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Does thinner right entorhinal cortex underlie genetic liability to cannabis use?

Subhadip Paul[1] and Sagnik Bhattacharyya[1,2]

[1]Institute of Psychiatry, Psychology & Neuroscience, King's College London, UK and [2]South London and Maudsley NHS Foundation Trust, Denmark Hill, Camberwell, London, UK

Original Article

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Author for correspondence:
Sagnik Bhattacharyya, E-mail: sagnik.2.bhattacharyya@kcl.ac.uk

Abstract

Background. Although alterations in medial temporal lobe structures have been previously associated with use of cannabis, one of the most widely used illicit drugs, whether such alterations are a cause or effect of cannabis use has been unclear.

Methods. In this cross-sectional observational study involving 404 twins/siblings, we have compared cortical thickness and surface area between groups of gender-matched sibling-pairs (concordant cannabis unexposed, concordant exposed and discordant for cannabis exposure) using permutation tests after controlling for potential confounds. Bi-variate polygenic model was used to assess the genetic and environmental contributions underlying cortical morphological phenotypes and frequency of cannabis use.

Results. Cortical thickness of the right entorhinal cortex was significantly lower in the concordant exposed siblings compared to both discordant unexposed and discordant exposed groups [false discovery rate (FDR)-corrected, $q < 0.05$]. The association between the right entorhinal cortex thickness and frequency of cannabis use is due to the contribution of significant shared additive genetic ($\rho_g = -0.19 \pm 0.08$; $p = 0.02$) factors but not unique environment ($\rho_e = 0.05 \pm 0.09$; $p = 0.53$). Significantly lower surface area of the right entorhinal cortex in discordant exposed group compared with the discordant unexposed group furnishes preliminary evidence in support of causal effect of cannabis use (FDR-corrected, $q < 0.05$). However, bi-variate polygenic model-based analysis did not show any significant effect.

Conclusions. Shared genetic liability may underlie the association between cannabis exposure and thinner right entorhinal cortex. Prospective longitudinal studies are necessary to definitively disentangle the cause–effect relationships of cannabis use.

Introduction

Worldwide, cannabis remains one of the most widely used illicit drugs (United Nations Office on Drugs & Crime, 2016) which is associated with a number of adverse mental and psychosocial outcomes (Moore *et al.* 2007; Crippa *et al.* 2009; Meier *et al.* 2012; Marconi *et al.* 2016; Patel *et al.* 2016; Schoeler *et al.* 2016*a*, *b*, *c*, *d*, *e*). Hence, effects of cannabis use on brain structure and function that may underpin its effects on cognition and behaviour are of particular interest, more so in light of the legislative changes governing its use, for example in the USA (Volkow *et al.* 2016). The residual cognitive effects of cannabis use have been most robustly observed in memory (Grant *et al.* 2003) and its various sub-domains (Schoeler *et al.* 2016*a*) suggesting that the medial temporal lobe (MTL) structures, which play a critical role in learning and memory (Eichenbaum *et al.* 2007), are of particular interest in terms of the effects of cannabis use on their structure and function. Pharmacological challenge studies offering greater experimental control consistently show acute effects of cannabinoids on MTL function using fMRI (Borgwardt *et al.* 2008; Phan *et al.* 2008; Bhattacharyya *et al.* 2009; Bhattacharyya *et al.* 2010; Bhattacharyya *et al.* 2012*a*; *b*; Bossong *et al.* 2013; Batalla *et al.* 2014; Bossong *et al.* 2014; Bhattacharyya *et al.* 2015*a*; *b*; Bhattacharyya *et al.* 2017; Bhattacharyya *et al.* 2018). However, studies investigating the long-term effects of cannabis use on brain structure and function have been less consistent in terms of effects on MTL structure and function [reviewed here (Batalla *et al.* 2013; Lorenzetti *et al.* 2014)], potentially because of modest sample sizes, differing degrees of cannabis exposure, genetic differences between participants and potential differences in strains of cannabis used by them (Bhattacharyya *et al.* 2010; Bhattacharyya *et al.* 2012*a*; Bhattacharyya *et al.* 2014; Bhattacharyya *et al.* 2015*b*). Furthermore, available evidence from cross-sectional studies do not help disentangle whether group differences in brain structure and function are a result of cannabis use or alternatively may predispose to cannabis use. In the absence of ethically impermissible randomised controlled studies involving long-term experimental treatment with cannabis or placebo or logistically complex longitudinal study designs with multipoint assessment over the life course (Schoeler *et al.* 2016*e*), cross-sectional observational studies using sibling or twin data offer the best possibility of disentangling cause and effect

