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Cannabis use in early adolescence: Evidence of amygdala hypersensitivity to signals of threat

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Abstract

Cannabis use in adolescence may be characterized by differences in the neural basis of affective processing. In this study, we used an fMRI affective face processing task to compare a large group

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(*n* = 70) of 14-year olds with a history of cannabis use to a group (*n* = 70) of never-using controls matched on numerous characteristics including IQ, SES, alcohol and cigarette use. The task contained short movies displaying angry and neutral faces. Results indicated that cannabis users had greater reactivity in the bilateral amygdalae to angry faces than neutral faces, an effect that was not observed in their abstinent peers. In contrast, activity levels in the cannabis users in cortical areas including the right temporal-parietal junction and bilateral dorsolateral prefrontal cortex did not discriminate between the two face conditions, but did differ in controls. Results did not change after excluding subjects with any psychiatric symptomology. Given the high density of cannabinoid receptors in the amygdala, our findings suggest cannabis use in early adolescence is associated with hypersensitivity to signals of threat. Hypersensitivity to negative affect in adolescence may place the subject at-risk for mood disorders in adulthood.

Keywords

Cannabis; Adolescence; Face processing; fMRI; Amygdala; Emotion; Faces

1. Introduction

Adolescence is a significant period of psychosocial development, with increases in novelty-seeking and risk-taking behaviors (Adriani et al., 1998; Romer et al., 2010; Trimpop et al., 1998). Experimentation with drugs of abuse – especially alcohol, tobacco, and cannabis, is typically initiated during this phase (Chen and Kandel, 1995). As cannabis becomes more available and public opinion trends towards acceptance, adolescents may have increased access to the substance.

Current rates of cannabis use among adolescents are high, with a quarter of all 10th graders, and over a third of all 12th graders in the US reporting trying cannabis at least once (SAMHSA, 2014). Chronic use also appears to be growing; in 2008, 5.5% of users aged 12 and up reported near daily use while in 2013 this rate had risen to 8.1% (SAMHSA, 2014). These increasing rates of use are consequential in that about 10% of those who try cannabis will become weekly users in adulthood (Hall and Pacula, 2003). Furthermore, adolescent beliefs about the risks associated with cannabis appear to be declining (Johnston et al., 2011).

Adolescence is also a period of marked neural development including gross volume changes, myelination, synaptic pruning, and receptor proliferation (Spear, 2000). These changes are especially large in the prefrontal cortex (PFC) (Gogtay et al., 2004; Whitford et al., 2007), amygdala, hippocampus, and striatum, and are governed in part by the endogenous cannabinoid system (Bossong and Niesink, 2010). Interestingly, the primary cannabinoid receptor, CB1, is found in high concentrations in these cognitive and affective regions of the brain (Glass et al., 1997; Herkenham et al., 1991; Katona et al., 2001), and appears to be fully expressed by adolescence (Belue et al., 1995; de Fonseca et al., 1993; Morozov and Freund, 2003; Romero et al., 1997). Studies have shown that exogenous cannabinoids can interfere with the endogenous system (Hoffman et al., 2007; Mato et al., 2004). Given the natural maturation occurring in the brain during adolescence, and the propensity towards cannabis use, the consumption of exogenous cannabinoids during

adolescence may disrupt typical neurodevelopment within the cognitive and affective neural systems.

Mounting evidence supports the relationship between early cannabis use and mood disorders (Wittchen et al., 2007), even with relatively low levels of use (Cheung et al., 2010). Hence, it is crucial to investigate the consequences of cannabis use on emotional development. Although numerous studies have associated cannabis use in adolescence with an increased likelihood of schizophrenia and/or other affective disorders (Arseneault et al., 2004; Degenhardt and Hall, 2006; Fergusson et al., 2006; Hall, 2006; Linszen and van Amelsvoort, 2007; Manrique-Garcia et al., 2012) there is relatively little research on the impact of cannabis use from a cognitive and affective neuroscience perspective.

