Associations between regular cannabis use and brain resting-state functional connectivity in adolescents and adults

Natalie Ertl[1,2], Will Lawn[3,4], Claire Mokrysz[5], Tom P. Freeman[5,6], Naji Alnagger[5], Anna Borissova[5], Natalia Fernandez-Vinson[5], Rachel Lees[6], Shelan Ofori[5], Kat Petrilli[6], Katie Trinci[5], Essi Viding[7], H. Valerie Curran[5], Matthew B. Wall[1,2,3*].

[1]Invicro London, Burlington Danes Building, Hammersmith Hospital, Du Cane Road, London, W12 0NN, UK

[2]Faculty of Medicine, Imperial College London, Hammersmith Hospital, Du Cane Road, London, W12 0NN, UK

[3]National Addiction Centre, Institute of Psychiatry Psychology and Neuroscience, King's College London, London, UK

[4]School of Mental Health and Psychological Sciences, Institute of Psychiatry Psychology and Neuroscience, King's College London, London, UK

[5]Clinical Psychopharmacology Unit, University College London, 1-19 Torrington Place, London, WC1E 7HB, UK.

[6]Addiction and Mental Health Group (AIM), Department of Psychology, University of Bath, Bath, UK

[7]Clinical, Educational, and Health Psychology Research Department, University College London, London, UK

***Corresponding Author:**

Matthew B. Wall

Burlington Danes Building

Hammersmith Hospital

Du Cane Road

London, W12 0NN, UK

Tel: 07801518566

matt.wall@invicro.co.uk

Abstract

Cannabis use is highly prevalent in adolescents however little is known about its effects on adolescent brain function. Resting-state functional Magnetic Resonance Imaging was used in matched groups of adolescents (16-17 years old, N=70, 35 users/35 controls) and young adults (26-29 years old, N=70, 35 users/35 controls). Pre-registered analyses examined the connectivity of cortical and sub-cortical brain networks. Cannabis users (across both age-groups) showed localised increases in connectivity in the default mode, executive, and limbic striatum networks. Localised decreases in connectivity were seen in the salience network, and the sensorimotor striatal network showed both localised increases and decreases in connectivity. Mean connectivity across entire networks was significantly decreased in the default mode network in cannabis users. There were no significant interactions found between age-group and user-group. Cannabis use is associated with changes to connectivity in cortical and sub-cortical brain networks, however adolescence does not appear to modify these effects.

Introduction

Cannabis is the most widely used illicit drug under international control, with 7.8% of 16-59 year olds reporting past year use in England and Wales. This statistic increases to 18.7% in 16–24-year-olds[1]. This pattern of high use in young people is reflected globally; data from 17 countries suggests the median onset of first use of cannabis is 18-19 years old[2]. There is some recent evidence that cannabis use during adolescence may alter brain development in a number of ways[3,4].

A common method used to study human brain function is resting-state functional Magnetic Resonance Imaging (rs-fMRI). While the brain is at rest there are communities of structures which are highly functionally connected; resting-state networks (RSN). The most commonly studied RSN is the default mode network (DMN), which is most active when the brain is not actively engaged in a task[5]. The antagonistic network to the DMN, the executive control network, is the community of brain regions most active while engaged in an external task[6,7]. The Salience network (SN) is thought to be the mediator between these two networks as well as facilitating attention and detection of emotional and sensory stimuli[8]. For further information on RSN see supplementary material.

As well as these cortical RSNs, other neural structures also show structured patterns of connectivity at rest. The functional connectivity of sub-cortical regions like the striatum and hippocampus can also be studied using fMRI[9]. The striatum can be sub-divided into three distinct regions based on functional and structural connectivity with the cortex: the limbic, associative, and sensorimotor divisions[10] which are involved in motivational processes, cognition and motor functions respectively[10,11].

Adolescence is a time of synaptic reorganisation and pruning in most mammals[12]. During adolescence, there is a rapid development of the endocannabinoid system which contributes to maturation of corticolimbic neuron populations[13]. Resting-state fMRI studies have shown that during adolescence, neuronal networks become more segregated, leading to the hierarchical organisation seen in adulthood[14]. Connectivity between frontal regions and the executive and salience networks increases with age and there is a migration of the DMN from central to more anterior

and posterior positions[15]. Animal literature has demonstrated CB_1Rs are at their highest levels during adolescence, and decline into adulthood[16], and dopamine receptors in the striatum are also over-produced prior to puberty and then heavily pruned during adolescence[17]. Given that the main psychoactive component of cannabis is Δ-9-tetrahydrocannabinol (THC), which is a partial agonist of the endogenous cannabinoid receptor 1(CB_1R)[18] there is cause for concern that regular use of cannabis in adolescents may alter corticolimbic development or other neurodevelopmental trajectories. One recent large study with a five-year follow-up period has shown a small association between adolescent cannabis use and cortical thinning in the medial prefrontal cortex[3]. Conversely, it has also recently been shown that there is no effect of age on reward anticipation and feedback processing in adolescent and adult cannabis users [19].

There are mixed findings about the effects of cannabis on striatal function[20], with lifetime cannabis use being previously associated with both increased[21] and decreased[22] striatal activation when reward tasks are used. A selective effect of THC in the limbic striatum has recently been supported by work with an acute cannabis challenge, suggesting effects of THC on limbic striatum connectivity can be ameliorated when administered in combination with cannabidiol[9]. In other brain regions and networks, THC can cause morphological changes in the hippocampus[23], amygdala[24] and cortex[25], all of which highly express CB_1R. Resting-state studies have shown decreased DMN connectivity in cannabis users compared to controls[26,27] while other studies have shown increases in functional connectivity in the core of the DMN but reduced connectivity with areas in overlapping networks[28]. Functional connectivity generally decreases in older adults, but cannabis use may increase functional connectivity in RSN in those over 60 years old, to similar levels as younger adults[29]; this suggests the age of the cannabis user may be an important factor.

No previous study has directly compared the effects of age (adolescents and young adults) and cannabis users vs non-users on resting state-networks. To examine the differences in cortical and sub-cortical RSN connectivity in adolescents versus adult cannabis users and age matched controls as well as the interaction between cannabis usage and age, we used rs-fMRI on 140 subjects: 70 users (35 adults, 35

adolescents) and 70 controls (35 adults and 35 adolescents). Using seed-based analysis (for more information on seed based analysis please see the supplementary material), we defined three striatal networks (associative, limbic, and sensorimotor) and three cortical RSNs (DMN, ECN, SN), plus the hippocampal network, in all subjects. We then used a whole brain approach to investigate significantly different user effect regions as well as a region of interest (ROI) approach to investigate global network differences, as pre-registered in our analysis plan on the Open Science Framework[30]. We hypothesised that connectivity will be reduced in cannabis users compared to controls. We also hypothesised that there will be an interaction between age-group and cannabis user-group, such that adolescent cannabis users will be more different to their age matched controls than adult cannabis users are to their age matched controls. Our final hypothesis was that cannabis use frequency will be positively correlated with measures of RSN connectivity in the cannabis users.

