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Subcortical Local Functional Hyperconnectivity in Cannabis Dependence

Peter Manza, Dardo Tomasi, and Nora D. Volkow

ABSTRACT

BACKGROUND: Cannabis abuse (CA) has been associated with psychopathology, including negative emotionality and higher risk of psychosis, particularly with early age of initiation. However, the mechanisms underlying this association are poorly understood. Because aberrant dopamine signaling is implicated in cannabis-associated psychopathology, we hypothesized that regular CA would be associated with altered resting-state functional connectivity in dopamine midbrain-striatal circuits.

METHODS: We examined resting-state brain activity of subcortical regions in 441 young adults from the Human Connectome Project, including 30 subjects with CA meeting DSM-IV criteria for dependence and 30 control subjects matched on age, sex, education, body mass index, anxiety, depression, and alcohol and tobacco usage.

RESULTS: Across all subjects, local functional connectivity density hubs in subcortical regions were most prominent in ventral striatum, hippocampus, amygdala, dorsal midbrain, and posterior-ventral brainstem. As hypothesized, subjects with CA showed markedly increased local functional connectivity density relative to control subjects, not only in ventral striatum (where nucleus accumbens is located) and midbrain (where substantia nigra and ventral tegmental nuclei are located) but also in brainstem and lateral thalamus. These effects were observed in the absence of significant differences in subcortical volumes and were most pronounced in individuals who began cannabis use earliest in life and who reported high levels of negative emotionality.

CONCLUSIONS: Together, these findings suggest that chronic CA is associated with changes in resting-state brain function, particularly in dopaminergic nuclei implicated in psychosis but that are also critical for habit formation and reward processing. These results shed light on neurobiological differences that may be relevant to psychopathology associated with cannabis use.

Keywords: Addiction, Basal ganglia, Emotionality, fMRI, Graph theory, Marijuana, Resting-state functional connectivity

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Cannabis is one of the most widely used addictive substances in the United States, with 44% of individuals older than 12 years of age reporting cannabis use at least once in their lifetime (1). Despite current efforts to legalize cannabis, little is known about the long-term effects of cannabis abuse (CA) on brain function and neuropsychiatric outcomes. Of particular concern has been the association between regular CA and psychiatric symptoms such as amotivation, negative emotionality (2,3), and a heightened risk for psychosis (4). Indeed, CA was associated with up to a sixfold increase in the risk of schizophrenia in early-onset users (5,6) and with the use of cannabis with high Δ^9-tetrahydrocannabinol (7). The increased risk remains after controlling for other substances of abuse and for familial risk of psychosis (8). Aberrant dopaminergic function in the midbrain-striatal circuitry, a hallmark feature of schizophrenia, may underlie this association (9). Accordingly, individuals with CA with genetic variants that confer high midbrain-striatal dopamine (DA), including the *DRD2* rs1076560 T allele, the *DAT1* 3′ 9-repeat allele, and the *AKT1* rs2494732 C allele, have an increased risk of psychosis compared with individuals with CA who do not have these genetic variants (10–12). However, the effects of chronic CA on the functional organization of subcortical regions modulated by DA and their relevance for psychiatric symptoms are poorly understood.

Resting-state functional magnetic resonance imaging (rsfMRI) offers a noninvasive method for probing the functional connectedness of neural circuits. By measuring correlations among spontaneous low-frequency blood oxygen level–dependent signals, studies have revealed the involvement of functional changes in subcortical circuits in psychiatric diseases, including schizophrenia. For instance, functional connectivity between reward processing regions, such as nucleus accumbens and orbitofrontal cortex, appears to be related to disrupted DA function and, as such, has clinical relevance: higher intrinsic connectivity correlated with amotivation syndrome (13) and with the duration that schizophrenia had been left untreated (14). Intriguingly, a similar pattern of nucleus accumbens–orbitofrontal cortex hyperconnectivity was reported in individuals with CA (15). However, the relevance of

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these effects for psychopathology in individuals with CA is unknown. Furthermore, prior investigations in CA have relied mainly on seed-based connectivity analyses.