The amygdala has a high density of CB1 receptors, notably in the basal and lateral nuclei (Katona et al., 2001). In adulthood, increased amygdala activity is associated with major depressive disorder (Drevets, 2001; Sheline et al., 2001), and generalized social phobia (Evans et al., 2008; Phan et al., 2006). In adolescence, the amygdala was found to yield stronger responses to fearful faces than adults (Thomas et al., 2001), and greater amygdala reactivity may account for adolescent vulnerability to mood disorders (Guyer et al., 2008a; Monk et al., 2008; Roberson-Nay et al., 2006). In consideration of the amygdala's role in the endocannabinoid system and affective processing, adolescent vulnerability to mood disorders and propensity for cannabis use, it is important to assess functional differences in this region in cannabis-using teenagers.

Using an animal model, Rubino and colleagues (2008), and Schramm-Sapyta and colleagues (2007) examined the relationship between anxiety and THC exposure in adolescent and adult rats. Findings indicate that adolescent rats exhibit elevated signs of anxiety, depression, and anhedonia when treated with THC compared to placebo. Translating these findings to humans may imply cannabis use in adolescence is related to differences in the generation and regulation of affect.

To examine the impact of cannabis use on brain regions subserving emotional processing, we conducted an fMRI study on 14-year old cannabis users vs. controls using affective face stimuli. Angry and neutral faces provide a robust probe of activity within the amygdala and PFC in adults (Morris et al., 1996; Pessoa et al., 2002; Whalen et al., 1998), as well as children and adolescents (Baird et al., 1999; Thomas et al., 2001). The differential activity of the amygdala to angry versus neutral faces is an excellent index of emotional processing and may relate to psychopathology. However, in order to prevent ceiling effects, we used a set of stimuli that was only mildly negatively valenced on the basis that they may provide a sensitive test of enhanced amygdala reactivity (Grosbras and Paus, 2006).

To date, few study have examined the relationship between cannabis and face processing. Phan and colleagues (2008) recruited healthy adults in a dual-session, double-blind, placebo-controlled study of THC intoxication and face processing using fMRI. Findings indicate THC attenuates the amygdala response to fearful faces. Similarly, Gruber and colleagues (2009) studied 15 chronic cannabis users vs. matched controls under fMRI during a masked affective face processing task. Results suggest chronic cannabis use is associated with

decreased reactivity in the anterior cingulate and amygdala. While both Phan and Gruber's findings suggest anxiolytic effects in intoxicated adults, these studies do not address whether the effects would replicate in users not intoxicated during scanning, nor does it address whether the effects would generalize to adolescents. Nonetheless, these studies provide evidence that cannabis use is associated with differences in affective processing.

In this relatively large fMRI study (N = 140), we investigated the impact of previous cannabis use (n = 70) compared to closely matched controls (n = 70) in early adolescence using a face processing task during fMRI. To date, there has been no previous research directly studying history of cannabis use with face processing, especially not from a developmental affective neuroscience perspective.

2. Methods

2.1. Participants

We identified a sample of cannabis-experimenting adolescents (n = 70) and matched controls (n = 70) from the IMAGEN dataset, a large multi-site longitudinal study of adolescent development (Schumann et al., 2010). The European School Survey Project on Alcohol and Other Drugs (ESPAD) item for lifetime history of cannabis use was used to identify the cannabis-experimenting group. Subjects provided a self-report based on a scale from 0 to 6, (1 = 1–2 times; 3 = 6–9 times; 6 = 40+ times; see supplementary Table S1 for complete distribution, and S2 for substance use age of onset distributions). Subjects who endorsed using other illicit substances were excluded, and any subject exhibiting signs of intoxication were excluded from scanning.

Given the relationship between amygdalar reactivity and psychopathology, subjects completed the Development and Well-Being Assessment (DAWBA; Goodman et al., 2000) to screen for psychopathology symptomology. DAWBA clinical rating scores were obtained from trained DAWBA clinicians who generated clinical rating scores by reviewing parent, teacher, and adolescent DAWBA responses. Final scores consisted of one of three categories: no-diagnosis, unsure, and, sure diagnosis, on any DSM-IV symptom class of psychopathology. From our sample, five of the controls and nine of the cannabis-experimenting group did not complete the DAWBA. Nonetheless, subjects were matched to the best of our ability on the DAWBA as indicated via chi-square analyses.