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relationships. Using this approach Pagliaccio *et al.* (2015) have recently shown that cannabis exposure was associated with smaller left amygdala volume, which was attributable to shared predispositional factors rather than being a consequence of cannabis use. In another large study, adolescent cannabis use was associated with lower mean cortical thickness (across the entire cortex) in males with a high genetic risk for schizophrenia (French *et al.* 2015) with the largest differences between those exposed and those unexposed to cannabis being in brain regions with high cannabinoid receptor expression, e.g. entorhinal and anterior cingulate cortex. However, conflicting evidence has emerged from smaller studies that have examined the relationship between cannabis exposure and cortical thickness measures especially in the entorhinal cortex (Jacobus *et al.* 2014; Jacobus *et al.* 2015).

Within the MTL structures that play a critical role in mnemonic processing, evidence suggests distinct contributions of different regions of the medial temporal cortex to memory. While the hippocampus plays a critical role in the recall of information along with the parahippocampal cortex, the entorhinal and perirhinal cortices are particularly necessary for familiarity-based recognition (Eichenbaum *et al.* 2007). In particular, the entorhinal and adjacent parahippocampal cortices are thought to be involved in the representation of object-related and spatial information during encoding (Navarro Schroder *et al.* 2015; Schultz *et al.* 2015). The entorhinal and adjacent parahippocampal cortices are of particular interest in terms of the effects of cannabis use as unimodal and polymodal neocortical projections that bring information to the hippocampus for this to be bound into an integrated memory trace, pass through them before they converge on the hippocampus (Munoz & Insausti, 2005; Navarro Schroder *et al.* 2015). Furthermore, the relationship between cannabis expsoure and hippocampal and amygdala structure has already been investigated using the HCP cohort in a couple of other studies. For example, Pagliaccio *et al.* (2015) did not find evidence of an association between cannabis use and hippocampal volume supportive of either causal or predispositional relationship. Another study by Orr and colleagues (Orr *et al.* 2016) has also investigated the parametric relationship between cannabis use measures and white matter intergity as well as volume and shape of cortical and subcortical structures using a larger dataset from the HCP cohort. They showed an association between number of times of cannabis use over lifetime and the shape of amygdala and hippocampus. On the other hand, the precise direction of change in the thickness and surface area of the entorhinal and parahippocampal cortices, in relation to cannabis use and whether such changes are a cause or consequence of cannabis use is unclear (Jacobus *et al.* 2014; Jacobus *et al.* 2015). Hence, the primary objective of the present study was to employ the sibling-pair design using data collected as part of the Human Connectome Project (HCP) to investigate the nature of the relationship between cannabis use and thickness and surface area of the entorhinal and parahippocampal cortices. While cortical volume alterations have often been the focus of investigations in the context of cannabis use (reviewed in Batalla *et al.* 2013)and (Lorenzetti *et al.* 2016), there has been growing acceptance that cortical volume is a composite measure that is principally determined by two dimensions of the cortical sheet, i.e. its surface area and thickness and focus on the cortical volume measure alone can obscure information about these two distinct determinants of cortical volume (Im *et al.* 2008; Raznahan *et al.* 2011; Tamnes *et al.* 2017) Specific focus on cortical thickness and surface area is particularly important as they capture the effects of distinct developmental, evolutionary, genetic and cellular processes (Panizzon *et al.* 2009; Raznahan *et al.* 2011; Chen *et al.* 2013; Geschwind & Rakic, 2013; Storsve *et al.* 2014; Wierenga *et al.* 2014; Lyall *et al.* 2015; Tamnes *et al.* 2017) as well as experiences (Sirevaag & Greenough, 1988; Park *et al.* 2009; French *et al.* 2015). Given the organisation of the cerebral cortex into columnar functional units (Mountcastle, 1997), it is thought that surface area of the cortex is influenced by the number of functional columns, while cortical thickness is influenced by the number of cells in these columns (Rakic, 1988, 1995, 2007). In light of previous evidence (summarised earlier), we hypothesised that changes in cortical thickness and surface area in the entorhinal and parahippocampal cortices would reflect a causal effect of cannabis use on these structures rather than reflecting shared predisposition. Specifically, we hypothesised that cannabis-exposed siblings from gender-matched sibling pairs discordant for cannabis exposure would have significantly thinner entorhinal and parahippocampal cortices and smaller surface area of these regions compared with siblings not exposed to cannabis from the same gender-matched sibling pairs discordant for cannabis exposure. We also hypothesised that the cortical thickness and surface area of these regions from both groups of siblings (i.e. cannabis-exposed and non-exposed) from sibling pairs discordant for cannabis exposure would not be significantly different from sibling pairs who were both exposed to or not exposed to cannabis.

