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ORIGINAL ARTICLE

Adverse Effects of Cannabis on Adolescent Brain Development: A Longitudinal Study

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Abstract

Cannabis is widely perceived as a safe recreational drug and its use is increasing in youth. It is important to understand the implications of cannabis use during childhood and adolescence on brain development. This is the first longitudinal study that compared resting functional connectivity of frontally mediated networks between 43 healthy controls (HCs; 20 females; age $M = 16.5 \pm 2.7$) and 22 treatment-seeking adolescents with cannabis use disorder (CUD; 8 females; age $M = 17.6 \pm 2.4$). Increases in resting functional connectivity between caudal anterior cingulate cortex (ACC) and superior frontal gyrus across time were found in HC, but not in CUD. CUD showed a decrease in functional connectivity between caudal ACC and dorsolateral and orbitofrontal cortices across time. Lower functional connectivity between caudal ACC cortex and orbitofrontal cortex at baseline predicted higher amounts of cannabis use during the following 18 months. Finally, high amounts of cannabis use during the 18-month interval predicted lower intelligence quotient and slower cognitive function measured at follow-up. These data provide compelling longitudinal evidence suggesting that repeated exposure to cannabis during adolescence may have detrimental effects on brain resting functional connectivity, intelligence, and cognitive function.

Key words: cannabis, cognition, development, functional connectivity, longitudinal

Introduction

The age of initiation of cannabis use is shifting back with younger children and adolescents reporting daily cannabis use. According to NIDA (2014), 16.4% of individuals age 12–17 and 51.9% of individuals age 18–25 years have used cannabis in their lifetime in the USA. While cannabis use seems to be increasingly accepted as a safe recreational drug (e.g., legalization in certain US states), it is important to better understand what are the implications of chronic cannabis use during critical periods of development such as adolescence.

Previous studies investigating the effect of cannabis on development suggest that there is a persistent effect on cognition and neuropsychological performance in individuals who initiate cannabis use during adolescence. Longitudinal data show that individuals with more persistent cannabis dependence have a pronounced intelligence quotient (IQ) decline, with significant impact on overall IQ (full-scale IQ) (Meier et al. 2012). Moreover, evidence suggests that overall IQ deficits do not fully recover after cessation of use (1 year), particularly in adolescent-onset cannabis users (Meier et al. 2012). In addition to its effects on intellectual ability, cannabis has been observed to have a negative impact on neuropsychological test performance in tasks that assess executive function and psychomotor speed (Bolla et al. 2002; Lane et al. 2007). Individuals that started using cannabis during adolescence have persistent neuropsychological deficits even after 10 months of abstinence (Schweinsburg et al. 2008). It is important to examine the specific neural changes underlying poor cognitive and neuropsychological performance in adolescents with cannabis use disorder.

Brain networks known to mediate cognition and executive function undergo critical maturation during adolescence (Lenroot and Giedd 2006). Neuroimaging studies have identified inconsistent alterations in brain activity within regions that mediate executive function in young adults with adolescent-onset cannabis use disorder (CUD) versus healthy controls (HCs).

There is evidence of "lower" task-related frontal activity in CUD (vs. controls). For example, CUD has shown (1) lower anterior cingulate cortex activity (vs. controls) during evaluation of responses and consequences in a gambling task (Wesley et al. 2011; De Bellis et al. 2013). There is also evidence of "increased" prefrontal functional connectivity in CUD (vs. controls) during response inhibition measured with tasks such as the Stroop task (Filbey and Yezhuvath 2013). A recent study reported that individuals with current cannabis use disorder show increased activity in a network including dorsolateral prefrontal cortex, anterior cingulate, parietal, and striatal regions when making the decision of purchasing cannabis (Bedi et al. 2015). Finally, a longitudinal study reported to have not found task-related (working memory) functional magnetic resonance imaging (fMRI) differences between individuals with cannabis use disorder and HCs (Cousijn et al. 2014). While most task-related fMRI studies mentioned above highlight alterations within brain regions known to mediate executive function, there are inconsistencies which may be related to differences in task requirement, engagement, and task difficulty.

The examination of brain fluctuations during rest allows us to investigate intrinsic neural network organization irrespective of cognitive processes related to task performance (Biswal et al. 1995). Temporal coherence or correlations between regions within resting-state networks represent brain functional connectivity, an index of brain function which has been found to be consistent across individuals and time (Damoiseaux et al. 2006; De Luca et al. 2006; Fox and Greicius 2010). Strength of resting functional connectivity has been directly associated with the quality of individual behavioral performance (Seeley et al. 2007; Mennes et al. 2010). Cross-sectional studies have examined resting fluctuations in adolescent CUD. One study reported that adolescents with CUD entering treatment have (1) reduced interhemispheric functional connectivity in cerebellum and superior frontal gyrus (SFG) and (2) higher fractional amplitude of regional low-frequency fluctuations in regions known to comprise right fronto-parietal and fronto-cerebellar networks (Orr et al. 2013). Another study reported that adolescents with high cannabis use who are not seeking treatment show a relationship between strength of resting functional connectivity within an executive function network (selected with independent components analysis) and cannabis use (Houck et al. 2013). These findings provide cross-sectional evidence of potential alterations in resting functional connectivity within networks known to mediate executive control in adolescents with CUD. A recent study reported that functional connectivity patterns within resting fMRI data can be used to classify male cannabis users versus HCs (Cheng et al. 2014). Cheng et al. (2014) used a connectivity-based MVPA analysis (multivoxel pattern analysis) to distinguish cannabis versus control subjects based on resting functional connectivity strength between frontal regions (middle frontal, cingulate, and superior frontal gyri) and posterior cingulate. While the above cross-sectional studies identify important functional connectivity differences related to cannabis use disorder, a longitudinal design is needed to further explore the trajectory of cannabis effects on development.