Controls were identified and matched on sex, handedness, age, verbal comprehension and perceptual reasoning IQ, pubertal development, socioeconomic status, and site. As cannabis use is highly correlated with alcohol and cigarette use (Hall and Pacula, 2003), which often makes it difficult to attribute group differences to the cannabis use *per se*, controls were also matched on lifetime alcohol and cigarette use. Chi-square tests were performed on the DAWBA, sex, and handedness; *t*-tests were performed on the remaining continuous measures (see Table 1 for subject information and *p*-values).

2.2. Task

Participants passively viewed a collection of video clips that contained either a person's face or a control picture (concentric circles). The task was designed and originally implemented

by Grosbras and Paus (2006) and required participants to passively view a series of short (2–5 s) black-and-white video clips showing a face that started from a neutral expression and progressively turned angry, or, progressively turned to a second neutral expression. The control pictures contained expanding and contracting concentric circles of various contrasts, roughly matching the contrast and motion characteristics of the faces. These control images were designed and originally implemented by Beauchamp and colleagues (2003) and were included to account for neural activity associated with viewing non-biological motion. All stimuli were presented as 18 s blocks, with 4–7 video clips per block during a face block. Each run was comprised of 5 blocks of neutral faces and 5 blocks of angry faces.

2.3. Imaging parameters

All MRI data were acquired using 3T MRI scanners made by several manufacturers (Siemens, Philips, General Electric, Bruker) in the eight IMAGEN assessment sites (London, Nottingham, Dublin, Mannheim, Dresden, Berlin, Hamburg, and Paris). Important scanning parameters were identical across sites (i.e., field of view, flip angle and matrix; see Schumann et al., 2010) and followed an extensive program of cross-site standardization. Although our groups were matched on site, each participant's site was modeled as a nuisance covariate in the statistical analyses. In the present task, 160 volumes per subject were obtained, each comprising 40 slices. The slices were aligned to the connecting line between the anterior and posterior commissure (2.4 mm thickness, 1 mm gap, TR = 2.20 s, TE = 30 ms).

2.4. Imaging analysis

The pre-processing of the EPI data was done within SPM8 (Statistical Parametric Mapping, http://www.fil.ion.ucl.ac.uk/spm/). Time series data were first corrected for slice-timing, then corrected for movement (spatial realignment), non-linearly warped into MNI space (using a custom EPI template), and Gaussian-smoothed at 5 mm-FWHM. Activation maps were computed with SPM8, and regressed using a general linear model (GLM) with AR noise model (SPM default) against a design-matrix modeling each event of the stimulus presentation. Contrast images were obtained for the main effect of angry faces and neutral faces, as well as the differential activation for angry vs. neutral faces.

2.5. Preliminary analysis

A preliminary voxel-wise analysis directly comparing the cannabis-experimenting group to the control group was conducted using the AFNI toolbox (Cox, 1996). We subjected the data to a between-group *t*-test on the contrast image of angry minus neutral face processing. We detected greater differential activation to angry faces in the cannabis-experimenting group in small clusters spanning potentially interesting cortical and subcortical areas (dorsolateral prefrontal cortex, temporal parietal junction, fusiform, and right extended amygdala into the striatum). However, at a whole-brain uncorrected $p < .005$, the clusters were small and consequently prompted a functionally defined region-of-interest analysis.

2.6. Voxel-wise analysis

The central goal of the voxel-wise analysis was to find unbiased clusters of brain activation that discriminated between angry and neutral faces. All cannabis-experimenting and control subjects were combined and treated as one group in a *t*-test vs. zero using the angry vs. neutral contrast. Scanning site was used as a nuisance covariate to account for the variance associated with multisite data collection.

2.7. ROI selection

ROIs were defined based on the results from the above voxel-wise analysis. The alpha-level for cluster detection was determined by running Monte Carlo simulations using AFNI's 3dClustSim. The smoothness of the data was estimated using 3dFWHMx (details at http://afni.nimh.nih.gov/pub/dist/doc/program_help/). Based on a voxel-wise uncorrected alpha of $p = .005$, a minimum cluster extent was determined to be 112 contiguous voxels, so as to arrive at a corrected ROI-level alpha of $p = .01$. From these criteria, we identified seven regions that were significantly more active for angry faces relative to neutral faces.