Results

Participants

A summary of the participant characteristics (demographics, questionnaire scores, and drug history) can be found in Supplementary Table 1. A more detailed report of the statistics used can be found in the supplementary material.

User-group main effects: Whole brain analyses

Significant clusters for 'user effects' can be found in Supplementary Table 2. Regions were labelled using a mixture of Harvard-Oxford cortical and subcortical atlases[31] and expert knowledge of functional areas. All three resting-state networks showed significant user clusters. These are summarised in Figure 1. In the salience network (panels A-B), one large cluster was found around the sensorimotor cortex, this cluster had lower activation in the user compared to the control groups ($F(1,134)=60.84$, $P<0.001$). In the DMN (C) two clusters were found, the precuneus (D) and the cuneal cortex (E). Both were found to have greater activation in the user compared to control groups ($F(1,134)=14.54$, $P<0.001$), ($F(1,134)=22.45$, $P<0.001$). Users were found to have greater connectivity within three regions of the executive control network (F), these were: the frontal operculum (G), the cingulate (H), and the temporal parietal junction (I). No effects of user-group were seen in the analyses of the hippocampal network.

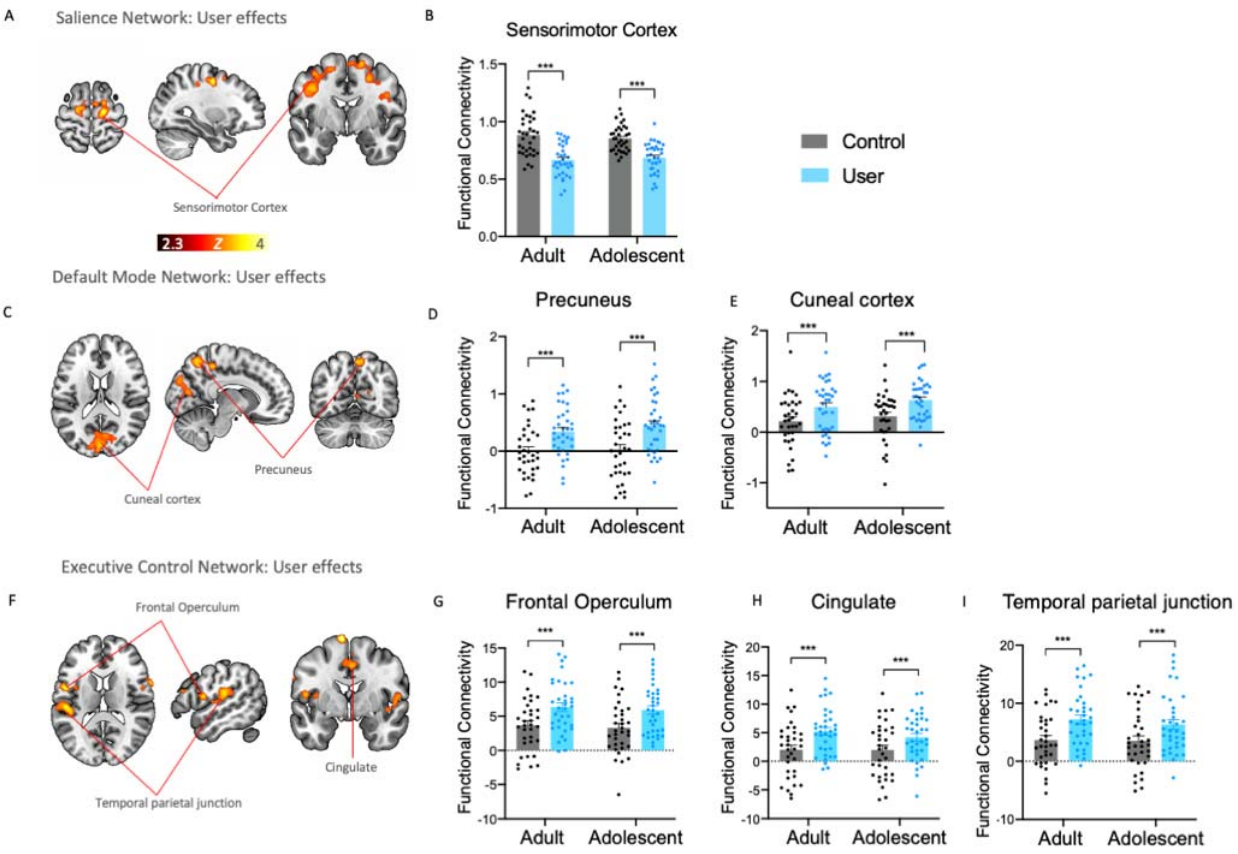

Figure 1. Significant user-group effects found in the cortical resting state networks. In the salience network a significant cluster was found in the sensorimotor cortex (A), activity was found to be lower in the user-groups compared to the non-user-groups ($F(1,134)=60.84$, $P<0.001$). (B) In the default mode network, two clusters were found: the cuneal cortex and the precuneus (C), the user-groups were found to have higher BOLD activation than the non-user-groups in both clusters: (D) the cuneal cortex ($F(1,134)=14.54$, $P<0.001$), and (E) precuneus ($F(1,134)=22.45$, $P<0.001$). In the Executive control network, three clusters were found (F): the frontal operculum, the cingulate and the temporal parietal junction. User-groups were found to have significantly more functional connectivity in these areas than controls, (G) Frontal operculum ($F(1,34)=16.97$, $P<0.001$, (H) Cingulate ($F(1,134)=12.67$, $P<0.001$, (I) temporal parietal junction ($F(1,134)=14.18$, $P<0.001$.

In the analyses of striatal networks, greater functional connectivity in users compared to controls was seen in the limbic striatum analyses, with a cluster in the far ventral extension of the precuneus cortex (figure 2 panels A-B). In the sensorimotor striatum network, significant effects of user-groups were seen in the left middle frontal gyrus, where there was significantly more activity in the user compared to the control group and the right intra-parietal sulcus where there was reduced activity in the user compared to the control group; (figure 2, panels C-E). No user effects were seen in the associative striatal network.