In contrast, local functional connectivity density (lFCD), the size of a local cluster of correlated voxels, is a data-driven method for identifying functional hubs in the brain (16). lFCD accounts for up to 70% of resting-state brain metabolism (17) and therefore is an index of local brain activity that has superior spatiotemporal resolution to positron emission tomography imaging. We recently used this method to identify functional connectivity changes that were associated with cognitive and mood-related behaviors in heavy drinkers (18). To our knowledge, no studies have examined the effects of CA on subcortical functional hub organization and their relevance to negative emotionality, which is elevated in individuals with CA (3) and schizophrenia (19). Intriguingly, recent studies using a very similar approach found subcortical hyperconnectivity in a cohort of 95 individuals with schizophrenia (20). We hypothesized that similar effects may be observed in individuals with CA. To test this hypothesis, we took advantage of the large dataset produced by the Human Connectome Project (HCP) (21). While the HCP does not have targeted measures that specifically assess psychosis, they do offer measures of negative emotionality, a symptom shared between CA and schizophrenia (2,22) that we have previously found to be associated with subcortical dopaminergic function in individuals with CA (3). Thus, while the present study does not directly study individuals with schizophrenia, negative emotionality is relevant in light of the emerging view that psychiatric disorders represent clusters of symptoms and traits that are elevated over a spectrum of normal functioning (23–26) and that elevated negative emotionality predicts development of psychosis (27). We were particularly interested in one aspect of negative emotionality—symptoms of alienation (beliefs of individuals that others wish them harm and that they are deceived by friends)—after our recent investigation demonstrated that this aspect may be particularly affected in individuals with CA and associated with aberrant brain function (2).

METHODS AND MATERIALS

Participants

We analyzed data from the S500 release (https://www.humanconnectome.org/study/hcp-young-adult/document/500-subjects-data-release) of the WU-Minn HCP (Washington University–University of Minnesota Consortium of the Human Connectome Project) Consortium (21). We included only participants who had 1) complete structural and rsfMRI imaging data that passed a quality assurance check and 2) complete measures of cognitive function and emotionality (N = 441 participants). The HCP initiative studied young adults 22 to 35 years of age from a wide range of backgrounds and behavioral profiles representative of the population at large. Thus, whereas all participants are considered generally healthy, participants with subclinical psychiatric symptoms and recreational drug use are included.

Of the 441 participants, 36 met the DSM-IV criteria for cannabis dependence (see Supplement for a description). Three participants were excluded for comorbid alcohol dependence, and one was excluded for anxiety and depression ratings >3 SD from the group mean. Recent studies have indicated that it is critical in studies of CA to select a well-matched control group, particularly on measures of alcohol and tobacco usage [e.g., (28)]. Therefore, we matched groups on age, sex, education, body mass index, anxiety, depression, and alcohol and tobacco usage [we calculated composite tobacco/alcohol usage the same way as a recent study of HCP data; see Supplement and (29)]. Two subjects from each group were excluded to ensure that groups were matched on tobacco usage (Supplement), and the final sample included 30 subjects with CA and 30 control subjects; demographics and statistical tests are presented in Table 1.

Behavioral Measures of Interest

We examined data related to cognitive function and negative emotionality, given the interest in potential chronic effects of cannabis use in these domains (30). Participants completed various National Institutes of Health Toolbox measures as part of the HCP. We were particularly interested in relating the current work to our previous findings that individuals with CA are vulnerable to feelings of alienation, i.e., the belief that others wish them harm and that they are betrayed or deceived by friends (2). However, our previous work used the Multidimensional Personality Questionnaire, and this was not administered as part of the HCP protocol. Therefore, we attempted to find analogous measures for the three main domains of the Multidimensional Personality Questionnaire: stress reactivity, aggression, and alienation. For stress reactivity, we used the perceived stress measure; for aggression, we averaged together the Z scores of anger hostility and anger aggression (i.e., one's own behavior in the anger and aggressive domains); and for alienation, we averaged together the Z scores of perceived hostility and perceived rejection (i.e., how one perceives others behaving toward them). We then averaged these stress, aggression, and alienation measures

Table 1. Demographics of Cannabis Abuse and Control Groups

	CA	CTRL	Statistical Value[a]
Age, Years	29.17 ± 3.07	30.23 ± 2.74	.161
Sex, Male, n	22	20	0.573[b]
Education, Years	14.6 ± 1.89	14.6 ± 1.92	1.000
BMI	27.17 ± 3.6	26.83 ± 4.89	.757
DSM Depression	0.03 ± 0.86	−0.13 ± 0.83	.452
DSM Anxiety	0.07 ± 1.04	−0.16 ± 0.95	.383
Alcohol Use (Composite Z)	0.27 ± 0.4	0.15 ± 0.38	.250
Tobacco Use (Composite Z)	0.57 ± 0.83	0.32 ± 0.85	.260

Values are reported as n or mean ± SD. Depression, anxiety, tobacco, and alcohol use values were converted to Z-scores based on the larger population of 441 participants. See Tobacco and Alcohol Usage in Supplement for a description of how the combined past and present use measures were derived.