Based on prior knowledge of the importance of the amygdala in affective face processing, left and right anatomically defined amygdala ROIs were also included in the analysis. Amygdala ROIs were obtained using the Eickhoff–Zilles macro label atlas in MNI space distributed within AFNI (Eickhoff et al., 2005). The voxels in the amygdala ROIs were then resampled to match the grid dimensions of the functional data.

2.8. ROI analysis

The seven functionally defined clusters, plus the left and right amygdala ROIs, were used to extract the mean BOLD signal from the angry face and neutral face contrasts for all subjects. The mean signal for each ROI were then subjected to a 2-by-2 (group × face type) analyses of variance using SPSS v. 22 (IBM Corp. Armonk, NY). All *p*-values reported were corrected for multiple comparisons using a modified Bonferroni procedure (Keppel and Wickens, 2004). For display purposes, the mean signal for face type by group was plotted using MATLAB v. R2014a (The MathWorks, Inc., Natick, MA). Lastly, we tested for any correlation between the mean signal per face type within all the ROIs with the level of cannabis use, and age of onset of cannabis, alcohol, and cigarette use.

3. Results

3.1. Subjects

As shown in Table 1, the two groups did not differ in sex, handedness, age, verbal or perceptual IQ, pubertal development, socioeconomic status, total (any) DSM-IV diagnoses, lifetime alcohol or cigarette use. Further, the cannabis-experimenting group's mean verbal and perceptual IQ did not significantly differ from the means of the entire IMAGEN sample ($N = 1849$) at $p < .05$.

3.2. Voxel-wise analysis results

Seven clusters were identified centered on the right and left middle temporal gyrus, right and left inferior frontal gyrus, bilateral anterior cingulate, left cerebellum, and right lingual gyrus (see Table 2).

3.3. ROI ANOVA results

As expected given how they were identified, all seven functionally defined ROIs, plus the amygdalae, exhibited a significant main effect of face type ($F(9,130) = 30.03$, $p < .001$). None showed a main effect of group but, instead, five of the nine had significant interactions between face type and group. These five were the left amygdala ($F(1,138) = 8.54$, $p < .001$); right amygdala ($F(1,138) = 8.54$, $p = .004$); right middle temporal gyrus with extent into temporal parietal junction ($F(1,138) = 5.28$, $p = .006$); left inferior frontal gyrus with extent into dorsolateral prefrontal cortex ($F(1,138) = 4.87$, $p = .008$); and right inferior frontal frontal gyrus with extent into dorsolateral prefrontal cortex ($F(1,138) = 5.71$, $p = .006$) (see Figs. 1–3 and Table 2).

Post hoc tests revealed that within the cannabis-experimenting group, there were significant differences in the bilateral amygdalae with greater activation for the angry faces (right amygdala $t(69) = 4.02$, $p < .001$; left amygdala $t(69) = 3.15$, $p = .002$) but no effect of face type on activity in the cortical ROIs.

Controls showed a different pattern; there were significant face type differences in all the cortical regions with greater activation for neutral faces, but no effect of face type on the BOLD signal in the amygdalae (right middle temporal gyrus $t(69) = -7.20$, $p < .001$; left inferior frontal gyrus $t(69) = -5.13$, $p < .001$; right inferior frontal gyrus $t(69) = -5.68$, $p < .001$: see Table 3 for all post hoc *t*-test results).

4. ROI Correlations with Other Drugs

To examine dosage–response effects, we investigated Pearson's correlation on frequency of cannabis use with the mean signal per face type within each region. Dosage effects within bilateral amygdalae and dlPFC were non-significant at $p < .05$. Interestingly, we detected a significant correlation within the right TPJ cluster with frequency of cannabis use. Both the mean signal related to angry faces ($r = -.25$, $p < .05$), and neutral faces ($r = -.26$, $p < .05$), was correlated with frequency of cannabis use, such that, more frequent cannabis use is associated with less processing by the right TPJ during presentation of both face types.

We also investigated Pearson's correlation on age of onset of cannabis, alcohol, and cigarette use with the mean signal per face type within each ROI. However, we failed to detect any significant correlations at $p < .05$ between age of onset for any drugs of abuse with any of the ROIs.

