, HC had higher metabolism than CUD both in PL and MPH conditions, which might reflect the deleterious effects of chronic exposure to cannabis in the NAc or that pre-existing lower activity and reactivity of NAc might constitute a risk factor for cannabis consumption.

Concomitant metabolic changes (MPH-PL) in the thalamic seeds and their target ROIs

We also observed that MPH-induced metabolic changes in the motor and sensory thalamic seeds were associated with the cerebellar metabolic changes. However these associations were driven by the HC, for they were not significant in CUD. On the other hand, while metabolic changes due to MPH in NAc were not significant due to potentially larger individual variability in MPH effects, they were significantly correlated with the metabolic changes in the premotor thalamic seed in both HC and CUD. The significant correlation between the changes in metabolism even when the main effect of MPH was non-significant was most likely due to the inter-subject variability for MPH effects in NAc metabolism (i.e., MPH increased metabolism in some participants and decreased it in others) both for HC and CUD, though overall NAc metabolism was significantly higher for HC than for CUD.

Catecholaminergic system: dopaminergic versus noradrenergic

The distinct RFC patterns triggered by MPH in cerebellum versus those in NAc could reflect the fact that these brain regions are differentially modulated by noradrenaline and dopamine, both of which are enhanced by MPH. The cerebellum is predominantly influenced by noradrenergic signals from the locus coeruleus, which also extends afferent projections to the thalamus [47]. MPH

