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Depression, marijuana use and early-onset marijuana use conferred unique effects on neural connectivity and cognition

Osuch EA, Manning K, Hegele RA, Théberge J, Neufeld R, Mitchell D, Williamson P, Gardner RC. Depression, marijuana use and early-onset marijuana use conferred unique effects on neural connectivity and cognition.

Objective: Marijuana (MJ) use is common. Research shows risks for psychiatric illnesses, including major depressive disorder (MDD) and cognitive deficits with MJ use, particularly early-onset use. We investigated cognitive function, functional connectivity, and genetic risk with MDD alone and combined with MJ use, and differences between early-vs. late-onset/non-MJ use in youth.
Method: A total of 74 youth in four groups were studied: healthy control, MDD, frequent MJ use and current/past MDD plus frequent MJ use. Psychiatric symptoms, cognitive performance and demographics were measured. Default mode network (DMN) brain connectivity was determined. Risk alleles in six genes of interest were evaluated.
Results: DMN differences among groups in reward-processing and motor control regions were found; the effects of MJ use and MDD were distinct. Early-onset MJ use was associated with lower IQ and hyperconnectivity within areas of the DMN. Early-onset MJ use was associated with the BDNF risk allele.
Conclusions: Cognitive deficits linked with early-onset MJ use were present within several years after MJ use began and may result from, pre dispose to, or share a common cause with early-onset MJ use. The DMN was affected by MDD, MJ and their combination, as well as by early-onset MJ use. BDNF carrier state may predispose to early-onset MJ use.

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Key words: marijuana; youth; young adult; major depressive disorder; default mode network; cognitive function

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Significant outcomes

- Major depressive disorder (MDD) and frequent marijuana (MJ) use conferred independent effects on resting-state brain (rs-fMRI) connectivity in regions of motor planning, motor control and ventral emotion and reward-processing areas of brain. However, neither affected cognitive performance.
- Early-onset MJ use was associated with increased rs-fMRI connectivity within regions of the default mode network and reward-processing areas and was also associated with lower total and vocabulary IQ.
- Neither MDD nor MJ use alone or combined was associated with a genetic risk allele tested in this sample; however, early-onset MJ use was associated with the presence of the BDNF risk allele.

Limitations

- As mirrored in the larger population, participant groups were not matched for gender, with more males in the two MJ-using groups.
- Small sample size limits conclusions about the genetic findings identified here.
- The cross-sectional design limited conclusions about the cause vs. effect of the findings, including the cognitive differences and brain connectivity differences in the early-onset vs. late-onset/non-MJ-using groups.

Introduction

Marijuana (MJ) is the most commonly used illegal (or previously illegal) substance in North America and the world, with 3.2 million youth aged 18–25 using MJ in the United States alone (1). Research has found a relationship between MJ use and mental illnesses (2, 3); however, this relationship is complex and poorly understood. Growing evidence suggests MJ use, especially early in life, is associated with schizophrenia (4–6) and mood disorders (7–10). Youth in particular, who are at the age of greatest risk for the onset of psychiatric disorders (11) and drug experimentation (12), are therefore at increased risk to use MJ while having major depressive disorder (MDD). Yet the consequences (or benefits) of this combination remain unknown.

Impairment in cognition is associated with MJ use acutely (13), in the early abstinence phase (14), and longitudinally following early-onset use (15). An 11-year longitudinal study following 1265 children found that MJ use before the age of 21 was associated with declining educational achievement and income, increasing welfare dependence, greater unemployment, declining relationship satisfaction and declining life satisfaction (16). Cognitive impairment is a potential effect of MDD as well (17, 18).

Resting-state functional brain connectivity has been shown to be abnormal in MDD with connectivity increased, decreased or a combination of both between the default mode network (DMN) and other regions and/or among DMN nodes themselves (19–24). Far fewer studies of the DMN have been published in the context of MJ use, but showed reduced connectivity with the DMN (25). There are no published studies of the DMN in the context of either MDD plus MJ use or of early-vs. late-onset MJ use.

Genetic vulnerability has been shown to mediate the association between MJ use and schizophrenia (6, 26, 27) and also MJ use and MDD (28). Genetics also play a role in MDD generally, though in more complex ways (29–31).

This study was designed to investigate the associations of MDD and MJ use in youth aged 16–23 with psychiatric symptomatology, cognition, resting-state fMRI (rs-fMRI) connectivity and vulnerability genes for mental illnesses. We hypothesized that (i) the effects of combined MDD with frequent MJ use on cognitive deficits and rs-fMRI connectivity would be additive; (ii) early-onset MJ use would be associated with cognitive deficits and abnormal rs-fMRI connectivity, especially in areas of reward processing; and (iii) risk alleles of genes associated with brain development or mental illnesses would be associated with MJ use and early-onset MJ use.