BMI, body mass index; CA, cannabis abuse; CTRL, control.

[a] t test p values are reported except for male sex.

[b] χ^2_{59}.

together for a composite negative emotionality score. All three domains were included to examine if the effects were specific to alienation. More comprehensive descriptions of cognitive and emotional measures are available in the Supplement and at https://www.humanconnectome.org/study/hcp-young-adult/document/500-subjects-data-release.

MRI Acquisition and Preprocessing

All brain images were collected on a Siemens 3T Connectome Skyra scanner (Siemens Healthcare, Erlangen, Germany) with a 32-channel coil at Washington University in St. Louis, MO. T1-weighted and T2-weighted anatomical scans were acquired (field of view = 224 mm, matrix = 320, 256 slices, 0.7-mm isotropic voxels). rsfMRI scans were acquired with an echo-planar imaging sequence (multiband factor = 8, repetition time = 720 ms, echo time = 33.1 ms, flip angle = 52°, field of view = 208 mm, 104 × 90 matrix, 72 slices of 2-mm isotropic voxels, no gap). Two sessions were completed with two rsfMRI scans (one left-to-right and one right-to-left phase encoding) in each session. Each scan was 14:33 minutes, for a total scanning time of 54:15 minutes. For rsfMRI, participants were instructed to lie with eyes open, to relax and look at a white cross on a dark background, to think of nothing and to not fall asleep. For further details on image acquisition, see https://www.humanconnectome.org/storage/app/media/documentation/s500/hcps500meg2releasereferencemanual.pdf.

For analysis of rsfMRI data, we used the minimal preprocessing datasets (hp2000_clean.nii files), where preprocessing included 1) gradient distortion correction, 2) rigid body realignment, 3) field map processing, 4) nonlinear normalization to Montreal Neurological Institute space, 5) high-pass filtering with independent component analysis–based denoising, and 6) brain masking. In our own subsequent preprocessing, we removed time points that were severely affected by motion using a scrubbing approach (Supplement). Remaining motion effects on fMRI time series were regressed out using the six translation and rotation regressors. Finally, bandpass temporal filtering (0.01–0.10 Hz) was applied. lFCD was computed separately on each of the four runs of processed, unsmoothed data, masked by each participant's FreeSurfer (https://surfer.nmr.mgh.harvard.edu/) subcortical parcellation (wmparc.2.nii.gz), which included bilateral thalamus, caudate, putamen, pallidum, amygdala, nucleus accumbens, hippocampus, midbrain, and brainstem (see lFCD Analysis below). Finally, the four resulting lFCD maps (LR/RL; REST1/REST2) were averaged together, and averaged images were smoothed at 2-mm full width at half maximum.

lFCD Analysis

The Pearson correlation was used to assess the strength of functional connectivity, C_{ij}, between voxels i and j. A positive correlation threshold of $r = .2$ (sufficient to Bonferroni correction for the number of correlations performed in the subcortical mask, $p < 1 \times 10^{-4}$) was used to compute the binary connectivity coefficients, $a_{ij} = 1$ (if $C_{ij} > 0.2$) or $a_{ij} = 0$ (if $C_{ij} \leq 0.2$). This threshold was lower than previous investigations (16) to have sensitivity to detect effects in subcortical regions that have noisier signals than the neocortex and hence have weaker observed resting-state correlations (31). The lFCD (or local degree) for voxel i was computed as the size of a continuous cluster of voxels with $a_{ij} = 1$ that are connected by surface. A growing algorithm was used for time-efficient estimation of lFCD (16).

Seed-Based Functional Connectivity Analysis

To examine functional connectivity differences with other regions of the brain, we computed seed-based connectivity using the same methods as our previous work (32,33) (Supplement).

Statistical Analysis

Second-level statistical analyses were conducted using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/) for imaging data and GraphPad Prism 7.02 (GraphPad Software, Inc., La Jolla, CA) for behavioral data. First, to examine lFCD across the larger population, we conducted a one-sample *t* test of lFCD across all 441 participants. Next, to compare subjects with CA with the matched control subjects, we conducted a two-sample *t* test of lFCD between groups. These analyses were thresholded at $p < .001$ uncorrected, with a cluster-level correction of $p < .05$ familywise error corrected and a minimum cluster size of $k = 100$ voxels. To control cluster-level type I error rates (34), we calculated cluster corrections with the SnPM13 (5000 permutations) (http://warwick.ac.uk/snpm). Because lFCD has a power law distribution (16), we also conducted analyses with log-transformed lFCD values; this did not alter the findings, and so we report these data in the Supplement. We also conducted two-sample *t* tests on the volume of subcortical nuclei (from FreeSurfer output) as well as measures of cognition and negative emotionality. To examine if subcortical lFCD had relevance for aberrant cognition and/or negative emotionality in CA, we conducted correlation analysis between lFCD in regions showing significant group differences and in behavioral measures showing significant group differences.