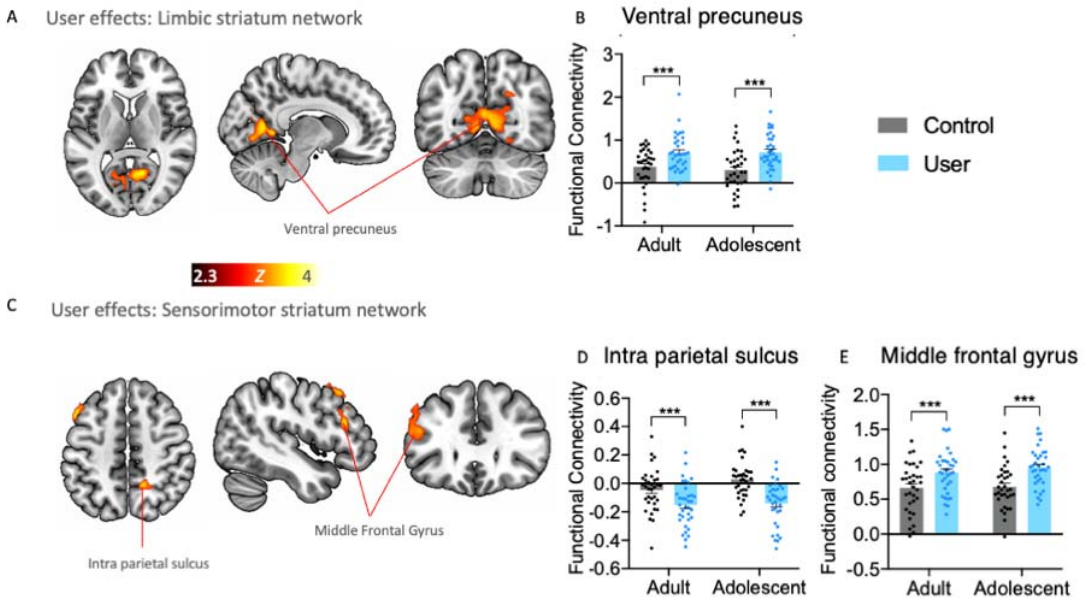

Figure 2. In the striatal networks, significant user effects were found in the limbic striatum and sensorimotor striatum analyses. One user-group effect cluster was identified from the limbic striatum seed in the ventral precuneus (A), activity in the precuneus was found to be higher in the users than the non-user-groups ($F(1,134) = 24.45$, $P < 0.001$). Two clusters were identified from the sensorimotor striatum seed, in the middle frontal gyrus and the intraparietal sulcus (C). Activity was found to be higher in the middle frontal gyrus in the user compared to non-user-group (D) ($F(1,134) = 22.35$, P <

0.001), while activity was lower in the intraparietal sulcus in the user-groups compared to non-user-groups (E) (F (1,134) = 30.02, P < 0.001).

User-group main effects: Network ROIs

The group-mean (all subjects, all scans) results were used to produce whole-network masks, with data from these masks providing a mean connectivity measure across each network, for analysis using conventional statistical methods. Results from these analyses are shown in supplementary figures 2 and 3, with the derived ROI masks shown in supplementary figure 4. The overall pattern seen for all the networks in these group-mean analyses conforms to previous work[32 33], so these analyses also serve to validate the acquisition and analysis methods used.

From the seven networks tested (Salience, DMN, ECN, associative striatum, limbic striatum, sensorimotor striatum, and hippocampus) only the DMN showed a significant decrease in overall connectivity in the user-groups compared to non-user-groups (Figure 3), (F(1,134)=5.73, P=0.018). There were no other significant effects found in any of the other whole network analyses; Bayesian *post hoc* tests all had Bayes factors BF_{01} <0.55 indicating moderate evidence for the null.

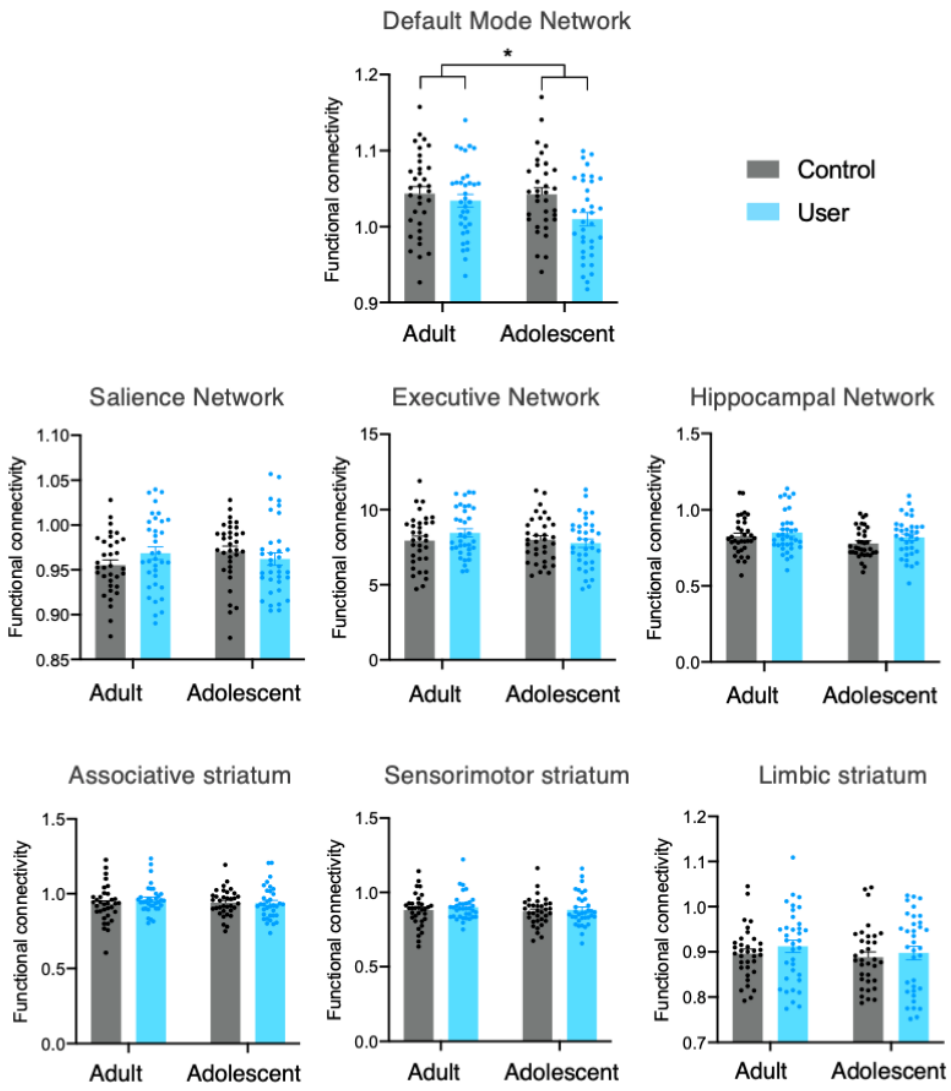

Figure 3: Cannabis users have reduced overall connectivity in the default mode network while at rest compared to controls. DMN was defined from a group mean of a posterior cingulate cortex (PCC) seed-based analysis. An ANOVA was used to test for age and user effects. A significant effect of user-group was found $F(1,1) = 5.73$, $P = 0.018$., * $P < 0.05$, $N = 138$.