Materials and methods

Participants

All data were obtained from the S900 release (December, 2015) of data collected as part of the HCP (https://db.humanconnectome.org/), which plans to recruit 1200 twins and non-twin siblings excluding individuals whose siblings have neuropsychiatric, neurologic or severe neurodevelopmental disorders, but including persons with history of alcohol or recreational substance use who did not need inpatient treatment for ⩾2 days or specialist treatment [for detailed exclusion/inclusion criteria please see (Van Essen *et al.* 2012)]. For the present analyses, participants were excluded if pertinent interview/questionnaire/neuroimaging data were missing or where there was discrepancy between self-report information and related test result (e.g. positive urine test result for tetrahydrocannabinol with negative self-report) or in the absence of a gender-matched sibling. HCP data collection and public release were approved by the Institutional Review Board (IRB) of University of Washington in St. Louis (IRB # 201204036; Title: 'Mapping the Human Connectome: Structure, Function, and Heritability').

Information on Cannabis use

Cannabis exposure was assessed using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) (Bucholz *et al.* 1994). Lifetime exposure to cannabis was coded as a dichotomous categorical variable (1 = yes, 0 = no). Ordinal coding schemes were used to represent the reported age of first use of cannabis and information on frequency of lifetime cannabis use (defined as total number of times of reported cannabis use over lifetime). HCP codes for these two measures were as follows: age of first use (⩽14 year = 1; 15–17 year = 2; 18–20 year = 3; ⩾21 year = 4) and frequency (never = 0; 1–5 = 1; 6–10 = 2; 11–25 = 3; 26–50 = 3; 51–100 = 3; 101–999 = 4; 1000–2000 = 5; >2000 = 5).

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Neuro-anatomical data

T1-weighted brain images were acquired using a customised 3 T Siemens Skyra scanner employing a 3D MPRAGE sequence with the following pulse-sequence parameters: FOV = 224 mm, matrix size = 320, number of sagittal slices = 256, voxel dimension = 0.7 mm isotropic, TR = 2400 ms, TE = 2.14 ms, T1 = 1000 ms, FA = 8 degree, BW = 210 Hz per pixel, Echo Spacing = 7.6 ms, GRAPPA factor = 2. MR gradient nonlinearity-induced distortions of the individual T1-weighted images were corrected using FreeSurfer's package (gradient_nonlin_unwarp) (Fischl, 2012). T1-weighted images were aligned by rigid registration (degree of freedom = 6) using FMRIB's Linear Image Registration Tool (FLIRT) from FMRIB Software Library (FSL) (Jenkinson *et al.* 2012) followed by FNIRT (FMRIB's Nonlinear Image Registration Tool) based non-linear registration. After intensity normalisation, images were processed using the appropriate FreeSurfer pipeline (recon-all) to estimate cortical thickness and surface area of the regions of interest (left and right parahippocampal gyri and left and right entorhinal cortices). Brain regions were defined as per Desikan–Killiany atlas (Desikan *et al.* 2006). MR image acquisition and image processing methods are detailed in (Glasser *et al.* 2013).

Causal, graded liability and predisposition hypotheses

Gender-matched sibling pairs were classified into four groups based on their reported lifetime cannabis use. Sibling pairs who were both unexposed to cannabis were assigned to the '*Concordant Unexposed*' (*CU*) group. *Discordant Exposed* (*DE*) and *Discordant Unexposed* (*DU*) groups included those members of the sibling pairs discordant for cannabis exposure who were exposed to or were not exposed to cannabis, respectively. Where both members of sibling pairs were exposed to cannabis, they were assigned to the *Concordant Exposed* (CE) group.