RESULTS

Demographics and Behavioral Measures

Demographics and lifestyle factors with descriptive statistics are presented in Table 1. The groups did not significantly differ on any of the DSM-oriented scales, including depression, attention-deficit/hyperactivity disorder, panic disorder, agoraphobia, anxiety, and somatic problems (all $p > .15$), except that the CA group reported higher levels of antisocial behavior ($p = .05$) and more childhood conduct problems ($p = .008$). Cognitive scores and measures of negative emotionality are presented in Table 2. Notably, whereas there were no obvious differences in cognitive performance, the CA group showed significantly higher levels of negative emotionality ($t_{58} = 2.14$, $p = .036$), particularly alienation ($t_{58} = 2.34$, $p = .023$), in line with our previous work (2,3).

Subcortical Volume

Volumetric data and descriptive statistics are reported in Supplemental Table S1. In line with recent work (28,29), no subcortical regions showed significantly different volume

Table 2. Scores on Measures of Cognition and Negative Emotionality in Cannabis Abuse and Control Groups

	CA	CTRL	Statistical Value[a]
Cognition (Composite *Z*)	0.02 ± 0.49	−0.05 ± 0.49	.572
Episodic Memory	−0.33 ± 1.16	−0.11 ± 0.99	.440
Working Memory	−0.07 ± 0.9	0.17 ± 1.08	.344
Flexibility	0.17 ± 0.96	−0.05 ± 0.82	.343
Inhibitory Control	0.03 ± 0.97	0.09 ± 0.93	.799
Processing Speed	0.1 ± 0.85	−0.09 ± 1.25	.494
Delay Discounting	0.02 ± 0.85	−0.28 ± 1.06	.229
Fluid Intelligence	0.28 ± 0.79	−0.03 ± 0.96	.183
Spatial Orientation	0.22 ± 0.98	0.01 ± 0.96	.393
Verbal Episodic Memory	−0.24 ± 1.04	−0.18 ± 0.93	.799
Negative Emotionality (Composite *Z*)	0.35 ± 0.74	−0.05 ± 0.71	.036[b]
Aggression	0.42 ± 0.96	0.14 ± 0.82	.240
Alienation	0.43 ± 0.85	−0.1 ± 0.92	.022[b]
Stress	0.2 ± 1.05	−0.2 ± 1.02	.138

Values are reported as mean ± SD. Raw values for each measure were converted to *Z*-scores based on the larger population of 441 participants.

CA, cannabis abuse; CTRL, control.

[a]*t* test *p* values are reported.

[b]Significant at the $p < .05$ threshold.

between subjects with CA and control subjects. However, subjects with CA did show a trend toward smaller volume of the left hippocampus ($p = .068$), consistent with findings of structural hippocampal abnormalities by prior studies in individuals with CA (35).

IFCD Analyses

We first conducted a voxelwise one-sample *t* test of lFCD across all 441 participants. Results showed widespread lFCD; to summarize, peaks were observed in ventral striatum (VS), hippocampus, amygdala, midbrain, and posterior-ventral brainstem (Figure 1; see Supplemental Figure S2 for maps restricted to CA and control groups). We then examined group differences in lFCD between subjects with CA and matched control subjects. In voxelwise two-sample *t* tests, CA demonstrated significantly higher lFCD in the VS, dorsal midbrain (including substantia nigra and ventral tegmental area), brainstem, and lateral thalamus (all $p < 1 \times 10^{-5}$) (Figure 2 and Table 3). Motion estimates were highly similar between the CA and control groups (mean framewise displacement across all images for subjects with CA 0.171 ± 0.05 and for control subjects 0.163 ± 0.05; $t_{58} = -0.557$, $p = .580$); results were nearly identical when including motion or FreeSurfer-estimated subcortical volume as covariates in the model. Because the lFCD values across these four regions of interest were highly correlated across subjects (mean bivariate correlation: $r = .78$), we averaged together the lFCD values across the four regions of interest to increase statistical power; subsequent analyses refer to this averaged value. This averaged lFCD value did not significantly correlate with FreeSurfer subcortical volume estimates across subjects ($r = -.10$, $p = .432$). In whole-brain functional connectivity analysis using the four clusters from Figure 2A as seed regions, no significant between-group differences emerged at an exploratory threshold of $p < .005$ uncorrected.