Brain functional connectivity in networks involved in executive function are defined and delineated during critical developmental stages such as childhood, adolescence, and young adulthood (Kelly et al. 2009). Kelly et al. (2009) identified indices of brain maturation manifested as significant age-related shifts in functional connectivity within 5 anterior cingulate cortex (ACC) resting-state networks. ACC is a critical prefrontal region associated with self-regulatory mechanisms (Posner 2007). There is cross-sectional evidence that functional connectivity of a frontal network that includes ACC during task stop-signal task performance (assessing inhibitory control) is disrupted in CUD versus nondependent cannabis users (Filbey and Dunlop 2014). Lower ACC in active CUD versus controls has been associated with CUD's inability to recognize an error or monitor behavior, a deficit that may contribute to maintenance of drug abuse (Hester et al. 2009). ACC activity (during Stroop task performance) recorded before treatment has been found to be positively correlated to treatment outcome 1 year after rest fMRI scan: CUD with higher ACC activity during task performance had better treatment outcome (Kober et al. 2014). Effects of cannabis use on ACC network maturation and its relationship to treatment outcome needs to be further investigated. Given that previous studies that reported differences in resting functional connectivity and task-evoked activity in ACC in CUD were cross-sectional, it is crucial to investigate the trajectory of brain functional connectivity change across time in CUD. Moreover, the relationship between resting functional connectivity changes and clinically relevant measures such as cognition and treatment outcome needs to be explored. The current study is the first longitudinal neuroimaging study that examines changes in functional connectivity across time in abstinent CUD. Based on the above literature, the current study focused on examining changes in ACC resting-state networks involved in self-regulatory control (Kelly et al. 2009) at 2 timepoints after adolescents with CUD had completed treatment.

The study aims and hypotheses were: (1) to determine whether there are differences in the trajectory of ACC functional connectivity across time between adolescents with CUD and HC. Due to the effects of drug addiction on brain functional connectivity (Camchong et al. 2014) and cannabis on brain maturation (Hester et al. 2009; Kober et al. 2014), we hypothesized that CUD would show a significant drop in functional connectivity across time when compared with HC. (2) To investigate whether baseline functional connectivity measures could be used to predict cannabis use during the interscan interval. Based on previous findings (Camchong et al. 2013), we expected to find that lower ACC functional connectivity connectivity would predict the amount of cannabis use during the observation period. (3) To explore whether the degree of cannabis use during the interscan interval was related to (i) intelligence and (ii) executive function given recent evidence of an adverse cannabis effect on cognitive development. We hypothesized that higher cannabis use during the interscan interval would be negatively associated with measures of intelligence and executive function.

Materials and Methods

Participants

Eighty-seven participants (age: 10–21 years) were recruited under an approved Institutional Review Board protocol at the University of Minnesota (Kumra et al. 2012; Epstein and Kumra 2014). For subjects under age 18, informed consent was obtained from at least one parent, and assent was obtained from the subjects themselves. Participants over age 18 consented to their own participation, and their parents consented for a collateral interview and substance use history. To ensure that any group differences were not due to recent cannabis use, 18 subjects were excluded from analysis because they tested positive for cannabis on the day of MRI scanning. Data from 3 additional subjects were eliminated due to excessive MRI motion artifacts (see fMRI Image Analysis section). Usable data were available for 65 participants: 22 abstinent adolescents with CUD (8 females; age at study entry $M = 17.6 \pm 2.4$; range 13–23 years old) and 43 HC (20 females; age at study entry $M = 16.5 \pm 2.7$; range 10–22 years old).

Table 1 Demographics for adolescents with CUD and HC

	Time 1		Time 2	
	CUD ($N = 22$)	HC ($N = 43$)	CUD ($N = 22$)	HC ($N = 43$)
Gender (% females)	36.4%	46.5%	–	–
Mean age (SD)	17.0 (2.0)	15.9 (2.9)	18.6 (2.0)	17.4 (2.9)
Parental SES	2.1	1.9	–	–
Number of months between MRI scans	19.2 (3.2)	17.5 (4.6)		
WASI full scale IQ***	99.4 (14.4)	116.4 (8.8)	102.2 (15.2)	119.0 (9.8)
Mood*	13.6%	0	31.3%	6.5%
Externalizing***	36.4%	0	–	–
Anxiety	13.6%	2.3%	4.5%	2.3%
Nicotine***	45.5%	0	54.5%	0
Lifetime alcohol (SD)*** (number of days used)	203.68 (257.15)	2.23 (7.50)	372.95 (349.36)	15.70 (44.37)
Lifetime cannabis (SD)***(number of days used)	1049.86 (637.55)	0	1202.45 (604.49)	12.32 (59.31)

*Significant differences between groups: CUD and HC, $P < 0.05$.
***Significant differences between groups: CUD and HC, $P < 0.001$.