Age-group main effects: Whole brain analyses

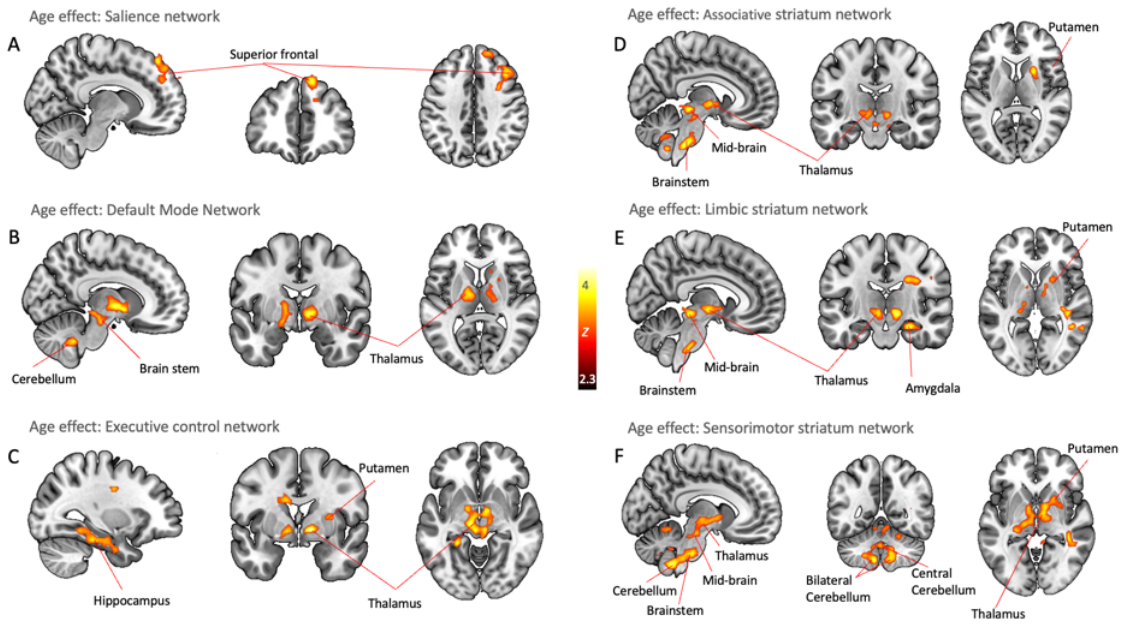

Figure 4: Significant clusters found from the age ANOVA analyses in the cortical networks (A-C) and the striatal networks (D-F).

There were many significant clusters showing the effect of age-group on functional connectivity, particularly in the analyses of striatal networks. As above, ROIs were defined from significant clusters in the whole brain analyses in order to further investigate the direction of the effects. Twenty-four significant age effect clusters were defined as ROIs from six networks (only the hippocampal network showed no significant clusters). Of these 24, only one (the superior frontal region from the anterior insula seed) showed a significant increase in adolescents compared to adults, the rest all showed a decrease. A summary of results is shown in supplementary table 3, and images of the significant clusters can be seen in figure 4.

Interaction (age x user-group) effects

There were no significant interaction effects between age and user-group found in the whole-brain analyses (no significant clusters in the group-level comparisons). There were also no significant interaction effects present in the analyses of the network ROI data.

Correlation with cannabis use

Correlation analyses were conducted to investigate the relationships between cannabis use frequency, and each network-ROI derived data, in the cannabis user-group. There were no significant correlations present in these analyses.

Discussion

We identified differences in resting-state functional connectivity associated with cannabis use in a large cohort of adolescent and adult cannabis users and age- and gender-matched controls. Whole brain analyses showed regional differences between cannabis users and non-users in the limbic and sensorimotor striatum, the DMN, ECN and the salience network (i.e. all networks except the hippocampal and associative striatum networks). The general pattern in these results was an increase in functional connectivity in these regions in cannabis users compared to controls. The exceptions were for the salience and one small cluster in the sensorimotor network, which showed a decrease in connectivity with sensorimotor regions and the middle frontal gyrus respectively. Examining overall network changes using network-mask ROIs showed significantly lower DMN connectivity in users compared to the controls, but no overall effects in any other network. Crucially, there were no significant age-group by user-group interaction effects, suggesting the relationship between regular cannabis use and RSN functional connectivity is not different in adolescents and adults, contrary to our original hypothesis. Our study does not provide any evidence that adolescent cannabis users are more vulnerable to the putatively harmful impacts of chronic cannabis use on the brain's functional connectivity. Also contrary to our hypotheses, there were no significant relationships found between use frequency and functional connectivity measures.

Effects of age-group were also evident, in many of the networks (see figure 4 and the supplementary material for details). The majority of regions identified are sub-cortical (striatum, thalamus, mid-brain) and show relatively decreased connectivity in adolescents; consistent with a developmental/maturational trajectory of increasing resting-state functional connectivity in these regions[34] and other work suggesting differentiations in the reward system in adolescents and adults[35]. As these results are largely confirmatory and were not the focus of this study or analysis, they will not be discussed further.

The DMN is comparatively well-studied, but some previous authors have reported decreases in connectivity associated with cannabis use[27], while others have reported

increases[28]. The current results show a general pattern of localised increases in connectivity associated with cannabis use. The most straightforward interpretation of this is that these increases reflect adaptive compensatory processes which may be residual effects of the acute disruptive effects of cannabinoids (particularly THC, which strongly reduces connectivity) on these networks[9,36]. Previous work has shown that CB_1 receptors are highly plastic, with normalisation of cannabis-related downregulation after just two days of abstinence[37]. The finding in the whole-network ROI analyses of a general *decrease* in connectivity within the DMN, while not being logically inconsistent with the localised *increases* found in the whole-brain results, is somewhat at odds with this simple interpretation.

Previous work suggests cannabis use can cause the DMN to become less active during rest and more active while engaging in a task, with the reduced deactivation during an active task correlating with lower performance[38]. In fact, the regions that showed significant increases in connectivity with the DMN were the dorsal precuneus and cuneal cortex. These areas (while close to the PCC) are not usually associated with the DMN, suggesting within-DMN connectivity may be decreased, but combined with an abnormal increase in connectivity with these non-DMN areas, which may suggest dysfunction. A related interpretation is that these results represent a decrease in the brain's modularity. Modularity is the tendency of the brain to form well-defined networks with relatively strong connectivity within networks and relatively weak connectivity between networks. This might explain the relative decrease in overall DMN connectivity, but the acute increases in connectivity with the non-DMN associated areas: the dorsal precuneus and the cuneal cortex. This is an emerging concept in work on classic psychedelics, has been shown with acute challenge studies[39], and may also be related to clinical anti-depressive effects of psychedelic therapy[40]. Further work will be needed with specialised analysis techniques to investigate this possibility.