Material and methods

Participants

Approval was obtained from the Research Ethics Board at the University of Western Ontario, London, Ontario, Canada, and all safeguards for human research were respected. Participants were recruited from the local community and through the local early intervention programme for affective disorders. After a complete description of the study and any questions were answered, written informed consent was obtained from willing participants.

Data were collected from 74 youth, age 16–23. Participants included healthy controls (HC; $n = 20$), youth with current MDD without frequent MJ use (MDD; no more than four times MJ use per month for the last year; $n = 18$), youth with frequent MJ use (MJ; at least 4 times per week for at least the 3 months preceding study; $n = 20$) and youth with frequent MJ use and either current or past MDD (MDD + MJ; $n = 16$) (32). All subject had a structured diagnostic interview, and diagnoses in the two clinical groups were verified by the treating psychiatrist, when available. MJ use was assessed by self-report and confirmed by positive urine screen. Participants in the HC and MDD groups had negative MJ urine screens. Participants were excluded for head injury or significant medical illness.

Questionnaires

Participants were administered diagnostic and symptom measures including the Structured Clinical Interview for Diagnosis (SCID-I) (33), Youth Risk Behavior Surveillance System (YRBS) (34), Hamilton Depression Rating Scale (HDRS) (35), Beck Depression Inventory (BDI) (36), Spielberger State Anxiety Inventory (STAI) (37), Adult ADHD Self-Report Scale (ASRS) (38), Snaith–Hamilton Pleasure Scale (SHPS) (39), Emotion Regulation Questionnaire (ERQ) (40) and Trauma History Questionnaire (THQ) (41). Cognitive assessments included the Wechsler's Abbreviated Scale of Intelligence (WAIS) (42) Vocabulary and Matrix Reasoning subtests; Wechsler Memory Scale (WMS-III) (43), eight subtests (and four composites); three Delis–Kaplan Executive Function System (DK) (44) tests (Verbal Fluency, Sorting and Tower Tests).

Ages of onset of MJ and alcohol use were determined from the YRBS, which included seven answer options for each question, then dichotomized into two groups: onset before vs. after their 17th birthday/never, based on a median split of responses to the age of onset of MJ use question. Rates of lifetime alcohol use, age of first alcohol use, and presence or absence of regular tobacco use and last 30-day tobacco use were also from the YRBS. Timeline Follow-Back (45) quantified MJ and alcohol use 28 days prior to study.

Of the 34 participants in the two MDD groups, 29 had a current depressive episode while six had a past episode by SCID (five in the MDD + MJ group, three of whom had received professional treatment; one in the MDD group, in early partial remission). Eight participants in the MDD group were medication free; nine were taking antidepressant(s) (SSRI, SNRI or bupropion) and one only a sleep aid (trazodone). In the MDD + MJ group, 11 participants were medication free, two were taking an antidepressant(s) (SSRI) and two were on non-antidepressant psychiatric medication (over-the-counter sleep aid and a psychostimulant). The doses of all medication were stable for 3 weeks prior to scanning.

No participants in the HC or MJ groups were on any prescription medications other than possibly oral contraceptives, and none met criteria for a current or past psychiatric illness except related to MJ use in the MJ group. Youth in the HC group were free from family history of all psychiatric illnesses in first- and second-degree relatives. Participants were not excluded based on alcohol use. Seven participants met SCID criteria for alcohol abuse (one MDD, four MJ, two MDD+MJ); two met criteria for alcohol dependence (MJ group).

Imaging

All magnetic resonance imaging (MRI) was performed using a 3.0 Tesla MRI scanner (Siemens Verio, Erlangen, Germany) at the Lawson Health Research Institute, with a 32-channel phased array head coil (Siemens). Whole-brain T1-weighted anatomical images with 1 mm isotropic spatial resolution were acquired and used as reference for spatial normalization and to select the orientation of functional MRI images 6 degrees coronal to the AC-PC plane. Resting-state fMRI scans consisted of a single-shot gradient echo, T2*-weighted, echo planar pulse sequence (volume acquisition time TR = 3 s; 60 transverse slices; voxel size 2 mm isotropic, FOV 25.6 cm × 25.6 cm, flip angle = 90°, parallel imaging acceleration factor = 4) for a total time of 5 min 42 s (four automatically discarded steady state volumes and 110 brain volumes). Two series were acquired for a total of 220 functional volumes collected for each participant. Participants were instructed to lie comfortably with eyes closed and let their minds wander without falling asleep. All participants reported compliance with instructions.