Early onset of CA in life is associated with a higher risk for poor neuropsychiatric outcomes (36). Therefore, we ran

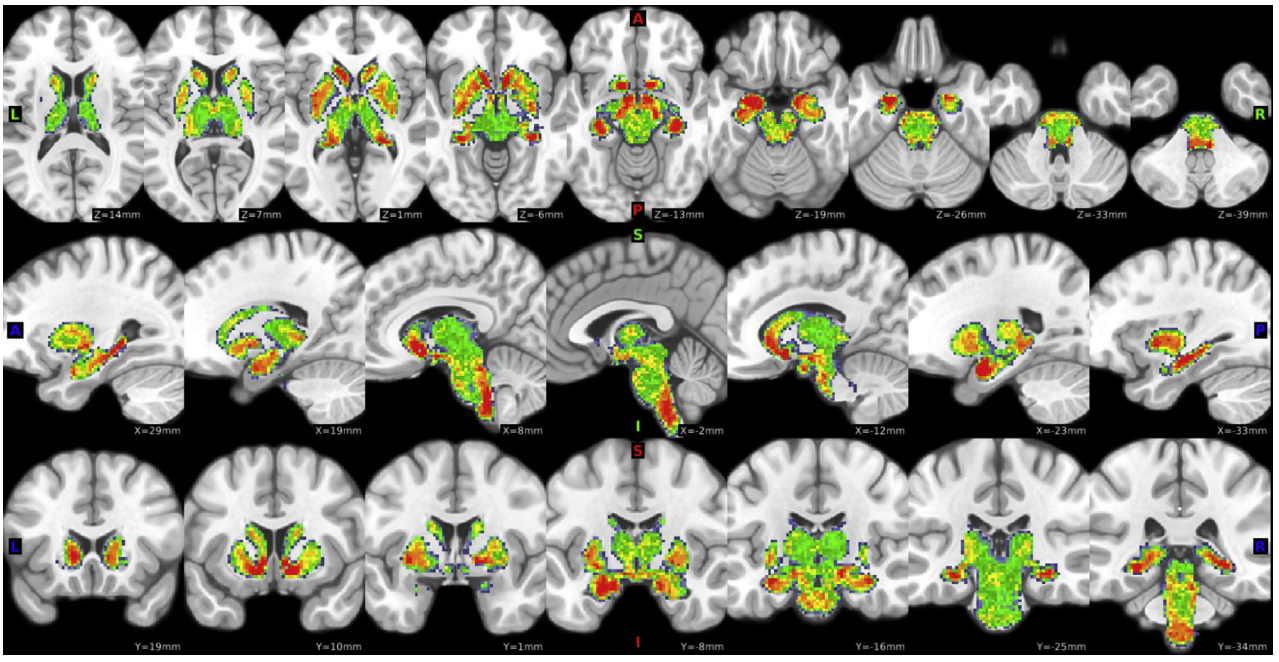

Figure 1. Subcortical local functional connectivity density results across the larger population of 441 participants from the Human Connectome Project. Maps are thresholded at $T > 10$, for visualization. Hot colors indicate regions with high local connectivity density. A, anterior; I, inferior; L, left; P, posterior; R, right; S, superior.

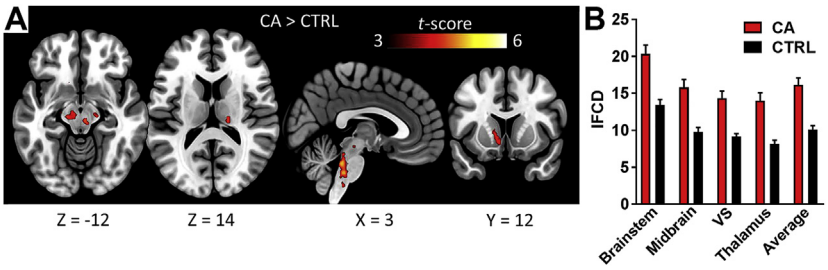

Figure 2. **(A, B)** Subcortical regions where local functional connectivity density (lFCD) was significantly higher in subjects with cannabis abuse (CA) than in control (CTRL) subjects (two-sample *t* test, CA > CTRL). Results are thresholded voxelwise at $p < .001$, with a nonparametric cluster-level threshold of $p < .05$ familywise error corrected, using SnPM13. See Table 3 for coordinates of each cluster in Montreal Neurological Institute space. Error bars represent SEM. VS, ventral striatum.