The *Causal Hypothesis* tested whether cannabis exposure caused changes in cortical thickness or surface area. As siblings reared together share 50% of their genes and a similar rearing environment, any observed differences in brain structure between *Discordant Unexposed* and *Discordant Exposed* groups were likely to provide preliminary support for the causal hypothesis, which would need confirmation by comparing monozygotic twin pairs discordant for cannabis use. The *Graded liability* and *Predisposition* hypotheses tested whether shared genetic or familial factors predispose to both cannabis use and changes in cortical thickness or surface area. To test the *Graded liability* hypothesis, *Discordant Unexposed* and *Discordant Exposed* groups were separately compared with the *Concordant Exposed* group. Significant differences would imply that the *Concordant Exposed* sibling pairs were at altered liability compared with the discordant pairs, as both siblings were exposed to cannabis in the *Concordant Exposed group* compared with only one member of the sibling pairs in *Discordant* group. To test the *Predisposition* hypothesis, *Concordant Unexposed* group was compared with all the other three groups to test whether the differences in neuro-anatomical measures and cannabis use were related to a shared predisposition, but that liability did not differ on the basis of concordance or discordance for cannabis exposure.

Following extreme-outlier removal (Tukey, 1977) there were $N = 152$, $N = 51$, $N = 51$ and $N = 174$ siblings in the *Concordant Unexposed*, *Discordant Unexposed*, *Discordant Exposed* and *Concordant Exposed* groups, respectively, constituting total 214 gender-matched sibling pairs (84 monozygotic, 69 dizygotic and 61 non-twin sibling pairs). A total of 214 siblings have reported exposure to cannabis out of total 404 siblings included in this study. Further details about these sibling groups are available in online Supplementary Table S1.

Potential confounds

Gender (Lv *et al.* 2010), ethnicity, zygosity, age (Thambisetty *et al.* 2010), height, weight, total cortical surface area, average cortical thickness, total household income, picture vocabulary measure (as a proxy of IQ) (Menary *et al.* 2013), handedness (Li *et al.* 2015), NEO Five Factor Inventory (NEO-FFI) personality measures (Wright *et al.* 2006) (conscientiousness, extroversion/introversion, neuroticism, openness, agreeableness), delayed discounting (impulsivity/self-regulation) (Schilling *et al.* 2013), depressive symptoms (Lev-Ran *et al.* 2014), childhood conduct problem (Hyatt *et al.* 2012) and alcohol (Wagner & Anthony, 2002) (drinks/ day), nicotine (Wagner & Anthony, 2002; Kuhn *et al.* 2010) (cigarettes smoked/day) and other illicit drug (Wagner & Anthony, 2002) (number of times used during lifetime) use were considered as confounders (additional details in online Supplementary Information).

Potential confounding variables were compared between the groups using Fisher's exact test for categorical and unpaired *t* tests for other variables and those significantly ($p < 0.05$) different between the groups were included as confounders for group comparisons of neuroanatomical measures using the Permutation Analysis of Linear Models (PALM) tool (Winkler *et al.* 2014). Hypothesis-specific confounders that we controlled for during the comparison of means of cortical thickness and surface area are listed here: *Causal Hypothesis* (use of alcoholic drinks, cigarette use, illicit drug use), *Graded liability hypothesis* (Zygosity, Ethnicity, Delay-discounting, use of alcoholic drinks, cigarette use, illicit drug use) and *Predisposition hypothesis* (Zygosity, Ethnicity, Delay-discounting, use of alcoholic drinks, cigarette use, illicit drug use). As average whole brain cortical thickness was also significantly ($p < 0.05$) different between the groups, the average whole brain cortical thickness variable was entered as an additional confounder while comparing cortical thickness between the groups. The following confounds were significantly ($p < 0.05$) associated with cortical thickness and surface area: *Cortical thickness* (Age, Zygosity, Ethnicity, Picture vocabulary, Delay-discounting, use of alcoholic drinks, cigarette use, illicit drug use, average cortical thickness); *Surface area* (Age, Zygosity, Ethnicity, Picture vocabulary, Delay-discounting, use of alcoholic drinks, cigarette use, illicit drug use, total cortical surface area). Hence, we have controlled for these confounds while computing the correlation between cannabis use variables and neuro-anatomical data.