Genetics

Genomic DNA was isolated from saliva using Oragene kits (DNA Genotek, Kanata, ON, Canada). Sixty-seven samples passed quality control and were suitable for genotyping. Candidate genes were selected by consultation with local experts on relevance to cannabinoid exposure and mental illnesses (46) and confirmed for relevance by literature review (47). Selected candidate gene markers were single nucleotide polymorphisms (SNPs): *AKT1* encoding v-AKT thymoma viral oncogene homologue 1 rs2494732 (C allele is risk allele) (48); *BDNF* encoding brain-derived neurotrophic factor rs6265 (T allele) (49); *CNR1* encoding cannabinoid receptor 1 (brain) rs1406977 (C allele) (50); *DRD1* encoding dopamine receptor D1 rs4532 (T allele) (27); *RPS6KB1* encoding ribosomal protein S6 kinase 70-kDa polypeptide 1 rs8071475 (C allele) (51); and *SLC6A4* encoding sodium-dependent serotonin transporter rs1042173 (A allele) (28). Reagents for these SNP genotypes were purchased from Life Technologies (Mississauga, ON, Canada), and TaqMan genotyping was performed on a Viia7 Real Time PCR instrument (Life Technologies), using manufacturer-suggested reagents and Viia7 allele discrimination software.

Data analysis

All statistical analyses of demographic, cognitive and behavioural variables were conducted using SPSS v23. A series of univariate analyses were used for demographic, clinical and cognitive measures. Corrections for multiple comparisons within each variable category employed Bonferroni calculations and were two-tailed except for genetic analyses. Variables defining substance use were not normally distributed across the sample so non-parametric statistics were used for those analyses. Due to differences in gender across groups, clinical and cognitive continuous variables were compared using the general linear model with gender as a covariate, except those related specifically to gender differences. *Post hoc* differences among groups are reported.

Analyses of genetic associations with phenotypes, specifically chi-square and contingency

analyses of alleles and genotypes with discrete traits, were performed using SAS version 9.2. Observed genotype frequencies in the entire sample did not deviate significantly from those predicted by the Hardy–Weinberg equation. Genetic analysis assumed that the risk alleles would be associated with the 'disease state' (MJ use, early-onset MJ use or MDD), so one-tailed significance testing was employed.

One subject declined to be scanned (HC); one subject was removed from the rs-fMRI analysis due to excessive motion artifacts (MJ group). Both these subjects were female. Seventy-two scanned subjects remained, in four groups: 19 HC, 18 MDD, 19 MJ and 16 MDD + MJ. Raw data were transformed into NIfTI format and preprocessed using SPM8. Standard preprocessing steps included brain extraction, motion correction (INRIAlign), 8 mm FWHM spatial smoothing with a 3D Gaussian kernel and registration into standard Montreal Neurological Institute (MNI) space. Independent component analysis was run on all temporally concatenated subject data to identify the average DMN using FMRIB Software Library (FSL). A model order of 20 extracted components was chosen to describe the variance in the rs-fMRI data. Dual regression algorithms were used to back-reconstruct the group average DMN into single-subject-specific networks. These were input into a randomized statistical analysis using general linear model to compare the groups through an independent t-test, while correcting for gender. These output threshold-free cluster *P*-value maps that were corrected for multiple comparisons related to the number of voxel signals being compared through t-tests (52). As threshold-free cluster enhancement generally gives better sensitivity than arbitrary selection of a minimal cluster, spatial extent threshold voxels were considered significant with a *P*-value < 0.01.

Results

The mean age of participants was 19.8 ± 1.62 (range 16–23), including 34 males and 40 females. Gender was not evenly distributed, with more males in the two MJ-using groups (Table S1).

Effects of combined Major Depressive Disorder and marijuana use

Differences across groups for depression and substance use variables were as expected based on group selection and other behavioural variables differed as expected, related to the presence of MDD or MJ use (Table S1). The MDD + MJ group was no different than the MDD group on any depression measure (three measures, corrected $P < 0.017$). Group comparisons of demographic, other symptom or behavioural (5 measures, $P < 0.01$) and cognitive variables (WAIS, one test, $P < 0.05$; Wechsler, eight subtests, $P < 0.006$; D-K, three subtests, $P < 0.017$) are illustrated in Table S1 and indicate differences consistent with group assignment. Substance use variables (five variables, $P < 0.01$) differed predictably by MJ use. There was no difference on cognitive testing among the four participant groups (threshold corrections shown in Table S1).

There was a main effect of group on rs-fMRI (Fig. 1a and Table 1). Three areas of brain showed rs-fMRI differences among groups. The effects of MDD and MJ appeared to be additive only in the left culmen/fusiform gyrus (BA20/37). There were more complex main effects across groups in the right medial frontal gyrus (BA6) and an area encompassing the right caudate/temporal gyrus/parahippocampal gyrus (BA30). *Post hoc* comparisons are shown in Fig. 1b–d.

Effects of early-vs. late-onset/non-MJ use

When divided into early-onset vs. late-onset/non-MJ use, there were 36 and 37 in each group respectively (age of onset was missing for one subject in the MJ group, thus, $n = 73$ for these analyses). Demographic, symptom, behavioural and cognitive variables across groups were compared, with corrected significance level thresholds indicated in Table S2. More males stared MJ use early.