Abstinent CUD were recruited from treatment settings for chemical dependency in the Minneapolis and St. Paul metro areas. Upon study recruitment abstinent CUD had an average of 7 days of having completed treatment at the Time 1 scanning session. Adolescents were selected who reported cannabis as their drug of choice with significant cannabis exposure (>50 exposures to cannabis; age of cannabis use onset $M = 13 \pm 2.2$; range 8–18 years old), and who did not meet lifetime criteria for abuse or dependence of other illicit drugs with the exception of alcohol abuse or nicotine dependence. HC were recruited from the same geographic area through flyers and word of mouth to closely match the patient group on age, sex, and handedness.

Diagnostic Screening

Substance use disorder diagnosis was made using the Structured Clinical Interview for *DSM-IV* Axis I Disorders at both timepoints. Exclusion criteria for all subjects included any contraindication to MRI, positive pregnancy test, history of a *DSM-IV* diagnosis of mental retardation, neurological disorder and head injury with loss of consciousness (>30 s) or neurological illness. MRI scans were examined by a neuro-radiologist to exclude any gross anatomical abnormalities, which also served as an exclusion criterion. CUD inclusion criterion: current DSM IV dependence on cannabis. HC inclusion criteria: no current or past "DSM-IV" diagnosis. HC exclusion criteria: prior or current treatment with psychotropic medications, history of psychological counseling, history of >5 lifetime exposures to any illicit drug, and/or history of schizophrenia.

We excluded CUD subjects with a lifetime diagnosis of bipolar disorder or a schizophrenia-spectrum disorder and/or patients who were either depressed (Hamilton Depression Rating Scale, Williams 1988) or anxious (Hamilton Anxiety Rating Scale, Bruss et al. 1994) at baseline. To enhance program retention, 5 of 22 CUD subjects had been prescribed psychotropic medication to target irritability, affective dysregulation or sleep disturbance (Depakoate, Aripiprazole, Quetiapine) at the time of scanning.

The modified Time-Line Followback (Sobell and Sobell 1996) was administered to all subjects at 3 timepoints (baseline at treatment completion, 9 and 18 months after study entry) to obtain detailed information regarding cannabis use: age at first use, time since last use in days, average frequency of use measured as average days of use per month, duration of regular use in months and estimated cumulative lifetime dose (i.e., number of days of use).

All subjects had MRI data at 2 timepoints (at baseline and 18 months after study entry; days between scans: $M = 553.3 \pm 130.2$). Additionally, subjects were administered demographic, substance use history, and clinical and neuropsychological assessments at each timepoint (Table 1). Urine toxicology tests were performed on the day of the MRI scan using the K012B 12 Panel Drug Screen Test from Drug Test Systems (Dover, NH, USA) to confirm self-report of drug use. Subjects that tested positive after urine test were not scanned that day. At the second MRI scan visit 7 CUD had sustained abstinence and 15 resumed to cannabis use (number of days using cannabis between scans: $M = 471.8 \pm 202.9$).

Intelligence and Executive Functioning Metrics

The Weschler Abbreviated Scale of Intelligence (WASI, Weschler 1999) was administered to all subjects to estimate general intelligence at both timepoints. The Attention Network Test (ANT Fan et al. 2002), which assesses 3 aspects of attention (alerting, orienting, and executive attention) was administered to all participants at each timepoint. A description of this task has been detailed in Fan et al. (2002).

Imaging Data Acquisition

At both timepoints, all participants underwent a 6-min resting-state fMRI scan and were instructed to be as still as possible, keep their eyes closed and stay awake. Images were collected using a Siemens TIM Trio 3T scanner (Erlangen, Germany). Sequence parameters: gradient-echo echo-planar imaging (EPI) 180 volumes, repetition time (TR) = 2 s, echo time (TE) = 30 ms, flip angle = 90°, 34 contiguous AC–PC aligned axial slices with an interleaved acquisition, voxel size = 3.4 × 3.4 × 4.0 mm, matrix = 64 × 64 × 34. Participants were debriefed at the end of the scan to determine whether they had stayed awake. A high-resolution T_1-weighted anatomical image was acquired using a magnetization prepared rapid gradient-echo sequence. A field map acquisition was collected and used to correct the fMRI data for geometric distortion caused by magnetic field inhomogeneities (TR = 300 ms, TE = 1.91 ms/4.37 ms, flip angle = 55°, voxel size = 3.4 × 3.4 × 4.0 mm).

FMRI Imaging Analysis

Individual-Level Analyses

All individual-level analyses were conducted using procedures reported in our previous study (Camchong et al. 2014). The

following pre-statistics processing was applied for each subject using FEAT (FMRIB's Software Library [FSL]): first 3 volumes deleted to account for magnetization stabilization, motion correction, B0 field map unwarping, slice-timing correction, non-brain removal, spatial smoothing (with a 6-mm full-width half-maximum kernel), grand mean scaling, high-pass temporal filtering (100 Hz) to remove correlations associated with slow trends scanner noise and registration of all images to Montreal Neurological Institute (MNI) 2 × 2 × 2 mm standard space.