a one-way analysis of variance between subcortical lFCD and self-reported age of first use. Indeed, subcortical lFCD was significantly different across age of first use ($F_{4,55}$ = 4.13, p = .005) such that higher lFCD was associated with earlier age of cannabis use onset (Figure 3A). In a two-way analysis of variance including group and sex as factors, there was no significant main effect of sex on lFCD ($F_{1,56}$ = 0.49, p = .488), and there was no significant group-by-sex interaction ($F_{1,56}$ = 0.09, p = .761). Finally, because subjects with CA reported significantly higher feelings of alienation than control subjects, in line with our previous work (2), we ran an across-subject correlation between the alienation scores and subcortical lFCD. Subjects with CA showed a significant correlation between lFCD and alienation scores (r = .43, p = .019), whereas the control subjects did not (r = −.09, p = .615) (Figure 3B). The correlation among subjects with CA may be most strongly driven by lFCD near the midbrain; see Supplemental Figure S3 for a voxelwise regression analysis. These results remained significant when conducting a partial correlation to control for the FreeSurfer-estimated subcortical volume of each subject (subjects with CA: r = .43, p = .04; control subjects: r = .02, p = .895). The difference in slopes between subjects with CA and control subjects was significant ($F_{1,56}$ = 5.95, p = .018).

Table 3. Subcortical Regions Where lFCD Was Significantly Higher in Cannabis Subjects Than Control Subjects (Two-Sample *t* Test, CA > CTRL)

Cluster Size (mm^3)	FWE-Corrected p Value	Peak t Value	MNI Coordinates (mm)			Identified Region
			x	y	z	
2544	.008	5.20	20	−20	6	Right thalamus
		4.15	18	−22	−6	
		4.11	6	−26	−12	
3880	.002	4.77	2	−36	−30	Brainstem
		4.70	2	−34	−40	
		4.69	−8	−34	−30	
1968	.002	4.58	−16	−20	−10	Midbrain (including SN/VTA)
		4.18	−22	−12	−6	
		4.06	−28	−12	0	
976	.017	4.36	−10	20	−6	VS
		3.94	−8	12	−8	
		3.84	−12	12	2	

Results are thresholded voxelwise at $p < .001$, with a nonparametric cluster-level threshold of $p < .05$ FWE corrected, using SnPM13. Note that there were no significant results for the reverse contrast (i.e., CTRL > CA).

CA, cannabis abuse; CTRL, control; FWE, familywise error; lFCD, local functional connectivity density; MNI, Montreal Neurological Institute; SN/VTA, substantia nigra/ventral tegmental area; VS, ventral striatum.

DISCUSSION

Despite the high prevalence of cannabis use, little is known about potential chronic effects of CA on brain function and behavior. In this article, we demonstrate that heavy CA is associated with a marked increase in subcortical lFCD, including the midbrain (where the main DA nuclei are located) and the VS, relative to a well-matched control group. These effects are not explained by volumetric differences, and they are associated with critical features of CA: hyperconnectivity was most pronounced in individuals with early-onset CA, a demographic that is particularly vulnerable to the harmful effects of CA (36), and in individuals reporting the highest levels of negative emotionality, particularly alienation. These findings indicate that the resting-state functional organization of the subcortical regions is altered in CA, and this may have relevance for some of the adverse effects of early-onset CA, including emotional disturbance and increased risk for psychosis.

Increased lFCD in the VS and midbrain, including regions where the substantia nigra and ventral tegmental area are located, may be related to hyperdopaminergia in individuals with CA. Indeed, using positron emission tomography and [^{11}C]raclopride to measure DA-induced changes to methylphenidate, we found that subjects with CA when compared with control subjects showed increased DA release in the midbrain, though they showed an attenuated response in striatal regions (3). Functional connectivity between VS and ventral tegmental area is higher in patients with schizophrenia with symptoms of hyperdopaminergia, such as auditory and visual hallucinations, than in patients who do not experience these symptoms (37). Furthermore, in healthy adults and in rats, drugs that increase (levodopa) and decrease (haloperidol) DA signaling have been demonstrated to increase and decrease functional connectivity of these regions, respectively (38,39). However, it is important to note that the findings from these seed-based connectivity studies are likely network specific, as abnormal DA levels attenuate the connectivity between different resting-state networks (33,39). This may explain why individuals with CA show hypoconnectivity