There was no difference in incidence of MDD between early-vs. late-onset/non-MJ users ($\chi^2[1, N = 72] = 1.20$, $P = 0.352$), nor in any other psychiatric symptom or demographic variable measured (Table S2). In this sample, early-onset MJ use was not associated with current or lifetime MDD, other psychiatric variables measured or other demographic characteristics except gender. Substance use variables were higher in the early-onset users with the exception of alcohol use within the past 4 weeks, which was non-significant (Table S2).

Early-onset MJ users had significantly lower total WAIS IQ scores compared to late-onset/non-users (105.8 ± 9.6 and 111.9 ± 11.5, respectively; $F[1,72] = 7.92$, $P = 0.006$). *Post hoc* analysis showed that this was driven by WAIS Vocabulary subscale scores, with early-onset users scoring lower than later-onset/non-MJ users (52.0 ± 8.8 and 58.6 ± 8.3, respectively; $F[1,72] = 5.91$, $P = 0.004$). There were no differences in WAIS Matrix subscale scores between groups ($P = 0.580$). There were no other differences between these groups on other cognitive tests (Table S2).

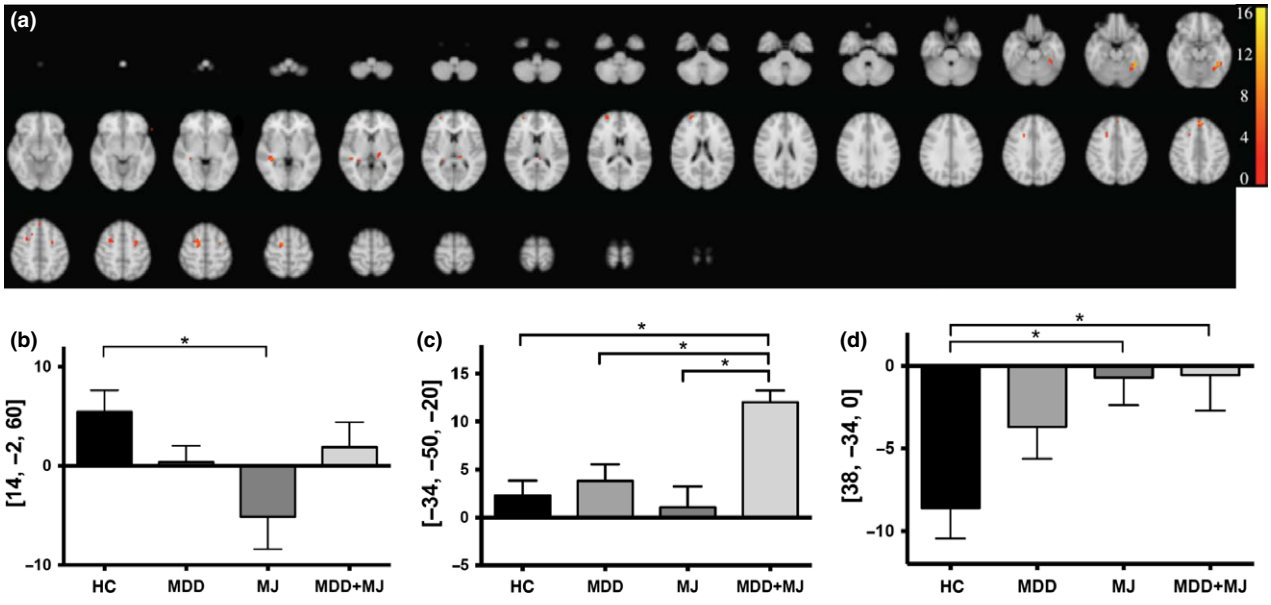

Fig. 1. Main effects of group (HC, Health Controls; MDD, Major Depressive Disorder group; MJ, frequent marijuana using group; MDD + MJ, combined MDD and frequent MJ use group) rs-fMRI difference in the default mode network (DMN). (a) Transverse sections are oriented as per standard radiological convention where the left side of the image represents the right side of the brain, and colour bar indicates *F*-statistic scale. (b–d) Graphs of mean z-scores at regions of significant main effect differences by group, *significant by *post hoc* Tukey's tests, corrected for multiple comparisons (see also Table 1). Peak coordinates of each region are indicated on each *y*-axis; individual participant groups indicated on the *x*-axis. Error bars indicate standard error of the mean (SEM). (b) Right medial frontal gyrus (BA 6). (c) Left culmen/fusiform gyrus (BA 20/37). (d) Right caudate/temporal/parahippocampal regions (BA30).