Data Denoising

Noise correction procedure used was chosen to remove all major sources of artifacts while preserving the integrity of the continuous time series. Independent Component Analysis was used to decompose individual preprocessed 4D data sets into different spatial and temporal components (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/MELODIC). Independent components defined by ICA (FSL MELODIC) were classified as noise using spatial and temporal characteristics detailed in the MELODIC (FSL) manual (http://www.fmrib.ox.ac.uk/fslcourse/lectures/melodic.pdf) and based on previous methodological reports that describe identification of individual sources of artifact (Kelly et al. 2010; Xu et al. 2014) representing head motion (i.e., "rim-like" artifacts around the brain, spikes in timeseries), scanner artifacts (i.e., slice dropouts, high-frequency noise, field inhomogeneities), physiological noise (i.e., respiration, cardiac frequencies, white matter signal, ventricular/cerebrospinal fluid fluctuations, frontal air cavities, ocular structures). Signal from noise components were regressed (subtracted out) from the preprocessed data. Two CUDs and one HC were excluded from the study because motion correction output showed more than 1.8 mm of movement (translation) or >50% of the ICA components were identified as movement related (e.g., "rim-like" artifacts around the brain, spikes in timeseries).

ROI Selection and Seed Generation

To examine longitudinal changes in resting functional connectivity in networks known to mediate self-regulatory control, we examined functional connectivity of 5 anterior cingulate (ACC) networks defined in Kelly et al. (2009): caudal, dorsal, rostral, perigenual, and subgenual ACC. A spherical seed with 3.5 mm radius was placed at each seed (Kelly et al. 2009; Camchong et al. 2011). We extracted the time series from these seeds for each participant by computing the mean intensity for all voxels within the seed region for each timepoint in the denoised residual data.

Resting-State Individual-Level Analysis

The average time series was extracted for each ACC seed for each participant in each group. A multiple regression analysis on the denoised data was performed between the extracted average timeseries from the seed and all voxels in the brain. This generated a map with a correlation coefficient (r) for each voxel, for each individual, for each seed, for both Time 1 and 2. Correlation coefficients (r) were transformed to standardized z values. Resulting standardized z-maps showed the degree of correlations with the corresponding ACC seed averaged time-series for each seed for each participant for both Time 1 and 2.

Group-Level Analyses

Interaction (Group × Time) Effects—Whole-Brain Analysis

To investigate whether CUD and HC had different trajectories of functional connectivity change across time, we conducted a mixed-effects analysis of variance (ANOVA; whole-brain analysis) using AFNI (3dLME). ANOVA examined: main effects of Group (CUD vs. HC), main effects of Time (Time 1 vs. Time 2) and interaction effects (Group × Time). Analysis of variance (ANOVA) was conducted with and without age, alcohol, and nicotine use as covariates. A threshold/cluster method derived from Monte Carlo simulations (AlphaSim, AFNI) was applied to the t-statistics map to control for false-positive findings. Monte Carlo simulations (1000 iterations) accounted for the full-width half-maximum Gaussian filter (6 mm FWHM; 3dFWHMx) and with a connectivity radius of 7.1 mm. On the basis of these simulations, the family-wise α of 0.025 was preserved with an a priori voxel-wise probability of 0.005 and 3-dimensional clusters with a minimum volume of 2352 μL. Clusters that showed a significant interaction and survived correction for multiple comparison were identified (Figs 1A and 2A) and used as masks from which individual z-scores were extracted for line graphs (Figs 1B and 2B) and for exploration of functional connectivity correlates.

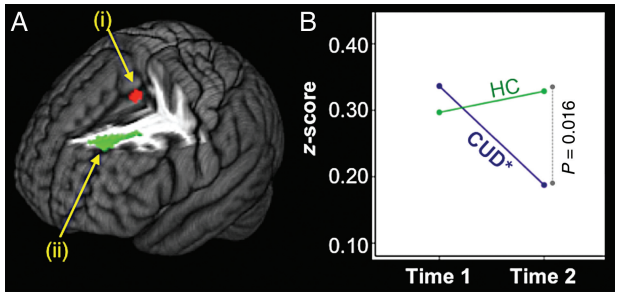

Figure 1. (A) Three-dimensional MNI brain with slices cut at $x = -12$ $y = 9$, $z = 43$ showing regions with lower functional connectivity in adolescents with CUD than healthy controls HC: (1) caudal ACC (red cluster) and (2) left DLPFC (BA 9; green cluster). (B) Line graph showing trajectory of functional connectivity from Time 1 scan to Time 2 scan for the CUD (purple line) and HC (green line) groups. CUD had significant decrease in functional connectivity across time ($P = 0.040$), HC did not. CUD showed significantly lower functional connectivity than HC at Time 2 ($P = 0.016$).

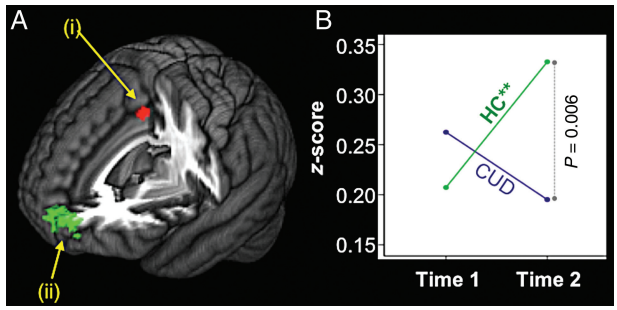

Figure 2. (A) Three-dimensional MNI brain with slices cut at $x = -12$ $y = 9$, $z = 11$ showing regions with lower functional connectivity in adolescents with CUD than HC: (1) caudal ACC (red cluster) and (2) left SFG (BA 10; green cluster). (B) Line graph showing trajectory of functional connectivity from Time 1 scan to Time 2 scan for the CUD (purple line) and HC (green line) groups. HC had significant increase in functional connectivity across time ($P = 0.003$), CUD did not. CUD showed significantly lower functional connectivity than HC at Time 2 ($P = 0.006$).