4.1. Psychopathology Symptomology

The DAWBA clinical rating scores revealed 14 cannabis-experimenters and 10 control subjects ($X^2(1,122) = 1.19$, $p > .05$) were identified as having a "sure" DSM-IV symptom class diagnoses. Chi-square analyses revealed the only symptom class that significantly

differed between the two groups was conduct disorder: $X^2(1,122) = 5.55$, $p < .05$. This finding is consistent with previous studies reporting an association between conduct disorder and cannabis use initiation during adolescence (Castellanos-Ryan and Conrod, 2011; Hopfer et al., 2013).

4.2. Influence of Psychopathology

To examine if the conduct disorder finding was related to our results, we first excluded the five subjects with a conduct disorder diagnosis and re-ran the ANOVA and post hoc *t*-tests. Both the ANOVA and post hoc *t*-tests results remained the same as the initial analysis with all subjects included. We then tested to see if conduct disorder in the cannabis-experimenting group was correlated with the BOLD signal in any of the ROIs, but failed to detect any significant correlation at $p < .05$.

Lastly, to test if *any* psychopathology influenced the dataset, we excluded all 14 cannabis-experimenting and 10 control subjects with a strong probability of a DSM-IV category diagnosis from the ANOVA and post hoc *t*-tests, and reran the analyses. When correcting for multiple comparisons, the left and right amygdala and right TPJ maintained significance on the ANOVA face type × group interaction. Nonetheless, the same five regions that initially survived correction for multiple comparisons for the full sample analysis still passed significance at an uncorrected *p*-value of <.05. Additionally, the post hoc *t*-test results remained the same. Consequently, with minor exceptions regarding correction for multiple comparisons, results remained largely the same even when analyzed on sub-groupings devoid of any mental health symptomology. Hence, these findings suggest that mental health symptomology was not contributing to the full sample group differences.

5. Discussion

In this study, we examined the functional neurobiology of angry and neutral face processing in a group of cannabis-experimenting adolescents vs. matched controls using fMRI. We found group-by-face type interaction effects in bilateral amygdala and three clusters of activation that span the right TPJ and bilateral dlPFC. Decomposing these results by face type, we found the cannabis-experimenting group exhibited increased activity to angry faces in the amygdala. Conversely, the control group exhibited increased activity to neutral faces in the cortical regions. Therefore, cannabis use during early adolescence is associated with hypersensitivity to negative affect in the amygdala. While we stress that this study does not permit us to conclude cannabis-experimentation caused the observed functional neurobiological differences, we are confident these differences are associated with the cannabis use status of the participants due to our relatively large sample size ($N = 140$), carefully matched control group (who did not differ on sex, pubertal development, IQ, site, psychopathology, or alcohol and cigarette use), and a conservative criteria to meet statistical significance.

With regard to the cortical findings, the right TPJ and bilateral dlPFC showed greater activation to neutral faces than angry faces in the control group. The right TPJ has been implicated in theory of mind, social processing, and face processing (Allison et al., 2000; Saxe and Kanwisher, 2003; Saxe and Powell, 2006). Furthermore, the right superior

temporal gyrus encodes biologically relevant motion (Grossman et al., 2000; Puce and Perrett, 2003; Saygin, 2007). Therefore this cluster may represent a signal of social salience related to the moving face stimuli. In contrast to controls, post hoc *t*-test results show the cannabis-experimenting group fails to process angry faces differently from neutral faces within the right TPJ (see Fig. 2). As this region was also the only region to exhibit significant dosage effects, a higher degree of cannabis experimentation may contribute to a departure from healthy social processing. Interestingly, as none of the regions exhibited a significant correlation with age of onset for any drugs of abuse, we are unable to make claims regarding face processing and cannabis use in relation to age of onset with other drugs.

Considering that the cortical clusters spanned the temporal, parietal, and bilateral frontal lobes, we suggest that the neutral faces demanded more cognitive resources. The neutral faces had greater ambiguity and variability in their content, such as nose twitching, mouth movements, and eye-blinks. Furthermore, all stimuli video clips started from neutral and transitioned to angry or neutral faces. The stimuli that transitioned to angry faces were more explicit during the shift to threat, whereas the transition to another neutral face may have required more cognitive strategies to decode. Hence, the neutral faces may have demanded a greater degree of attention and interpretation by these cognitive systems.