Striatal connectivity has been less frequently examined in previous work, despite a high expression of CB_1 receptors in the striatum and other sub-cortical regions[41]. Positron Emission Tomography (PET) studies have shown that cannabinoids may act as a modulator of dopamine release in the striatum[42,43] and that cannabis users may have reduced dopamine synthesis capacity[44]. However, other similar studies

have shown no effect on dopamine release[45] and that life-time use of cannabis is not associated with dopamine receptor availability in the limbic striatum[46]. Further understanding of the long-term effects of cannabis on the striatum (and by implication, dopaminergic systems) is essential because of the key role these areas play in cannabis-related addiction and psychosis[47]. In the present results, the limbic striatal network showed regional increases in connectivity with cannabis use in the ventral precuneus, a large and functionally heterogeneous brain region[48]. The sensorimotor striatum showed increased connectivity in the cannabis user-group in the left middle frontal gyrus and right intra-parietal sulcus; both relatively small and unilateral clusters. No significant effects of cannabis use were seen in the hippocampal network, or the associative striatum network.

These results clearly indicate that cannabis users have somewhat different patterns of functional connectivity to controls, however there were no significant interactions between cannabis usage and age-group, or in other words, the differences between users and controls were similar in adults and adolescents. This is contrary to our original hypotheses and rationale for the study; that cannabis may affect cortico-striatal development and may present greater disruption of networks compared to non-user controls in the adolescent group. In addition, we also found no significant correlations between cannabis frequency of use and any derived connectivity measure. Recent systematic reviews focussed on the question of cannabis use in adolescence have been somewhat inconclusive, based on the weak-to-moderate available evidence[49] and the small number of available studies[50]. Pre-clinical work[51] showing later deficits associated with adolescent exposure is clearly still a cause for concern, however it appears that in humans the effects of adolescent and adult usage are not as clear-cut (at least, on the measures used here).

This study has a number of strengths. It is the largest investigation into cannabis related functional connectivity using neuroimaging to examine groups of users and controls to date. Furthermore, the design is highly novel; no existing fMRI studies have directly compared adolescent and adult cannabis users with age-matched controls. Additionally, our controls were also matched on sex and the adult/adolescent cannabis users were matched on cannabis use frequency, at ~4 days/week. Drug and alcohol abstinence were biologically verified.

Interaction effects are generally smaller and require more statistical power to substantiate [52]. It is possible that the sample is still under-powered to detect a hypothetical interaction, of unknown effect size, between age and user-groups. The conservative correction for multiple comparisons used in the whole-brain analyses may have reduced our ability to detect an interaction, if it did exist. However, mitigating against this are the results from the overall network-mask analyses, which also showed no interaction effects. These overall measures of connectivity within a network are somewhat crude in that they are a single summary measure averaged over an entire network, however they are sensitive in that the conservative correction for multiple comparisons required for whole-brain analyses is not necessary. Another important limitation is related to the observational and cross-sectional nature of the study design. We cannot establish causality, and ideally, additional longitudinal analyses are needed to rule out alternative explanations for the effects seen (e.g. possibly confounding effects of genetics and/or environmental factors[53]). Our cannabis-using group was heterogeneous in terms of frequency of usage at 1-7 days/week, however significant correlations with use frequency were not found.

To summarise, we found a significant decrease in overall DMN connectivity in cannabis users compared to controls. We also found significant regional increases in functional connectivity associated with cannabis-use across most of the other networks examined, including striatal networks. This complex pattern of regional and network-level increases and decreases in functional connectivity in the non-intoxicated state may reflect adaptive allostatic or compensatory processes, arising in response to the regular acute disruption of these networks by regular cannabis use. No interactions between age and cannabis-use groups were found, suggesting that these effects are similar in both age-groups, and adolescents are not hyper-vulnerable to cannabis-related alterations to resting state networks. No correlations were present between network function and cannabis use frequency. Regular cannabis use appears to have significant long-term effects on resting-state brain function and future work should focus on substantiating these results further with longitudinal studies and investigating the implications of these changes for general cognitive and emotional function, as well as the development of pathological states.

Methods

The data derives from the longitudinal arm of the 'CannTeen' study. Readers are directed to the full study protocol[54] for further specification of aims, data collection procedures, tasks, and power calculations for the full project. Other recent manuscripts report the full study and have focussed on cognitive effects and clinical symptoms in this cohort[55,56]. Participants attended five behavioural sessions, one every three months, over the course of one year. Approximately half of the participants (see below) attended two MRI sessions, one at the start of the study, and the second a year later. The current data is a cross-sectional analysis of the baseline fMRI resting-state data. Our analysis plan was pre-registered (prior to any analysis taking place) here: https://osf.io/jdvq7/ [30].

Ethical approval

Ethical approval was obtained from the University College London (UCL) Research Ethics Committee, project ID 5929/003. The study was conducted in line with the Declaration of Helsinki, and all participants provided written informed consent to participate.

Participants

There were two between-subjects factors with two levels: user-group (users and controls) and age-group (adolescents and adults). Participants were 70 current cannabis users and 70 age and gender-matched non-using controls, with an equal split of 35 adults and 35 adolescents in each group. In each of these subgroups there were 18 males and 17 females, except for the adult user-group which included 18 females and 17 males. Participants were recruited from the Greater London area via school assemblies, physical posters and flyers, and Facebook, Instagram, and Gumtree advertisements. Key inclusion criteria are displayed in Table 1. For a full list of all inclusion/exclusion criteria for the CannTeen study, please see the main study protocol.

	Users		***Controls***	
	Adolescents (n = 35)	**Adults** (*n* = 35)	**Adolescents** (*n* = 35)	**Adults** (*n* = 35)
Inclusion criteria	**All participants** Age 16-17 years or 26-29 years			
	Users Cannabis use at least one day per week, averaged over the past three months		**Controls** Tobacco or cannabis use at least once in lifetime	
Exclusion criteria	**All participants** • Personal history of a diagnosed psychotic episode or disorder • Current daily use of psychotropic medication • Past month treatment for any mental health condition, including cannabis dependence • BMI <18.5 or >34.9 (adults), or at <2nd or >99.6th percentile (adolescents) • Any one illicit drug used more than two days per month, averaged over the past three months (except laughing gas) • Use of laughing gas more than once per week, averaged over the past three months • MRI contraindications • Left-handed			
	Adult users Weekly or more frequent cannabis use before the age of 18, over a period of at least three months		**Controls** More than 10 days of cannabis use in lifetime Any past-month cannabis use More than one day of cannabis use in past three months	

Abbreviations: BMI = Body Mass Index, MRI = Magnetic Resonance Imaging

Table 1. Inclusion and exclusion criteria at baseline.

Data acquisition

Participants completed an MRI session shortly after their baseline behavioural session, at the Invicro clinical research facility, Hammersmith hospital, London, UK. The resting state scan was eight minutes long and was acquired towards the beginning of the scanning session, after the anatomical scans, and a stop-signal task (reported elsewhere).