Exploration of Cumulative Effects of Cannabis—Whole-Brain Analysis

To investigate the cumulative effect of cannabis use on ACC functional connectivity. We conducted an ANOVA comparing functional connectivity collected at Time 2. We focused on examining differences at the Time 2 scan ("endpoint"; Wang and Duan, in preparation) because it represents cumulative effects on brain functional connectivity related to cannabis use during the

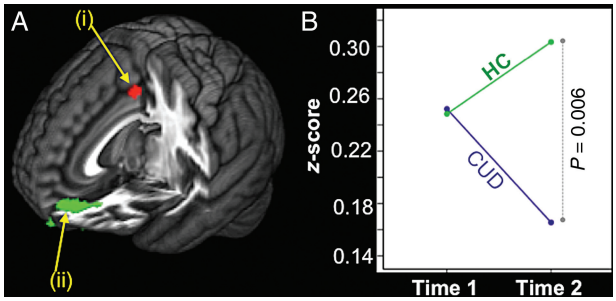

Figure 3. (*A*) Three-dimensional MNI brain with slices cut at x = −12 y = 9, z = −16 showing regions with lower functional connectivity in adolescents with cannabis use disorder (CUD) than HC: (1) caudal ACC (red cluster) and (2) left OFC (BA 11; green cluster). (*B*) Line graph showing trajectory of functional connectivity from Time 1 scan to Time 2 scan for the CUD (purple line) and HC (green line) groups. CUD showed significantly lower functional connectivity than HC at Time 2 ($P = 0.006$).

Table 2 MNI coordinates of clusters (bolded) in which resting functional connectivity of bilateral caudal ACC seed (Kelly et al. 2009; Camchong et al. 2011) showed a significant Group (adolescents with cannabis use disorder "CUD" vs. HCs) × Time (Time 1 vs. 2) interaction

	Hemisphere	BA	x	y	z
Dorsolateral prefrontal cortex	Left	9	−23	37	42
Superior frontal cortex	Left	10	−9	67	10
OFC	Left	11	−12	42	−19

Exploratory analysis showed that CUD showed lower functional connectivity than HCs at Time 2 (correcting for Time 1) between caudal ACC and regions that showed a significant interaction as well as between caudal ACC and OFC.

lifetime and during the interscan interval. A threshold/cluster method derived from Monte Carlo simulations (AlphaSim, AFNI) was applied to the *t*-statistics map to control for false-positive findings. Monte Carlo simulations (1000 iterations) accounted for the full-width half-maximum Gaussian filter (6 mm FWHM; 3dFWHMx) and with a connectivity radius of 7.1 mm. On the basis of these simulations, the family-wise α of 0.025 was preserved with an a priori voxel-wise probability of 0.005 and 3-dimensional clusters with a minimum volume of 3928 µL. Clusters that survived correction for multiple comparison were identified (Figs 1A, 2A, and 3A) and used as masks from which individual z-scores were extracted for line graphs (Figs 1B, 2B, and 3B) and for exploration of functional connectivity correlates.

Cannabis Use Prediction During Scanning Interval Using Baseline Functional Connectivity Measures

To investigate whether functional connectivity at Time 1 can be used to predict subsequent cannabis use (between Time 1 and 2), cannabis use during the interscan interval (number of days of cannabis use between Time 1 and Time 2) was subjected to hierarchical regressions. The regression model entered lifetime substance use at Time 1 (cannabis and alcohol) on step 1, covariates on step 2, and functional connectivity strength at Time 1 within identified clusters that showed significant functional connectivity differences between CUD and HC (Table 2) on step 3. Covariates included full-scale IQ and age at Time 1.

Exploratory Analyses

Given that chronic cannabis use may have long-term adverse effects on brain maturation (Meier et al. 2012), we explored whether amount of cannabis use could predict ACC functional connectivity. We assessed the influence of cannabis use during the interscan interval on functional connectivity at Time 2 using hierarchical regressions, in clusters showing significant functional connectivity differences at Time 2. The regression model entered Time 1 functional connectivity (for each of the 3 clusters identified in Table 2) on step 1, age at Time 1 step 2, and substance use on step 3. Substance use included cannabis use and alcohol use during interscan interval and cumulative lifetime cannabis and alcohol use.

Because adolescence is a sensitive period of cognitive development, we explored the relationship between cannabis use and intelligence measures. We assessed the influence of cannabis use during the interscan interval on cognition (WASI full-scale IQ) at Time 2 above and beyond IQ at Time 1, by conducting hierarchical regressions. The regression model entered Time 1 IQ scores on step 1, age at Time 1 on step 2, and substance use on step 3. Substance use regressors were same as above.

Given that ACC is known to mediate cognitive function, we explored the relationship between cannabis use and ANT (Attention Network Task) performance by conducting similar hierarchical regression analysis as above using ANT performance (reaction time and accuracy) as dependent variables.

Results

Group × Time Interaction

Mixed-effect ANOVA revealed 2 significant interactions (1) between caudal ACC and dorsolateral prefrontal cortex (DLPFC, BA 9; Fig. 1) and (2) between caudal ACC and SFG (BA 10; Fig. 2; Table 2). Interactions were significant "before" (DLPFC: $F = 6.43$, $P = 0.014$; SFG: $F = 7.35$, $P = 0.009$) and "after" (DLPFC: $F = 14.13$, $P = 0.00039$; SFG: $F = 9.48$, $P = 0.003$) correcting for the effects of age, alcohol use, and nicotine use.