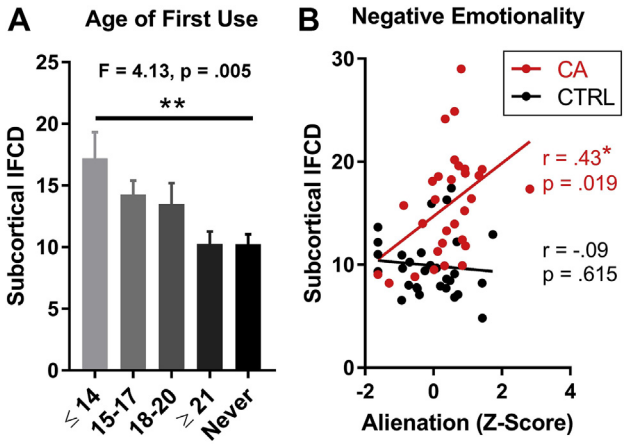

Figure 3. Associations between subcortical local functional connectivity density (lFCD) and **(A)** age at first use of cannabis and **(B)** self-reported feelings of alienation. The difference in slopes between cannabis abuse (CA) and control (CTRL) groups was significant (p = .018).

between nodes of the mesolimbic reward network and nodes of the salience network, e.g., between the dopaminergic midbrain and insula (40) and between nucleus accumbens and dorsal anterior cingulate cortex (41). Interestingly, our seed-based connectivity analysis from these regions did not yield significant group differences. Thus, whereas previous studies have observed long-range subcortical-cortical connectivity alterations in CA, the current results appear to be confined to local hub differences in subcortical circuits. There are at least two possible explanations for this. First, this study carefully controlled for factors such as alcohol and tobacco usage, which may have influenced findings from previous studies. Second, the HCP uses a high-resolution sequence with an aggressive multiband factor, and this contributes to lower subcortical signal-to-noise ratio than is observed with low-resolution sequences. lFCD is more resilient to noise than seed-voxel correlations because lFCD capitalizes on locally shared synchrony and high sampling rate, which makes it possible to reach significant correlations in the absence of significant long-range synchrony. Nevertheless, our lFCD results seem to be broadly in line with previous studies using the FCD technique, although evidence is limited. For instance, subcortical global FCD [a measure that is highly correlated with lFCD (42)] is increased in patients with schizophrenia relative to healthy control subjects (20) [but see (43), where the dopaminergic medication status of the patients was unknown].

Increased lFCD in the VS and midbrain may be a general consequence of pathology to these circuits, as this pattern is observed in various conditions with aberrant DA signaling. Subcortical lFCD is increased in aging (44), attention-deficit/hyperactivity disorder (45), and cocaine use disorder (46), and dopaminergic function is implicated in all these conditions (47–49). These results are also generally in line with the notion that altered connectivity in high-cost hubs is linked to neuropsychiatric disease burden (50). An important next step is to examine how tonic, resting-state subcortical hyperconnectivity may have consequences for phasic DA-dependent processes that are altered in individuals with CA, such as punishment-based learning. Individuals with CA show altered subcortical activations and impaired learning from nondrug rewards and punishment (51,52). If higher resting-state subcortical lFCD is indeed due to higher tonic DA transmission, this increased baseline activity would confer weaker ability to generate the phasic decreases in activity necessary to learn from negative outcomes, in line with extant models of dopaminergic function (53). Future studies with combined positron emission tomography and fMRI could examine this possibility.

We also observed heightened lFCD in subjects with CA relative to control subjects in the pulvinar nucleus of the thalamus and in the brainstem, regions critical for sensory processing and maintenance of autonomic functions, respectively. CA is hypothesized to increase thalamic neuronal excitability, disrupt burst firing patterns, and impair thalamocortical connectivity, leading to impaired sensory processing (54). Correspondingly, we found increased local thalamic connectivity, whereas others found decreased thalamocortical connectivity in individuals with CA (55), and both are exacerbated in individuals with early-onset CA. In addition, individuals with CA show hyperactive thalamic responses to cannabis cues, which correlate with subjective craving of cannabis and are thought to contribute to sensorimotor deficits (56,57). There has been comparatively less attention focused on changes to brainstem function in individuals with CA, perhaps because this region has lower concentrations of cannabinoid receptors than the basal ganglia (58). Yet CA impacts functions regulated by the brainstem region identified here, which includes the ventral raphe nuclei extending into the nucleus of the solitary tract. For instance, regular CA disrupts rapid-eye-movement sleep and increases insomnia (59,60) and negatively influences mood (61). Interestingly, in individuals with posttraumatic stress disorder, sleep disturbance is associated with heightened brainstem glucose metabolism (62), a measure that strongly correlates with lFCD (17). More work is needed to describe how changes to brain functional organization in individuals with CA have relevance for sensory and autonomic functions.