Table 1. Main effects of groups: four-group analysis based on group assignment and two-group analysis based on age of onset of MJ use. These regions had significantly higher connectivity with the default mode network across/between groups

Region (Brodmann Area)	Cluster size	Peak MNI coordinates (*x, y, z*)	*F*-statistic
Main effect of group: four-group analysis of HC, MD, MJ and MDD + MJ groups ($P \leq 0.005$)			
Right medial frontal gyrus (BA 6)	14	14, −2, 60	10.51
Left culmen, fusiform gyrus (BA 20/37)	13	−34, −50, −20	11.57
Right caudate, temporal gyrus, parahippocampal gyrus (BA 30)	5	38, −34, 0	11.22

Region (Brodmann Area)	Cluster size	Peak MNI coordinates (*x, y, z*)	*t*-statistic
Main effect of group: two-group analysis; early MJ users > late or non-MJ users ($P \leq 0.01$)			
R temporal, fusiform cortex; culmen; precuneus; bilateral occipital cortex (BA 20/21/22/39)	4541	34, −62, −24	4.09
R anterior cingulate cortex (BA 24)	265	6, 30, −4	4.39
L temporal, occipital, fusiform cortex (BA 37/20)	42	−26, −58, −16	4.17
L superior frontal gyrus (BA 10)	20	−26, 54, 8	4.51

There were no significant main effects of group where late/non-MJ users > early MJ users.

Brain imaging revealed that DMN connectivity was significantly different between early-onset and late-onset/non-MJ users in four regions (Fig. 2, Table 1). These areas are part of the DMN itself including right and left temporal, occipital and fusiform cortices, right precuneus and culmen; right ACC (BA24); and left superior frontal gyrus (BA10). These were all significantly more functionally connected to the DMN in early-onset than late-onset/non-MJ users. There were no significant correlations between WAIS IQ Vocabulary subscale score and any of the four clusters of significance in *post hoc* analysis.

Genetic effects

There were no significant differences among HC, MDD, MJ and MDD + MJ groups related to any of the six selected gene frequencies tested here (corrected $P < 0.016$, one-tailed).

However, *BDNF* rs6265 allele and genotype frequency (but no others) differed significantly between early-vs. late-onset/non-MJ users (Table S2). We assumed a dominant genetic model as the primary interest was in the risk allele carrier state of the individual youth participating in the study, rather than the presence of the risk allele in the genes themselves.