CUD: Functional Connectivity Decrease Across Time

Within-group repeated-measures t-test on extracted z-scores (from Table 2 clusters) revealed that CUD (not HC) had a significant decrease ($P = 0.04$) in functional connectivity between caudal ACC and DLPFC (BA 9) from Time 1 to Time 2 (Fig. 1B).

HC: Functional Connectivity Increase Across Time

Within-group repeated measures t-test on extracted z-scores revealed that HC (not CUD) had a significant increase in functional connectivity between caudal ACC and SFG (BA 10) ($P = 0.003$) from Time 1 to Time 2 (Fig. 2B).

Cross-sectional Exploratory Findings—Cumulative Effects of Cannabis on Functional Connectivity

ANOVA on Time 2 z-maps for each ACC seed (correcting for Time 1 functional connectivity) revealed that when compared with HC, CUD had lower functional connectivity between caudal ACC seed and same 2 frontal regions identified above: dorsolateral prefrontal cortex (DLPFC; BA 9) ($P = 0.016$; Fig. 1A) and SFG (Brodmann area: BA 10) ($P = 0.006$; Fig. 2A). In addition, results showed that lower functional connectivity between caudal ACC and orbitofrontal cortex (OFC) at Time 2 in CUD when compared with HC (OFC; BA 11) ($P = 0.006$; Fig. 3A) (Table 2 bottom row). A separate analysis of variance did not find significant group differences within these clusters at Time 1. Functional connectivity differences remained significant after controlling for alcohol and nicotine abuse.

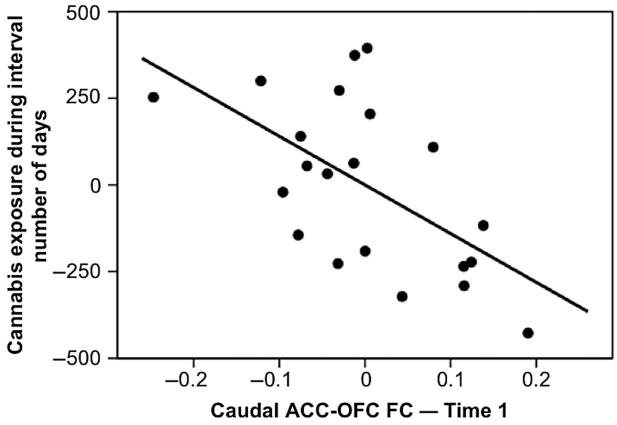

Figure 4. Partial regression plot. Values in *x* and *y* axes are residuals that illustrate the relationship between Caudal ACC–OFC functional connectivity at Time 1 and cannabis exposure during the scanning interval (18 months) after removing the linear effects of other independent variables in the hierarchical linear regression model: lifetime substance use (cannabis and alcohol) up to Time 1 (step 1), age at Time 1 (step 2), and functional connectivity between Caudal ACC and DLPFC and SFG (step 3). ACC, anterior cingulate cortex; functional connectivity, functional connectivity; DLPFC, dorsolateral prefrontal cortex; SFG, superior frontal gyrus.

Prediction of Cannabis Use During Scanning Interval Using Baseline Functional Connectivity Measures

To determine whether functional connectivity at Time 1 can be used to predict cannabis use during the following 18 months (between Time 1 scan and Time 2 scan) above and beyond covariates, hierarchical regression analyses were conducted on cannabis use during the scanning interval (Bava et al. 2013). Regression models entered lifetime cannabis and alcohol use up until Time 1 scan on step 1; age at Time 1 on step 2; and functional connectivity strength at Time 1 within the 3 identified frontal clusters (Table 2) on step 3. Lower functional connectivity between caudal ACC and OFC (BA 11) at Time 1 predicted more days of cannabis use during the interscan interval ($F_{6,20} = 2.19$, $P = 0.11$. $\beta = -1.08$, $P = 0.02$), above and beyond variability attributable to lifetime substance use up to Time 1 and age at Time 1 (Fig. 4).

Cannabis Consumption During Interscan Interval Predicts IQ at Time 2

To determine the influence of substance use (cannabis and alcohol) over the interscan interval on IQ at Time 2, hierarchical regression analyses were conducted on Full-scale IQ scores at Time 2. Regression model entered the Time 1 IQ measure on step 1; age at Time 1 on step 2; and cannabis use during the interscan interval on step 3. Greater cannabis use over the interscan interval predicted low full-scale IQ at Time 2, $F_{3,21} = 35.13$, $P < 0.001$; $\beta = -0.19$, $P = 0.044$, above and beyond variability attributable to the equivalent Time 1 IQ measure and Time 1 age (Fig. 5A). When adding alcohol use during the interscan interval as a covariate, findings of cannabis effects become a trend (4,21) = 27.40, $P < 0.001$; $\beta = -0.17$, $P = 0.087$. Alcohol use during interscan interval alone, however, was not a significant predictor of IQ at Time 2 ($\beta = -0.11$, $P = 0.243$).