MRI data was collected with a 3.0 T Siemens Magnetom Verio scanner using a 32-channel phased array head coil. Standard shimming was applied throughout, and 'dummy' scans acquired before T1 stabilization had been reached were discarded automatically by the scanner. T_2* images were acquired using a multiband gradient echo Echo-Planar Imaging (EPI) sequence (TR = 1250 ms, echo time, TE = 30 ms,

flip angle = 62°, parallel imaging factor = 2, multiband acceleration factor = 2, GRAPPA = 2, bandwidth = 1906Hz/pixel). A total of 384 volumes were collected for each subject, with a field-of-view of 192 mm and a matrix size of 64 x 64 mm, yielding an in-plane resolution of 3 x 3 mm. Slice thickness was also 3 mm, resulting in isotropic voxels. Forty-four slices were collected using an interleaved acquisition. Phase encoding direction was anterior to posterior. The forebrain, midbrain, and hindbrain (including the cerebellum) were covered. T_1-weighted structural images were acquired using a Magnetization Prepared Rapid Gradient Echo (MPRAGE) sequence (TR = 2300 ms, TE = 2.98 ms, flip angle = 9°, parallel imaging acceleration factor = 2), with a spatial resolution of 1 mm isotropic.

Analysis

All analysis procedures broadly followed the procedures for seed-based functional connectivity analyses used in previous independent datasets by [9,36,57,58].

Pre-processing

Pre-processing and analyses of fMRI data was performed in FSL (FMRIB Software Library v6.0, Analysis Group, FMRIB, Oxford, UK), with the fMRI Expert Analysis Tool (FEAT; [59,60])

Structural high resolution (anatomical) images were pre-processed using the fsl_anat function, which implements brain extraction, bias field correction, normalisation, and tissue segmentation. Pre-processing of the functional data consisted of head motion correction (with MCFLIRT), brain extraction (with BET) temporal filtering (100s), and spatial smoothing (6mm FWHM Gaussian kernel). The functional images were then normalised to MNI-152 (Montreal Neurological Institute) space with FNIRT (FMRIB's non-linear registration tool), using a 10 mm warp resolution and 12 degrees of freedom.

In order to ensure the quality of the data, each subject's raw functional image series was inspected for severe motion (>3 mm max displacement) and other artifacts. Outcomes of the registration process were also considered. Two subjects were excluded for excessive head motion after these checks leaving the final sample of N = 138. One adult non-user and one adolescent non-user were excluded at this stage.

First-level analysis

First-level analyses used seed-based functional connectivity methods, where a time-series from a particular region (the 'seed') is used to interrogate data from the rest of the brain in order to identify other areas that have correlated time-series; the implication being that areas with similar temporal characteristics are functionally connected. The regions for the PCC and Anterior Insula seeds were the same as those used in[36]. These were derived from automated meta-analytic data on http://neurosynth.org/ using the 'default mode' and 'salience' terms. In a divergence from our pre-registered analysis plan, we used an additional region in the dorsolateral prefrontal cortex (as recommended by[61]) as the seed-region for the executive control network, after testing showed this gave a better definition for the ECN that was more similar to previous work. This region was also derived from http://neurosynth.org/, using the "executive control" term. These meta-analysis maps were thresholded at an appropriate level to achieve anatomically plausible regions, and the PCC and anterior insula clusters isolated and binarized for use as image masks. The hippocampus seed region, denoting the hippocampal network, was defined anatomically using the Harvard-Oxford subcortical atlas (see supplementary figure 1A).

Masks for the three striatal networks (associative, limbic, and sensorimotor) were the same as those used in [9] and are defined according to the original parcellation by Martinez[11] and using the atlas provided by Tziortzi[62]. The associative mask includes the precommissural dorsal caudate, the precommissural dorsal putamen, and postcommissural caudate. The limbic mask includes the ventral caudate and substantia nigra, and the sensorimotor mask comprises the postcommissural putamen (see supplementary figure 1B).

All these standard (MNI152) space mask images were coregistered to each individual subject's functional space, thresholded at 0.5, and binarised to produce the final individualised mask images. The mean time series from each mask was extracted from the functional image series for each participant's scan. These time-series were then used as regressors in individual (one per region) first-level analysis models. Each of these regressors of interest were used in separate first level models

for each participant. This produces individual maps of activation for each participant for each functional network, defined by their relationship to activity in the seed region.

Mean white matter (WM) and cerebro-spinal fluid (CSF) masks were also produced as part of the anatomical image processing, by FSL's FAST algorithm. These masks were also co-registered to each subject's functional space and thresholded at 0.5. Mean signals from these masks were extracted and included in each model as regressors of no-interest to reduce noise, along with an extended set of motion parameters including temporal derivatives and quadratic versions of the six (three translations, and three rotations) basic motion parameters. Functional connectivity with each seed region was assessed using a positive contrast for the regressor of interest (the time series from each seed-region) in each model.

Second level analysis

All second level analyses used FMRIB's local analysis of mixed effects (FLAME); a two-step process using Bayesian modelling and estimation. FLAME uses a weighted least-squares approach and does not assume equal variance between groups. All group-level analyses used cluster-level analyses with a cluster-defining threshold of $Z = 2.3$ and a multiple test corrected cluster-extended wise threshold of $p < 0.05$, in order to account for multiple comparisons.

As an initial validation of the methods and analysis approach, a simple mean group-level analysis was computed for all seven seed-regions. This analysis collapsed across all subjects and age/user-groups, and the results (see supplementary figures 2 and 3) were compared to derived networks in previous similar work[9,36] as a validation step. Next, between-group analyses were conducted on the whole brain data using a voxelwise fully factorial two-way between-subjects ANOVA design to test the effects of age-group, user-group, and their interaction. F tests were used in the contrasts of this model to reveal significant differences between groups, and significant interaction effects. Whole-brain analyses of this type produce maps of F statistics which (unlike t statistics used for simple contrasts) are non-directional (always positive) and are therefore uninformative as to the direction of the effects. Therefore, the significant clusters resulting from these ANOVA analyses were

defined as ROIs, and mean values were extracted from these regions for each participant. These values were then plotted to determine the precise pattern and direction of the effects across the four groups.

ROI analyses

The mean network maps produced by the initial group-mean validation analyses were also used to define a broad ROI for each functional network, facilitating additional analyses. Maps were thresholded at $Z = 10$ to define a plausibly anatomically-constrained set of regions. Parameter estimates (single values, representing overall connectivity within the network) were then extracted for each subject, and for each network (as in[9,36]). These individual-level connectivity measures were then analysed using ANOVA to test for main effects of age, user status, and an interaction in the overall network-level results. These extracted parameter estimates from each resting-state network were also correlated with cannabis use frequency in the cannabis using groups, in order to address our final hypothesis. The alpha threshold for these correlations was reduced to 0.007 to reflect the seven tests (across the seven networks) being conducted.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request

Author contributions

Conception and methodology: WL, CM, TF, EV, HVC, MBW
Funding acquisition: HVC, TF
Data collection: WL, CM, NA, AB, NFV, RL, SO, KP, KT
Data analysis: NE, MBW, KT
Supervision: HVC, MBW
Manuscript first draft: NE, MBW
All authors edited and approved the final manuscript.