With regard to the amygdala findings, it is unclear whether amygdala hypersensitivity preceded cannabis use or was a consequence of use since this was a cross-sectional study. If amygdala hypersensitivity preceded use, which might seem most plausible given the low levels of reported use, then it's possible that these individuals may have been inclined to self-medicate for the drug's acute anxiolytic effects (Phan et al., 2008). Consistent with this interpretation, recent evidence has identified altered angry face processing in the ventromedial PFC (vmPFC) to predict future binge drinking (Whelan et al., 2014) and the vmPFC is part of a brain circuit that attenuates amygdala activity (Banks et al., 2007; Urry et al., 2006). If, however, the amygdala hypersensitivity is a consequence of cannabis use, then it is likely that this is due to exogenous stimulation of the endocannabinoid system. If confirmed, these findings would raise concerns regarding the risks associated with cannabis consumption and emotional health in adolescent users. Animal studies suggest exogenous cannabinoids inhibit GABAergic neurotransmission in the amygdala (Katona et al., 2001). Interestingly, this effect is magnified when the animal is given THC and placed in a threatening environment (Patel et al., 2004). Together these findings suggest that cannabinoids may compromise the major neuronal inhibitory mechanism within the amygdala and lower the threshold for activation, especially during signals of threat. Consistent with this interpretation, the angry faces used in the task were not exceptionally potent signals of threat yet the cannabis-experimenting group showed a heightened reactivity to them, an effect that is not observed in healthy controls viewing the same stimuli (Grosbras and Paus, 2006).

The amygdala's role in affective processing serves an important role in evolutionary biology as it directs attention towards aversive stimuli. However, mounting evidence suggests that over-recruitment of the amygdala is associated with various mood disorders. Greater signal change in the amygdala, specifically during affective face processing, is exhibited by

children with anxiety (Thomas et al., 2001), and adults with major depressive disorder (Drevets, 2001; Fu et al., 2008; Sheline et al., 2001) and generalized social phobia (Evans et al., 2008; Phan et al., 2006). Thus, cannabis use in adolescence may contribute to the etiology of mood disorders in adulthood. Moreover, relatively light use by an early age may contribute to an early marker of maladaptive affective processing. Nonetheless, major longitudinal studies are needed to illuminate these hypotheses as the current study is unable to infer causality.

The results reported here are inconsistent with those of Phan and colleagues (2008) and Gruber and colleagues (2009) who both found attenuated amygdala reactivity to threat signals in adults following acute THC administration, and chronic non-intoxicated users, respectively. In contrast, we report trait-related increased amygdala reactivity to threat signals in adolescence. Hence, we report divergent effects in adolescents compared to adults. As previous research demonstrates divergent findings between adolescents and adults during affective face processing (Guyer et al., 2008a,b), we do not hypothesize adolescent data to mirror the adult data. Indeed, our results support the notion that adolescence is period of sensitive affective development that can be perturbed even with very low levels of cannabis experimentation.

The current results are consistent with the animal models of cannabinoid exposure during adolescence (Rubino et al., 2008; Schramm-Sapyta et al., 2007) and suggest that more human research is needed on the long-term effects of cannabis use in adolescence. In consideration of the animal studies and the link between early cannabis use and mood disorders later in life, acute THC consumption effects in the adolescent brain may be different or, indeed, the long-lasting effects of repeated exposure may be different beyond the acute intoxication phase. As adolescents tend to be more reactive to emotional stimuli, especially face processing in the amygdala, the observed differences in adolescent cannabis-users may suggest evidence of maladaptive cognitive and affective systems related to psychosocial development.

Lastly, a notable feature of the present results is that our sample of cannabis users reported relatively low levels of use, but nonetheless exhibited significant differences in processing threat signals. Furthermore, due to the closely matched control group, we excluded a range of possible confounding factors, including mental health comorbidities, which may have accounted for the observed differences. As excluding subjects with mental health comorbidities failed to change the pattern of our results, the findings suggests that very low use of cannabis during early adolescence may compromise healthy emotional reactivity.