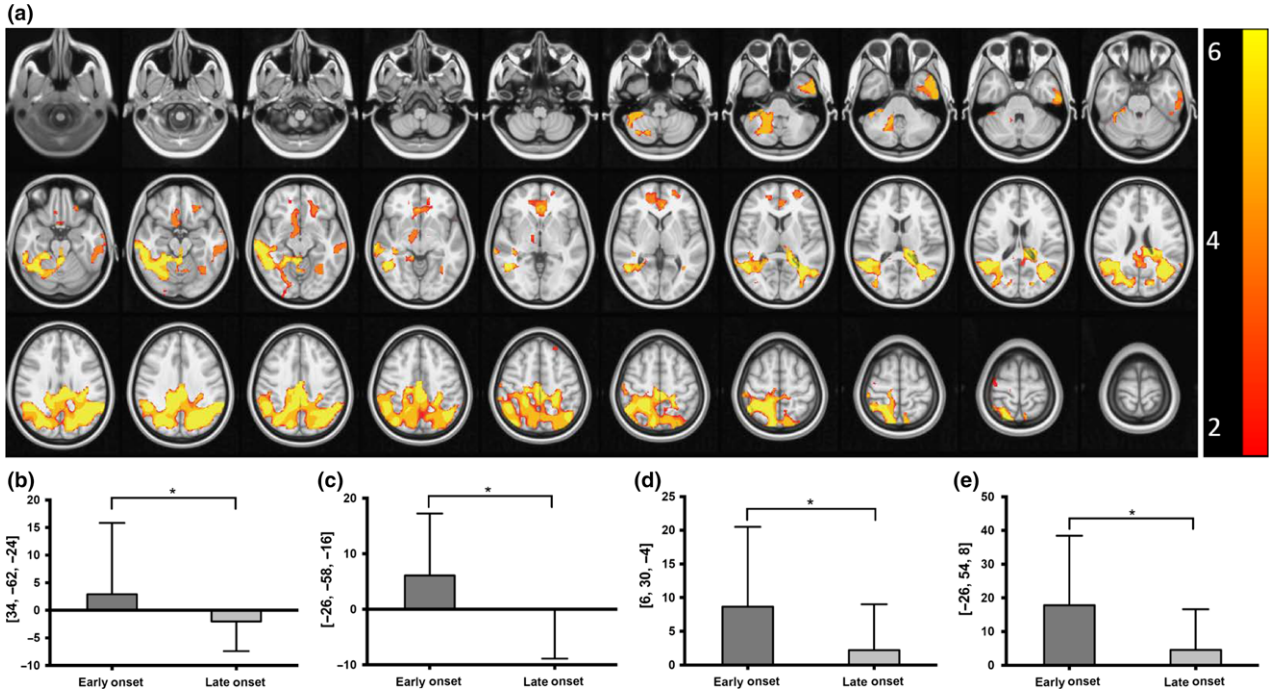

Fig. 2. (a) Comparison of resting-state DMN connectivity between early-onset marijuana (MJ) users vs. late-onset/non-MJ users; sections are oriented as in Fig. 1 (radiological convention); colour bar indicates *t*-score scale. (b–e) Graphs of mean t-scores at regions of significant main effect differences by group ($p \leq 0.01$; see also Table 1). Peak coordinates of each region are indicated on each *y*-axis; individual participant groups indicated on the *x*-axis. Error bars indicate SEM. (b) Right temporal/fusiform/precuneus/occipital cortex and culmen (BA 20/21/22/39). (c) Left temporal/occipital/fusiform cortex (BA 37/20). (d) Right ACC (BA 24). (e) Left superior frontal gyrus (BA 10). *Significance for the Tukey's post hoc testing is always $p < 0.05$.

Thus, all *BDNF* rs6265 Met66 carriers comprised 51.7% and 21.1% of individuals with early-vs. late-onset/no MJ use, respectively, with relative risk compared to Val66 homozygotes of 2.46 (95% confidence interval 1.21–4.99, $P = 0.009$).

Potential confounds for significant effects

There was no difference in WAIS total scores on the basis of gender ($F = 0.249$, d$f = 1[73]$, $P = 0.619$), the presence of the BDNF risk allele ($F = 0.435$, d$f = 2[64]$, $P = 0.728$) or ever having been depressed ($F = 0.344$, d$f = 2[73]$, $P = 0.710$). In addition, there was no difference in WAIS Vocabulary subscale score based on gender ($F = 0.054$, d$f = 1[73]$, $P = 0.817$), BDNF risk allele ($F = 0.320$, d$f = 2[64]$, $P = 0.728$) or ever having been depressed ($F = 0.178$, d$f = 2[73]$, $P = 0.837$). Again, gender was covaried within the rs-fMRI analysis to reduce the impact of this group difference on results.

In the group of 74 participants, 36 were current MJ users and 38 were not currently using MJ. Eight current non-MJ users had smoked MJ before age 17, and seven current MJ smokers had started only after their 17th birthday. Thus, being a current MJ user and being an early-onset user were strongly associated ($F[1,72] = 22.3$, $P < 0.0005$) but not identical. Current frequent MJ users did not differ from non-MJ users on total WAIS IQ score ($F = 1.52$, d$f = 1[73]$, $P = 0.226$). Additionally, current use did not relate to WAIS Vocabulary subscale scores ($F = 2.64$, d$f = 1[73]$, $P = 0.079$), although this was a trend, with current users having lower scores (52.7 ± 9.3 vs. 57.3 ± 8.8). There was no significant correlation between the total number of days having smoked MJ in the last four weeks and the WAIS Vocabulary score (Spearman's $\rho = -0.218$, $n = 73$, $P = 0.06$), although this also was a trend.

While approximately half of the group started MJ use before age 17, the majority of the youth first used alcohol before the age of 17 (71.6%, $n = 53$). Age of onset of MJ use was correlated with age of onset of alcohol use (Spearman's $\rho = 0.583$, $n = 70$, $P < 0.0005$). There was no difference in WAIS IQ ($F = 0.488$, d$f = 2$, $P = 0.616$), nor in the WAIS Vocabulary subscale score between participants who started alcohol use before vs. after age 17 ($F = 1.62$, d$f = 2$, $P = 0.205$). In addition, past 4-week alcohol use was not correlated with the WAIS Vocabulary subscale score (Spearman's $\rho = -0.042$, $n = 73$, $P = 0.72$). Thus, the difference in IQ with early-onset

MJ use did not appear to be strongly related to age of onset of alcohol use or current alcohol use.

Only 17% (n = 12 of 71 completed responses) of participants had ever smoked tobacco products regularly and all but one of those tobacco smokers started using MJ before the age of 17; the majority (68%, n = 24) of early-onset MJ users were never regular tobacco smokers. Small numbers made analyses of group differences using this variable unreliable. There was no correlation between past 30-day tobacco use and either total WAIS IQ (Spearman's $\rho = 0.113$, $P = 0.346$) or the Vocabulary subscale score (Spearman's $\rho = 0.014$, $P = 0.908$).

There were also very few participants in this study who endorsed other illicit drug use on the YRBS. For instance, 89% (all but eight) denied any cocaine derivative use (96% endorsed less than two lifetime uses of those substances); and 99% (all but 1) denied any illicit injectable drug use.