Cannabis Consumption During Interscan Interval Predicts Cognitive Function at Time 2

To determine the influence of substance use (cannabis and alcohol) over the interscan interval on Executive Control

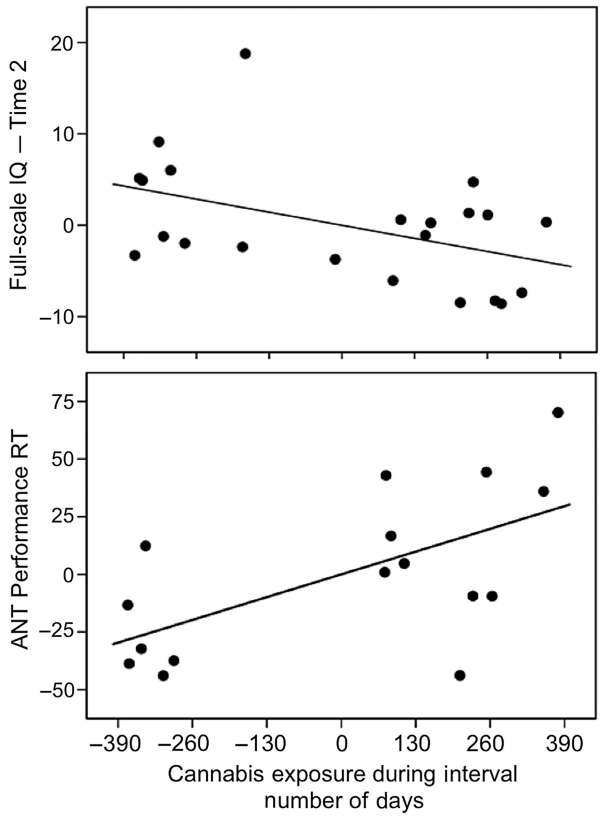

Figure 5. Partial regression plots. Values in *x* and *y* axes are residuals that illustrate the relationship between cannabis exposure during the scanning interval (18 months) and (*A*) full-scale IQ and (*B*) reaction time (RT) in the ANT at Time 2 after removing the linear effects of other independent variables in the hierarchical linear regression model: corresponding Time 1 measure of IQ or executive functioning (step 1) and age at Time 1 (step 2). IQ, intelligence quotient; ANT, attention network task.

performance as measured by ANT, hierarchical regression analyses were conducted on ANT reaction time scores at Time 2. Regression model entered the Time 1 ANT measure on step 1; age at Time 1 on step 2; and substance use during the interscan interval on step 3. Greater cannabis use over the interscan interval predicted slower reaction time during ANT performance at Time 2, $F_{4,15} = 37.62$, $P < 0.001$; $\beta = 0.28$, $P = 0.007$, above and beyond variability attributable to the equivalent Time 1 ANT measure and Time 1 age (Fig. 5B). Alcohol use during interscan interval, however, was not a significant predictor of IQ at Time 2 ($\beta = -0.16$, $P = 0.12$).

Discussion

This is the first study that examined longitudinal data to investigate the effects of cannabis on brain maturation during adolescence as indexed by resting functional connectivity of frontally mediated networks. We observed adverse effects of cannabis use during an 18-month period on IQ and executive functioning consistent with previous data demonstrating an adverse effect of cannabis use on adolescent cognitive development. This study extends the literature by identifying both cross-sectional and longitudinal differences in resting-state networks known to mediate executive function and regulatory control between adolescents with CUD and HCs. A crucial finding was the identification of a potential neural marker of relapse to cannabis use

characterized by lower resting functional connectivity between caudal anterior cingulate cortex (ACC) and OFC in adolescents with CUD.

Cannabis Effects on Brain Functional Connectivity

We found evidence of functional connectivity decrease across time between caudal ACC and 2 frontal regions known to mediate executive function (left dorsolateral prefrontal cortex (DLPFC) and OFC) in adolescents with CUD. A previous study examining anterior cingulate functional connectivity throughout development did not detect differences in caudal ACC functional connectivity across healthy children, adolescents, and adults (Kelly et al. 2009). Kelly et al. (2009) suggest that functional connectivity in this network undergoes rapid maturation and had presumably reached a plateau in their observed sample of healthy participants. Our data support this hypothesis, HC did not show functional connectivity change across time between these regions. While both groups (CUD and HC) showed similar levels of caudal ACC–DLPFC functional connectivity at Time 1 (Fig. 2*B*), the CUD group showed a significant functional connectivity decline across time. Interestingly, when separating the CUD into those that relapse or abstain during the interscan interval, only those that relapsed showed the significant functional connectivity decline. These findings may be related to alterations of underlying dopaminergic function in CUD that resumed cannabis use. Frontal functional connectivity has been previously reported in a previous cross-sectional study in which acute cannabis intake reduces frontal functional connectivity during task performance (Bhattacharyya et al. 2014) PET data show that acute cannabis use increases dopamine release in striatal regions which triggers a proportional decrease in dopamine D2 receptor (D2R) availability in the brain (Bossong et al. 2009). Reduced D2R availability reduces brain metabolism in ACC, OFC, and DLPFC (Volkow et al. 1993, 2001, 2007). Lower metabolism in these frontal regions may underlie impaired cognitive functioning and decision-making. Current findings in which cannabis consumption during 18-month interval was correlated with executive functioning performance support this hypothesis. CUD that reported more days of cannabis consumption during the interscan interval had slower reaction time when performing the ANT task at Time 2. These findings need to be confirmed with larger sample sizes and more data collection timepoints. In addition, while HC showed a significant increase in functional connectivity between caudal ACC and left BA 10, adolescents with CUD did not even show a trend. Current findings provide evidence for both a decline in functional connectivity as well as an attenuation in functional connectivity increase in adolescents in CUD (particularly those that relapse during the interscan interval) versus HC across time.