An alternative explanation regarding the observed differences in affective face processing may be attributed to unmeasured pre-existing differences in emotional functioning, which might have contributed to the adolescents' experimentation with cannabis. Indeed, we have previously shown that activation in response to these angry faces in the left PFC predicted binge drinking two years later, which would suggest altered emotional reactivity may precede use (Whelan et al., 2014). However, in the present analyses the measured psychiatric symptomology results failed to show elevated levels of any of the affective

disorders, therefore, it is unclear which preexisting differences, if any, might have been present in the cannabis-experimenting group.

Future studies will be performed on the follow-up (age 16 and 18) data of this project to identify predictive factors contributing to the cannabis use phenotype profile. As this was a cross-sectional study from the baseline IMAGEN dataset, we stress that we are unable to claim cannabis use caused amygdala hypersensitivity to negative affect. To investigate this question, longitudinal data analysis will inform whether hypersensitivity to threat signals precedes use or is a consequence of use, and assessments of psychopathology will clarify if early cannabis use and differences in face processing contribute to the generation of clinically relevant disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.dcn.2015.08.007.

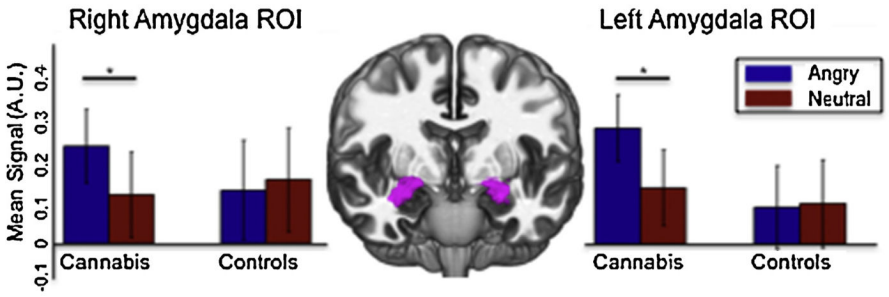

Fig. 1.

Mean activation for face type by group plotted for left and right amygdala. Asterisks indicate post hoc *t*-test differences significant at $p < .05$, corrected. Error bars represent the standard error of the mean.

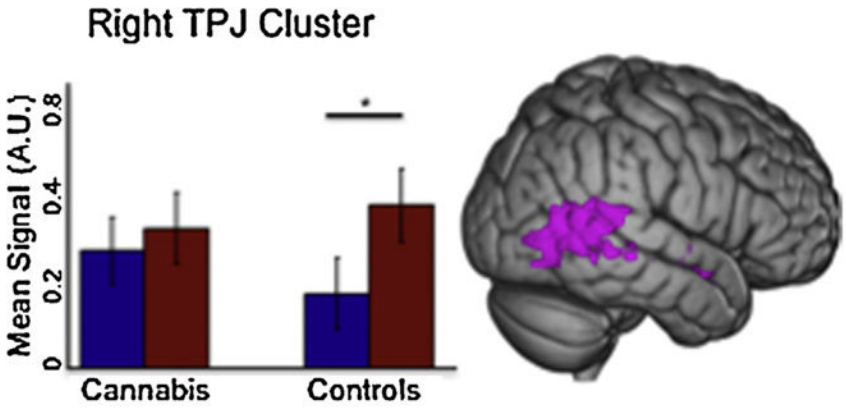

Fig. 2.

Mean activation for face type by group plotted for the cluster spanning the right temporal parietal junction. Blue bars represent angry faces, red bars represent neutral faces. Asterisks indicate post hoc *t*-test differences significant at $p < .05$, corrected. Error bars represent the standard error of the mean (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

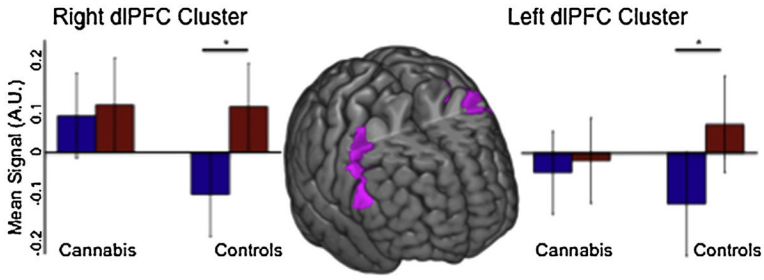

Fig. 3.