Funding

This study was funded by a grant from the Medical Research Council, MR/P012728/1, to HVC and TPF.

Competing interests

HVC has consulted for Janssen Research and Development. MBW and NE's primary employer is Invicro LLC, a contract research organisation that performs commercial research for the pharmaceutical and biotechnology industries. The remaining authors have no conflicts of interest to disclose.

References

1. Drug misuse in England and Wales - Office for National Statistics. https://www.ons.gov.uk/peoplepopulationandcommunity/crimeandjustice/articles/drugmisuseinenglandandwales/yearendingmarch2020 (2020).
2. Degenhardt, L., Stockings, E., Patton, G., Hall, W. D. & Lynskey, M. The increasing global health priority of substance use in young people. *The Lancet Psychiatry* **3**, 251–264 (2016).
3. Albaugh, M. D. *et al.* Association of Cannabis Use During Adolescence With Neurodevelopment. *JAMA Psychiatry* **78**, 1031–1040 (2021).
4. Jacobus, J. & Tapert, S. Effects of Cannabis on the Adolescent Brain. *Curr. Pharm. Des.* **20**, 2186–2193 (2014).
5. Raichle, M. E. The Brain's Default Mode Network. (2015) doi:10.1146/annurev-neuro-071013-014030.
6. Fox, M. D. & Raichle, M. E. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat. Rev. Neurosci.* **8**, 700–11 (2007).
7. Seeley, W. W. *et al.* Dissociable intrinsic connectivity networks for salience processing and executive control. *J. Neurosci.* **27**, 2349–56 (2007).
8. Goulden, N. *et al.* The salience network is responsible for switching between the default mode network and the central executive network: Replication from DCM. *Neuroimage* **99**, 180–190 (2014).
9. Wall, M. B. *et al.* Individual and combined effects of Cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC) on striato-cortical connectivity in the human brain. *J. Psychopharmacol.* 2020.11.20.391805 (2022) doi:10.1177/02698811221092506.
10. Joel, D. & Weiner, I. The Connections of the Dopaminergic System With the Striatum in Rats and Primates□: an Analysis With Respect To the Functional and Compartmental Organization of the Striatum. *Neuroscience* **96**, 451–474 (2000).
11. Martinez, D. *et al.* Imaging human mesolimbic dopamine transmission with positron emission tomography. Part II: amphetamine-induced dopamine release in the functional subdivisions of the striatum. *J. Cereb. Blood Flow Metab.* **23**, 285–300 (2003).
12. Blakemore, S. J. The social brain in adolescence. *Nat. Rev. Neurosci. 2008 94* **9**, 267–277 (2008).
13. Meyer, H. C., Lee, F. S. & Gee, D. G. The Role of the Endocannabinoid System and Genetic Variation in Adolescent Brain Development. *Neuropsychopharmacol. 2018 431* **43**, 21–33 (2017).
14. Stevens, M. C., Pearlson, G. D. & Calhoun, V. D. Changes in the interaction of resting-state neural networks from adolescence to adulthood. *Hum. Brain Mapp.* **30**, 2356–2366 (2009).
15. Solé-Padullés, C. *et al.* Intrinsic connectivity networks from childhood to late adolescence: Effects of age and sex. *Dev. Cogn. Neurosci.* **17**, 35–44 (2016).

16. Rodríguez de Fonseca, F., Ramos, J., Bonnin, A. & Fernández-Ruiz, J. Presence of cannabinoid binding sites in the brain from early postnatal ages. *Neuroreport* **4**, 135–138 (1993).

17. Teicher, M. H., Andersen, S. L. & Hostetter, J. C. Evidence for dopamine receptor pruning between adolescence and adulthood in striatum but not nucleus accumbens. *Dev. Brain Res.* **89**, 167–172 (1995).

18. Paronis, C. A., Nikas, S. P., Shukla, V. G. & Makriyannis, A. Δ9-Tetrahydrocannabinol acts as a partial agonist/antagonist in mice. *Behav. Pharmacol.* **23**, 802 (2012).

19. Skumlien, M. *et al.* ARTICLE Neural responses to reward anticipation and feedback in adult and adolescent cannabis users and controls. doi:10.1038/s41386-022-01316-2.

20. Skumlien, M. *et al.* The acute and non-acute effects of cannabis on reward processing: A systematic review. *Neurosci. Biobehav. Rev.* **130**, 512–528 (2021).

21. Nestor, L., Hester, R. & Garavan, H. Increased ventral striatal BOLD activity during non-drug reward anticipation in cannabis users. *Neuroimage* **49**, 1133–1143 (2010).

22. Van Hell, H. H. *et al.* Involvement of the endocannabinoid system in reward processing in the human brain. *Psychopharmacology (Berl).* **219**, 981–990 (2012).

23. Chan G. C. K., Hinds T. R., Impey, S., & Storm, D. R. Hippocampal neurotoxicity of Δ9-tetrahydrocannabinol. *J. Neurosci.* **18**, 5322–5332 (1998).

24. Heath, R. G., Fitzjarrell, A. T., Fontana, C. J. & Garey, R. E. Cannabis sativa: Effects on brain function and ultrastructure in rhesus monkeys. *Biol. Psychiatry* **15**, 657–690 (1980).

25. Downer, E., Boland, B., Fogarty, M. & Campbell, V. Δ9-Tetrahydrocannabinol induces the apoptotic pathway in cultured cortical neurones via activation of the CB1 receptor. *Neuroreport* **12**, 3973–3978 (2001).

26. Ritchay, M. M., Huggins, A. A., Wallace, A. L., Larson, C. L. & Lisdahl, K. M. Resting state functional connectivity in the default mode network: Relationships between cannabis use, gender, and cognition in adolescents and young adults. *NeuroImage Clin.* **30**, 102664 (2021).

27. Wetherill, R. R. *et al.* Cannabis, cigarettes, and their co-occurring use: Disentangling differences in default mode network functional connectivity. *Drug Alcohol Depend.* **153**, 116–123 (2015).

28. Pujol, J. *et al.* Functional connectivity alterations in brain networks relevant to self-awareness in chronic cannabis users. *J. Psychiatr. Res.* **51**, 68–78 (2014).

29. Watson, K. K. *et al.* Cannabis Use and Resting State Functional Connectivity in the Aging Brain. *Front. Aging Neurosci.* **14**, (2022).