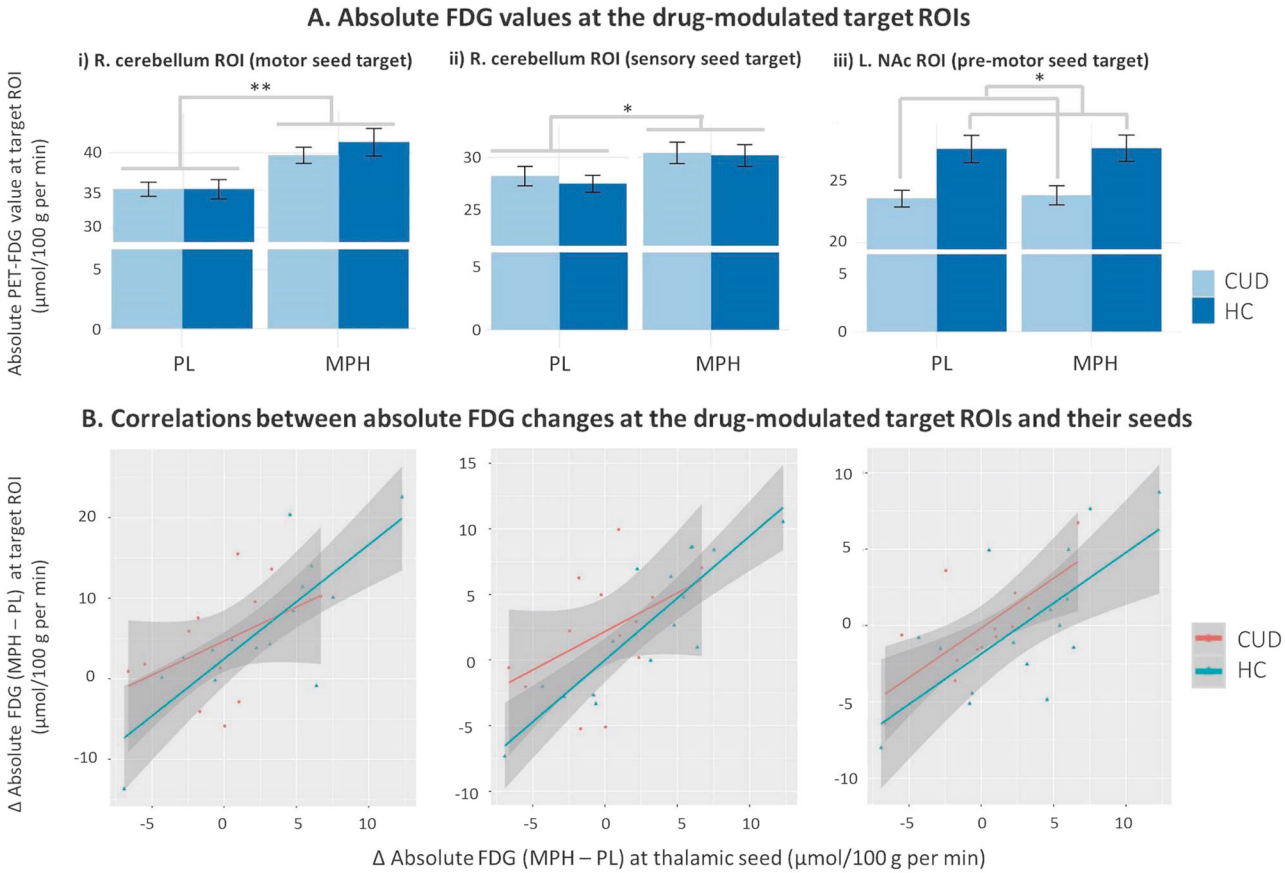

Fig. 3 MPH-induced metabolic changes at target ROIs and their corresponding correlations with thalamic seeds. Top row: absolute FDG-PET metabolic measures for PL and MPH on three target ROIs; (i) right cerebellum ROI formed by motor seed, (ii) right cerebellum ROI formed by sensory seed, and (iii) NAc ROI formed by pre-motor seed. Metabolism in the right cerebellum ROIs were significantly increased by MPH, independent of GROUP. Metabolism in NAc showed a main effect of GROUP (HC > CUD) but not an effect of DRUG. Bottom row: correlation between FDG metabolic changes (MPH-PL) in the thalamic seeds (*x*-axes) and in target ROIS (*y*-axes) shown for each GROUP for **a** right cerebellum ROI with motor seed, **b** right cerebellum ROI with sensory seed, and **c** NAc ROI formed with pre-motor seed

by blocking norepinephrine transporters (NET) and increasing noradrenergic signaling might have enhanced the connectivity between the thalamus and cerebellum. On the other hand, the NAc primarily receives dopaminergic inputs from the ventral tegmental area, which also projects to thalamic nuclei [48]. The differential effects of MPH's could also reflect that in the NAc both excitatory and inhibitory dopaminergic receptors co-exist (D1-like excitatory versus D2-like inhibitory). MPH could have modulated these receptors differently in HC and CUD accounting for the decreases in negative connectivity in CUD and the enhanced negative connectivity in HC. Interestingly, MPH normalized connectivity for CUD, reducing the negative connectivity to the levels in HC under placebo status, which we interpret to suggest that MPH might have alleviated the dopaminergic deficits in CUD [49–51]. This finding reiterates the pivotal role of NAc and the thalamus in stimulant drugs effects and in addiction [52]. Also, too much or too little dopaminergic signaling might lead to impaired cognitive performance and motivation, which might be mediated in part by thalamo-accumbens connectivity [53, 54]. Our results are consistent with recent preclinical findings that the inhibition of the paraventricular nucleus by D2Rs influences the NAc affecting cocaine-seeking behavior [21], and our finding of the disruption of thalamo-accumbens connectivity in CUD further support the relevance of this circuit in addiction. Its normalization by MPH also brings forth the possibility of its use as a potential therapeutic strategy to ameliorate connectivity deficits in CUD.

MPH's enhancement of both dopamine and noradrenaline signaling could also underlie the group differences in its metabolic effects. Specifically, if CUD had a predominant disruption of dopaminergic [55] over that of noradrenergic neurotransmission, then this could help explain the similarity in metabolic increases with MPH in CUD and HC in cerebellum, which is predominantly modulated by noradrenergic innervation [56]. A predominant disruption of dopaminergic neurotransmission in CUD [55], could also help explain their lower metabolism in the NAc (for both the PL and MPH conditions), which is mainly modulated by DA. In addition, an increase in metabolism in HC but not in CUD in the thalamus, might be due to its modulation by both dopaminergic and noradrenergic signals [57, 58].

Limitations

A major limitation in our study is the small sample of participants, which precluded us from assessing the associations between the main imaging findings and the effects of MPH on behavior and cognition. It also limited our ability to assess gender effects, which are relevant to both MPH effects but also CUD. It would have also been desirable to perform the PET and the fMRI measures at the same time rather that in a sequential design but we did not have access to an integrated PET/MRI instrument. Finally in our brain imaging findings in CUD participants we cannot distinguish between consequences of cannabis use and pre-existing characteristics that might have increased the risk for CUD.

CONCLUSION

We document significant effects of MPH in long-range thalamic connectivity and these effects differed between HC and CUD. We also showed aberrant negative thalamo-accumbens connectivity and reduced thalamic and NAc metabolism in CUD, which is consistent with disrupted dopaminergic signaling in CUD.

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ADDITIONAL INFORMATION

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