Mean activation for face type by group plotted for the cluster spanning the left and right dorsolateral prefrontal cortex. Blue bars represent angry faces, red bars represent neutral faces. Asterisks indicate post hoc *t*-test differences significant at $p < .05$, corrected. Error bars represent the standard error of the mean. Cutout: $y = 4$, $z = 48$ (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

Table 1

Subject information and statistics by group.

Measure	Group		*p*
	Cannabis (*n* = 70)	**Controls (*n* = 70)**	
Males/females (*n*)	50/20	41/29	.111
Left/right handedness (*n*)	6/64	5/65	.753
Age (*M*, SD)	14.765, 0.40	14.61, 0.655	.607
Perceptual reasoning IQ (*M*, SD)	104.219, 16.876	105.72, 13.879	.555
Verbal comprehension IQ (*M*, SD)	110.74, 16.84	110.43, 13.329	.905
Puberty development scale (*M*, SD)	3.60, 0.60	3.766, 0.63	.585
Socioeconomic status (*M*, SD)	18.45, 4.42	18.24, 4.70	.751
Any DSM-IV diagnoses (*n*)	10	14	.275
Conduct disorder diagnosis (*n*)	5	0	.019
Lifetime alcohol Use (*M*, SD)	3.71, 1.63	3.56, 1.32	.530
Lifetime cigarette use (*M*, SD)	3.106, 2.215	2.54, 2.215	.158
Lifetime cannabis Use (*M*, SD)[a]	1.70, 1.30	0, 0	.000

[a] Based on a self-report scale from 0 to 6. (1 = 1–2 times; 2 = 3–5 times; 3 = 6–9 times; 4 = 10–19 times; 5 = 20–39 times; 6 = 40+ times).

Table 2

Anatomically and functionally defined ROIs with group by condition interaction statistics. Rows in bold survived a modified Bonferroni correction for multiple comparisons.

Peak voxel location	Center of mass coordinate (MNI)	Cluster size	Group × condition interaction	
	x, y, z	*k*	*F*	*p*
Left amygdala		**120**	**8.54**	**.000**
Right amygdala		**139**	**5.56**	**.004**
Right middle temporal gyrus, cluster extends into temporal parietal junction (TPJ)	**−54, 47, 6**	**1333**	**5.28**	**.006**
Left inferior frontal gyrus, cluster extends into dorsolateral prefrontal cortex (dlPFC)	**54, −14, 28**	**417**	**4.87**	**.008**
Right inferior frontal gyrus, cluster extends into dorsolateral prefrontal cortex(dlPFC)	**−49, −14, 33**	**356**	**5.71**	**.004**
Left middle temporal gyrus, cluster extends into temporal-parietal junction (TPJ)	53, 51, 9	1181	2.19	.115
Left cerebellum	12, 78, −39	477	2.36	.096
Right lingual gyrus	−13, 79, −8	317	1.53	.219
Bilateral anterior cingulate, cluster extends into ventromedial prefrontal cortex	0, −45, 7	830	3.72	.026

Table 3

Post hoc *t*-test comparison for within-group differences. Cells in bold are significant at $p < .05$, corrected.

	Angry faces vs. neutral faces	
	Cannabis	**Controls**
Left amygdala	***t*(69) = 4.02, *p* < .001**	*t*(69) = −0.32, *p* = .750
Right amygdala	***t*(69) = 3.15, *p* = .002**	*t*(69) = −0.73, *p* = .470
Right middle temporal gyrus, cluster extends into temporal parietal junction (TPJ)	*t*(69) = −1.21, *p* = .231	***t*(69) = −7.20, *p* < .001**
Left inferior frontal gyrus, cluster extends into dorsolateral prefrontal cortex (dlPFC)	*t*(69) = −0.60, *p* = .551	***t*(69) = −5.13, *p* < .001**
Right inferior frontal gyrus, cluster extends into dorsolateral prefrontal cortex (dlPFC)	*t*(69) = −0.56, *p* = .576	***t*(69) = −5.68, *p* < .001**