Finally, subcortical hyperconnectivity was most pronounced in individuals with early-onset CA and correlated with feelings of alienation (especially in the midbrain). CA is thought to be particularly detrimental in adolescence because the brain is in a critical period of increased myelination and extensive synaptic pruning (63). Subcortical cannabinoid receptor development is ongoing at this time, and exogenous cannabis perturbs the normal development of the mesolimbic system, which is thought to contribute to psychopathology (64). It is well established that individuals with early-onset CA have poor cognitive and emotional outcomes (36), but the neural basis of this phenomenon is not well understood. Prior rsfMRI studies suggested that increased functional connectivity within cortical networks involved in self-awareness, including the salience and default mode networks, could lead to aberrant emotional and motivational processing (40). We also recently showed that glucose metabolism in inferior frontal gyrus negatively correlated with feelings of alienation in individuals with CA (2). These findings, together with the current data, provide convergent evidence supporting the notion that impaired prefrontal regulation of subcortical activity contributes to the negative emotionality seen in addictions (3,47).

The data presented here build on the small body of work in CA using HCP data. An initial investigation using structural MRI data found that effects of cannabis exposure on subcortical volumetry were minimal, but, critically, the investigators concluded that cannabis effects may be stronger in DA-rich regions, including the VS, and in individuals with the most frequent CA (65). Another diffusion tensor imaging study examined 466 individuals reporting at least one lifetime experience with cannabis and found that frequency of cannabis use was not associated with cortical volumes but was associated with changes to the shape of the amygdala and hippocampus (29). Most notably, the investigators observed that early-onset CA was associated with altered shape of the nucleus accumbens and loss of white matter integrity throughout the cortex. To our knowledge, the present study is the first to extend HCP investigations of CA to rsfMRI data. Because the HCP project is open access, there is a rich opportunity for further examination of the chronic effects of CA using a common dataset.

Limitations

The HCP does not have in vivo measures of subcortical DA release or receptor function, and so we could not directly assess the hypothesis of hyperdopaminergia and psychosis risk in individuals with CA. Resting-state IFCD is an indirect measure of neuronal activity, and the true neurobiological basis of this measure needs further exploration. Furthermore, how exactly hyperdopaminergia manifests at the neural level is disputed. While individuals with CA and psychosis do not show elevated striatal DA release, stimulant-induced changes in DA correlate with psychosis, suggesting that hyperdopaminergia may be more related to postsynaptic hypersensitivity than to total levels of synaptic DA (66,67). Nevertheless, the final release of the HCP will include single nucleotide polymorphism data for all participants; future studies should examine how genetic differences predicting $D_{2/3}$ receptor function, e.g., Taq1A and C957T single nucleotide polymorphisms, predict risk for CA and subcortical IFCD, which would help shed light on this issue. Additionally, it remains unknown whether emotional disturbance is directly caused by CA or if individuals use cannabis to self-medicate feelings of negative emotionality (30). Finally, we cannot rule out the possibility that subcortical hyperconnectivity may be associated with cannabis withdrawal and the extent to which it abates with prolonged abstinence, as has been observed with other functional connectivity abnormalities in individuals with CA (41).

Conclusions

Despite increased usage of cannabis worldwide, little is known about the neuropsychiatric effects of CA, especially in early-onset users. Here we show that resting-state connectivity of subcortical functional hubs, particularly within dopaminergic nuclei implicated in psychopathology, is greatly increased in individuals with CA. This pattern was exaggerated in individuals who began using in early adolescence and was associated with high levels of negative emotionality. Thus, subcortical functional connectivity may be a useful marker for tracking the development of psychopathology with prolonged CA.

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ARTICLE INFORMATION

From the National Institute on Alcoholism and Alcohol Abuse (PM, DT, NDV) and National Institute on Drug Abuse (NDV), National Institutes of Health, Bethesda, Maryland.

Address correspondence to Peter Manza, Ph.D., Laboratory of Neuroimaging, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, 10 Center Drive, Bethesda, MD 20892-1013; E-mail: peter.manza@nih.gov.

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