Discussion

This study investigated the behavioural, cognitive and rs-fMRI connectivity of MDD and frequent MJ use in young adults alone and in combination. It also investigated early-onset MJ use in the context of youth with and without MDD, as well as the genetic correlates of MJ, MDD and early-onset MJ use. Several of our hypotheses were confirmed, while others were not.

Being male was associated with both current MJ use and early-onset use. No other demographic variables were associated with MJ use or having had MDD. There was no difference in psychiatric symptom severity, including depression measures and others, nor cognitive performance, between MJ-using and non-MJ-using MDD groups, except criminal trauma exposure (Table S1). Thus, it did not appear that frequent MJ use either improved or worsened the severity of psychiatric or cognitive variables measured in this sample, although it increased endorsement of crime-associated trauma.

Nevertheless, there were differences in rs-fMRI DMN connectivity related to MJ use, MDD and the combination of both in three regions. These included right mPFC (BA6; Fig. 1b), a region implicated in multiple aspects of motor planning. Previous research has shown blunted activity in premotor cortex in MDD during a spatial working memory task (53), and a reduction in size on the right in melancholic depression (54), although the MDD groups did not account for group differences in this study. There is also prior evidence that MJ has an impact on BA6, with diminished activation in MJ users even after one month of abstinence in one study (55), but increased activation and a significant task-by-gender interaction using a different methodology in another (56). Differences in our participant groups were driven by HC's vs. the MJ group and suggested that the medial PFC was less functionally connected to the DMN in the MJ group compared with controls. This may relate to commonly found motor alterations in MJ users (57, 58). Thus, while our findings are compatible with previous research on MJ use, the effects of the combination of MJ use and MDD were not summative in this brain region, contradicting our hypothesis.

In contrast, the pattern of DMN connectivity in left culmen/fusiform gyrus was consistent with our hypothesis (Fig. 1c). Effects of MJ use on the cerebellum (including culmen) are well known and have been confirmed in a review of MJ use using 43 studies, including eight of adolescents (58). This region is involved in motor control and, interestingly, was found to show decreased activation in adolescent MJ users in a previous study of brain function during a motor task (57). In a recent meta-analysis of MDD, the cerebellum was identified as one of the primary regions associated with hypoactivation in this disorder as well (59). Our results did not demonstrate significant abnormalities in this region with MJ use or MDD alone, but showed greatly exaggerated connectivity to the DMN with the combination of both. Cerebellar network connectivity with the DMN may be particularly vulnerable to combined effects of frequent MJ use and MDD, which could have consequences for motor and other functions of this brain region.

The finding in right caudate/temporal/parahippocampal region was driven by differences between HC's and the two MJ-using groups (Fig. 1d). Yet these regions are known to be abnormal in MDD in youth (60). Interestingly, both temporal gyrus and parahippocampal gyrus volumes were decreased in MJ users slightly older than those studied here (61). Our results indicated that connectivity of this region to the DMN was normally very low (see Fig. 1d), indicating anticorrelation between the DMN and this region in health. This signal reduction then appeared to be diminished by MJ use, implying abnormally greater connectivity between the DMN and caudate, temporal and parahippocampal gyrus with MJ. As part of the ventral emotion- and reward-processing areas of brain, the abnormal connectivity in this region in these subject groups may relate to alterations in function of reward-processing networks, with hyperconnectivity to the DMN at rest. Interestingly, this region did not respond abnormally in the context of a rewarding stimulus in the same

subject groups in our prior analysis (32). However, the abnormality found here implicates MJ use alone and together with MDD as abnormally effecting reward processing at the level of the DMN, one of the most commonly identified intrinsic brain networks (62). This could relate to know alterations in reward processing in the context of MJ (63, 64) and is consistent with our hypothesis.

Testing our hypothesis about early-onset MJ use by dividing our sample by age of onset showed that early-onset MJ use was associated with lower scores on the total WAIS IQ, driven by lower scores on the Vocabulary subscale. A drop in IQ has been associated with early-onset MJ use in a previous longitudinal study (15). That study involved a several-decade gap in follow-up, such that subjects were 38 years old at the time of the finding. In the current study, lower IQ was already present in early-onset MJ users at a mean age of 20. It remains unclear whether this abnormality predated and potentially predisposed to the early-onset of MJ use, was a consequence of the early-onset use, or whether both were the result of some other variable. Importantly, the sociodemographic variables related to these youth and their parent's educational levels did not differ between our early-onset and late-onset/non-MJ users at the time of study (Table S2). The prospective, longitudinal study of Meier et al. (15) found no IQ differences in their youth prior to MJ use onset, so it is a consistent hypothesis that early MJ use led to the difference in IQ found here. However, future research should look more specifically at this possibility by measuring IQ before MJ use and at frequent intervals thereafter, as well as looking at potential common causal variables.