Potential Biomarker of Vulnerability to Relapse in Adolescents with CUD

Strength of functional connectivity between caudal ACC and OFC was found to predict severity of cannabis use for the following 18 months. This finding is in-line with findings from Kober et al. (2014) suggesting a relationship between pretreatment task-evoked caudal ACC activity and subsequent measures of abstinence in adult males with CUD: those with stronger Stroop-evoked caudal ACC activity reported more days of abstinence the subsequent year and more negative urine tests (Kober et al. 2014). Despite methodological and age differences with Kober et al. (2014), evidence points to the important role of caudal ACC in CUD treatment outcome.

Current findings of caudal ACC–OFC functional connectivity as a potential biomarker of relapse indicates that abstinence maintenance is associated with the strength of interactions between frontal regions. The role these 2 regions play in decision-making is closely related to behavioral processes altered in addiction. Caudal ACC activation has been reported in controls during stimulus–response selection, inhibitory control, attention and working memory tasks (Vogt et al. 1992; Bush et al. 2000). OFC activity has been related to evaluation of rewards, motivation/"drive", learning stimulus–reinforcement associations, and inhibition of emotional responses (Volkow et al. 2004). OFC damage both in non-human primates and in humans has been associated with an inability to reverse previously learned associations (Rolls 2000), a behavioral construct needed to achieve and maintain abstinence in addiction. Moreover, the role these 2 regions play on appropriate decision-making has been demonstrated in animal models examining the modulatory effects of cannabis (injection of cannabinoid agonist in animal models) on ACC and OFC synaptic transmission (Khani et al. 2015). Injection of cannabinoid agonist in ACC induces maladaptive decisions during an effort-based decision task (preference for immediate lower rewards over larger delayed rewards). Injection of a cannabinoid agonist in OFC may induce similar effects during a delay-based decision-making task (Khani et al. 2015). Given current findings and previous literature, a disruption in resting functional connectivity between caudal ACC and OFC may indicate a lack of integration of important behavioral constructs needed to sustain abstinence such as cognitive control (caudal ACC) over learned associations between rewards and behavior (OFC).

Exploratory Volumetric Analysis

Volumetric data suggest that cannabis exposure may affect cortical volume (Smith et al. 2014) and may exacerbate age-related decline of brain cortical volume (French et al. 2015). Moreover, there is evidence that preexisting orbitofrontal volumetric abnormalities identified during childhood may be related to subsequent cannabis use (Cheetham et al. 2012). A longitudinal study measuring brain cortical thickness reported specific frontal cortical thickness deterioration in young adulthood (measured at 18, 19, and 21 years old) related to ongoing cannabis use (Jacobus et al. 2015). Our findings of OFC predicting use in 18-month, may predict risk of use. Given above evidence, an exploratory analysis of volumetric differences was conducted. A general linear model with repeated measures design examining Group (CUD vs. HC) by Time (1 vs. 2) effects showed no significant interactions in gray matter volume within ACC, lateral orbitofrontal, medial orbitofrontal, pars orbitalis, rostral anterior cingulate, and rostral middle frontal regions. Independent samples *t*-test showed no significant cross-sectional group differences at Time 1 and Time 2 within these frontal regions ($P > 0.05$). Our findings are consistent with our previous report in which no prefrontal volumetric differences were detected (Kumra et al. 2012) and may suggest that cannabis effect on prefrontal cortex has functional rather than structural implications.

The present study has the following caveats. First, small sample size does not provide sufficient power to conduct proper categorical analyses separating between CUD that remained abstinent ($n = 7$) vs. CUD that resumed use ($n = 15$) during the interscan interval. Cannabis use during interval, however, is examined in the current study as a continuous variable. Second, although subjects were debriefed at the end of the resting fMRI scan to find out whether they remained awake, they were not

monitored with a periodic response or eye-tracking. Finally, to disentangle further the effects of chronic cannabis use during adolescence additional studies need to be conducted earlier during the disorder (e.g., before they enter treatment). Additional studies need to be conducted that address these issues.

Conclusions

The current study provides important longitudinal evidence of detrimental effects of cannabis use during adolescence on brain resting functional connectivity, intelligence, and executive function. The previous hypothesis of neural network imbalance in addiction proposed by Volkow et al. (2011) is expanded in the present study by providing evidence of the longitudinal trajectory of these neural network imbalances. Two patterns of dynamic changes of caudal ACC resting functional connectivity in individuals with cannabis use disorder (CUD) are reported: (1) decrease of resting functional connectivity with dorsolateral prefrontal and orbitofrontal cortices and (2) lack of increase of resting functional connectivity with SFG across time. Importantly, we identified a potential neural marker of relapse vulnerability in adolescents with CUD at treatment entrance characterized by lower resting functional connectivity between caudal ACC and orbitofrontal cortex. Finally, we provided evidence of adverse effects of cannabis use in adolescence during an 18-month interval on IQ and executive functioning. While these findings need to be replicated with larger samples and its generalizability to other addictions needs to be evaluated, this study provides crucial neurobiologically based evidence that could be used in campaigns to motivate cannabis abstinence in adolescents.

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