30. Ertl, N. *et al.* Function of major cortical and striatal resting-state networks in adult and adolescent cannabis users. 17 (2021) doi:none.

31. Desikan RS, Ségonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, Buckner RL, Dale AM, Maguire RP, Hyman BT, Albert MS, K. R. An automated labeling system for subdividing the human cerebral cortex on MRI

scans into gyral based regions of interest. *Neuroimage* **31**, 968–980 (2006).

32. Wall, M. B. *et al.* Dissociable effects of cannabis with and without cannabidiol on the human brain's resting-state functional connectivity. *J. Psychopharmacol.* **33**, 822–830 (2019).

33. Wall, M. B. *et al.* Individual and combined effects of Cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC) on striato-cortical connectivity in the human brain. *bioRxiv* 2020.11.20.391805 (2020) doi:10.1101/2020.11.20.391805.

34. van Duijvenvoorde, A. C. K., Achterberg, M., Braams, B. R., Peters, S. & Crone, E. A. Testing a dual-systems model of adolescent brain development using resting-state connectivity analyses. *Neuroimage* **124**, 409–420 (2016).

35. Telzer, E. H. Dopaminergic reward sensitivity can promote adolescent health: A new perspective on the mechanism of ventral striatum activation. *Dev. Cogn. Neurosci.* **17**, 57–67 (2016).

36. Wall, M. B. *et al.* Dissociable effects of cannabis with and without cannabidiol on the human brain's resting-state functional connectivity. *J. Psychopharmacol.* **33**, 822–830 (2019).

37. D'Souza, D. C. *et al.* Rapid Changes in Cannabinoid 1 Receptor Availability in Cannabis-Dependent Male Subjects after Abstinence from Cannabis. *Biol. Psychiatry Cogn. Neurosci. Neuroimaging* **1**, 60–67 (2016).

38. Bossong, M. G. *et al.* Default Mode Network in the Effects of Δ9-Tetrahydrocannabinol (THC) on Human Executive Function. *PLoS One* **8**, (2013).

39. Petri, G. *et al.* Homological scaffolds of brain functional networks. *J. R. Soc. Interface* **11**, (2014).

40. Daws, R. *et al.* Decreased brain modularity after psilocybin therapy for depression. *Prepr. (Version 1) available Res. Sq. [https//doi.org/10.21203/rs.3.rs-513323/v1]* 1–19 (2021).

41. Svíženská, I., Dubový, P. & Šulcová, A. Cannabinoid receptors 1 and 2 (CB1 and CB2), their distribution, ligands and functional involvement in nervous system structures - A short review. *Pharmacol. Biochem. Behav.* **90**, 501–511 (2008).

42. Bossong, M. G. *et al.* Δ9-Tetrahydrocannabinol Induces Dopamine Release in the Human Striatum. *Neuropsychopharmacology* **34**, 759–766 (2009).

43. Calakos, K. C. *et al.* Assessment of transient dopamine responses to smoked cannabis. *Drug Alcohol Depend.* **227**, 108920 (2021).

44. Bloomfield, M. A. P. *et al.* Dopaminergic function in cannabis users and its relationship to cannabis-induced psychotic symptoms. *Biol. Psychiatry* **75**, 470–478 (2014).

45. Stokes, P. R. A., Mehta, M. A., Curran, H. V., Breen, G. & Grasby, P. M. Can recreational doses of THC produce significant dopamine release in the human striatum? *Neuroimage* **48**, 186–190 (2009).

46. Stokes, P. *et al.* History of cannabis use is not associated with alterations in striatal dopamine D2/D3 receptor availability. *J. Psychopharmacol.* **26**, 144–149 (2012).

47. Curran, H. V *et al.* Keep off the grass? Cannabis, cognition and addiction. *Nat.*

Rev. Neurosci. **17**, 293–306 (2016).

48. Cavanna, A. E. & Trimble, M. R. The precuneus: a review of its functional anatomy and behavioural correlates. *Brain* **129**, 564–583 (2006).

49. Gorey, C., Kuhns, L., Smaragdi, E., Kroon, · Emese & Cousijn, J. Age-related differences in the impact of cannabis use on the brain and cognition: a systematic review. *Eur. Arch. Psychiatry Clin. Neurosci.* **269**, 37–58 (2019).

50. Blest-Hopley, G., Giampietro, V. & Bhattacharyya, S. Residual effects of cannabis use in adolescent and adult brains — A meta-analysis of fMRI studies. *Neuroscience and Biobehavioral Reviews* vol. 88 26–41 (2018).

51. Quinn, H. R. *et al.* Adolescent rats find repeated Δ9-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. *Neuropsychopharmacology* **33**, 1113–1126 (2008).

52. Marshall, S. W. Power for tests of interaction: Effect of raising the Type I error rate. *Epidemiol. Perspect. Innov.* **4**, 1–7 (2007).

53. Hicks, B. M. *et al.* Gene-Environment Correlation in the Development of Adolescent Substance Abuse: Selection Effects of Child Personality and Mediation via Contextual Risk Factors. *Dev. Psychopathol.* **25**, 119 (2013).

54. Lawn, W. *et al.* cannTEEN: How does long-term cannabis use affect teenagers' and adults' cognition, mental health and brains? 17 (2019) doi:none.

55. Lawn, W. *et al.* The CannTeen Study: Cannabis use disorder, depression, anxiety, and psychotic-like symptoms in adolescent and adult cannabis users and age-matched controls. *J. Psychopharmacol.* 1–12 (2022) doi:10.31234/OSF.IO/C7X95.

56. Lawn, W. *et al.* The CannTeen study: verbal episodic memory, spatial working memory, and response inhibition in adolescent and adult cannabis users and age-matched controls. *Psychopharmacol. 2022* **1**, 1–13 (2022).

57. Demetriou, L. *et al.* A comprehensive evaluation of increasing temporal resolution with multiband-accelerated sequences and their effects on statistical outcome measures in fMRI. *Neuroimage* **176**, 404–416 (2018).

58. Comninos, A. N. *et al.* Modulations of human resting brain connectivity by kisspeptin enhance sexual and emotional functions. *JCI insight* **3**, 0–10 (2018).

59. Smith, S. M. *et al.* Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* **23**, S208–S219 (2004).

60. Woolrich, M. W., Ripley, B. D., Brady, M. & Smith, S. M. Temporal autocorrelation in univariate linear modeling of FMRI data. *Neuroimage* **14**, 1370–1386 (2001).

61. Thomas Yeo, B. T. *et al.* The organization of the human cerebral cortex estimated by intrinsic functional connectivity. *J. Neurophysiol.* **106**, 1125–1165 (2011).

62. Tziortzi, A. C. *et al.* Connectivity-Based Functional Analysis of Dopamine Release in the Striatum Using Diffusion-Weighted MRI and Positron Emission Tomography. (2013) doi:10.1093/cercor/bhs397.