This sample was selected to be matched based on the presence/absence of current or past MDD in both the MJ and non-MJ groups, and significant psychiatric symptom differences were absent (including THQ crime exposure; Table S2) in the comparison of early-vs. late-onset/non-MJ users.

The comparison between the early-onset MJ users and the later-onset/non-MJ users revealed significant differences in rs-fMRI connectivity within DMN. These differences included nodes of the DMN in right temporal/precuneus/fusiform cortex, involved in visuo-spatial processing, episodic memory retrieval and self-processing (65, 66), and left temporal/fusiform gyrus, involved in memory processing, theory-of-mind and semantic processing (67). Interestingly, the differences involved greater connectivity within DMN regions in early-onset users, with no areas showing decreased connectivity relative to late-onset/non-users. A previous study showed increased functional connectivity in the DMN of chronic MJ users who started before age 16, which was associated with memory problems (68). Our analysis did demonstrate significantly lower scores on the WAIS Vocabulary subscale in our early-onset users, but on no other cognitive deficits. In *post hoc* analysis, no significant correlations were found between any regions of increased rs-fMRI connectivity in the early-onset MJ group and the cognitive tests administered. Excessive functioning of the DMN may be associated with reciprocal decreased functioning in the cognitive control network in the early-onset group, but that was not investigated here.

Increased DMN activation in MDD has been demonstrated using different subpopulations and paradigms (69), including in remitted youth with histories of MDD (70). Areas of increased connectivity include subgenual ACC (BA 24) and OFC (BA 10) as well as temporal/parietal regions, as found in our results (Fig. 2b–e). This hyperconnectivity has been suggestion to reflect that MDD involves a pathological inability of the DMN to regulate self-referential activity, with increased rumination (69, 70). In addition, the subgenual PFC has long been associated with MDD and depressive symptomatology (71). Although not associated specifically with MDD in this sample, our finding of increased rs-fMRI connectivity within the DMN in these regions in youth who stared MJ use early is cause for clinical concern. This may reflect an exaggerated propensity to engage in internal mental activity and a potential increased vulnerability to future depression, although whether these differences pre- or post-dated the onset of MJ use remains uncertain from the current study.

The genetic analyses performed here showed no main effect of group distinguishing any genetic variable tested related to MDD or MJ use, or the combination. Given the small sample size, this will need to be replicated in a much larger population to ensure that it does not represent a type II error.

Conversely, the presence of the BDNF risk allele associated with early-onset MJ use is a novel finding. There are associations between the BDNF risk allele and both neurocognitive dysfunction and psychiatric disorders (72), including smaller hippocampal volumes (73), medial-temporal cortex-based declarative memory processes (74) and reduced cognitive functioning in the elderly (75). The preliminary finding of targeted genetic risk in a small sample size found in the current study will need to be replicated in much larger samples to determine whether the BDNF risk allele truly

poses a risk factor for early-onset MJ use and what processes could be mediating this association.

Limitations of this study included the predominance of male subjects in the two MJ-using groups. Future studies could match groups better for gender. However, this mismatch does reflect the epidemiology of substance use (11, 76). The small sample size and the customary challenges related to non-experimental design are also limitations. Related to sample size, only a small number of risk alleles could be tested. The use of matched samples for depression reduced our ability to detect mood or other behavioural differences associated with early-onset vs. late-onset/non-MJ use. The cognitive differences identified here could imply a cause of early-onset MJ use, an effect of such use, or an association related to another causal factor. Future longitudinal studies may help clarify these relationships.

In summary, neither frequent MJ use at the time of study nor MDD, independently or together, led to measurable cognitive deficits on the tests administered to these young adults. The interaction of MDD and frequent MJ use on DMN connectivity was in some regions complex rather than summative, suggesting independent effects of these two factors. Early-onset MJ use exaggerated network connectivity in the DMN, which could be associated with some of the longer-term negative consequences found in longitudinal studies of early-onset MJ users, and early use was associated with lower WAIS total and Vocabulary IQ scores. There were no differences on prevalence of risk alleles of the six genetic variations studied here related to MDD, MJ and the combination. However, early age of onset of MJ use was associated with the BDNF risk allele.

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Declaration of interest

None of the authors of this work, including Elizabeth Osuch, M.D., Kathryn Manning, Robert A. Hegele, Jean Théberge, Ph.D., Richard Neufeld, Ph.D., Derek Mitchell, Ph.D., Peter Williamson, M.D., nor Robert C. Gardner, Ph.D. (emeritus), has any financial conflict of interest with this work.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Demographics and clinical variables; four-group comparisons (HC, healthy controls; MDD, Major Depressive Disorder group; MJ, frequent marijuana users; MDD+MD, combined MDD and frequent MJ user group) (N = 74); means are unweighted.

Table S2. Demographics and clinical variables; early versus late onset/non-marijuana users (N = 73); means are unweighted.