Statistical analysis

Frequency of cannabis use and age of onset of cannabis use measures were compared between the disconcordant cannabis-exposed siblings and concordant cannabis-unexposed sibling groups using unpaired *t* tests. Permutation tests were performed to assess the mean difference in neuro-anatomical measures between the groups after controlling for the potential confounding variables under the General Linear Model (GLM) framework using the PALM tool (Winkler *et al.* 2014) of FSL. During the permutation tests, two permutation blocks were defined according to

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the two groups of subjects and 20 000 random sign-flippings were used (Winkler *et al.* 2014). Spearman's partial rank correlation between neuro-anatomical and cannabis use measures in the cannabis-exposed individuals ($N = 214$) were computed after controlling for potential confound variables. Cannabis use measures between the siblings of concordant cannabis-exposed sibling pairs were correlated using Spearman's rank correlation.

As a preliminary test of the *Causal* hypothesis that cannabis use was causally associated with aletrations in the thickness and surface area of entorhinal and parahippocampal cortices, we compared the *Discordant Unexposed* and *Discordant Exposed* groups. As these groups were not significantly different, we did not carry out a confirmatory test for the causal hypothesis by comparing monozygotic twin pairs discordant for cannabis use. In order to test the *Graded liability* hypothesis, we compared *Discordant Unexposed* and *Discordant Exposed* groups separately to the *Concordant Exposed* group. Finally, we compared the *Concordant Unexposed* group with all the other three groups to test the *Predisposition* hypothesis.

Estimation of heritability and shared additive genetic effects

Heritability estimation was carried out for those neuroanatomical measures that satisfied tests for either causal, predisoposition or liability hypotheses as being related to cannabis use and included average cortical thickness, total cortical surface area and frequency of cannabis use and cortical thickness/surface area of the specific brain region of all sibling pairs. Phenotypic correlation (ρ_p) between the neuroanatomical measure and the frequency of cannabis use was estimated and decomposed into shared additive genetic (ρ_g) and individual environmental (ρ_e) factors using the bi-variate polygenic model (Blangero *et al.* 2013). We assumed that siblings with identical biological parents lived within a common environment. We employed the SOLAR (Sequential Oligogenic Linkage Analysis Routine)-Eclipse software (http://solar-eclipse-genetics.org/) for heritability estimations and bi-variate polygenic model-based analyses. Gender, age, ethnicity, total household income, personality measures, picture vocabulary test performance score and delayed discounting performance scores were controlled for during heritability analyses. Average cortical thickness and total cortical surface area were additionally controlled for while estimating heritability of regional cortical thickness and surface area, respectively. Heritability of the frequency of cannabis use was estimated after controlling for the use of alcohol, nicotine and other illicit drugs.

Results

Characteristics of the participants

Cannabis exposed individuals were significantly more open to experiences ($p = 0.0028$) than unexposed individuals (as indexed using NEO-FFI Openness) (Table 1). There was a significant association between cannabis exposure and race, with non-white individuals having 1.75 times greater odds of being exposed to cannabis than those from the white race ($p = 0.01$). Cannabis exposed individuals had 16.94 times greater odds of alcohol exposure ($p < 0.0003$), 6 times greater odds of cigarette smoking ($p < 10^{-16}$) and 21.22 times greater odds of exposure to other illicit drugs ($p < 10^{-18}$) than those not exposed to cannabis. Among the cannabis users, 49.53% reported onset of cannabis exposure under the age of 18 and 16.36% satisfied SSAGA criteria of cannabis dependence. The 31.31% of cannabis-exposed individuals use cannabis more than 100 times during their lifetime (Table 1).

Cannabis exposures in concordant and discordant siblings

Cannabis use started significantly ($p = 0.0067$) earlier in sibling-pairs who were both exposed to cannabis that those who were not (Fig. 1*a*). However, there was no significant difference between the two groups in terms of mean cannabis use frequency (Fig. 1*b*). Age of first use of cannabis was significantly correlated ($\rho = 0.49$, $p = 2.8 \times 10^{-6}$) between the sibling pairs of the concordant exposed groups. Frequency of cannabis use was also significantly ($\rho = 0.41$, $p = 1 \times 10^{-4}$) correlated between these sibling pairs.

Cortical thickness

Cortical thickness of the right entorhinal cortex was significantly reduced [false discovery rate (FDR)-corrected, $q < 0.05$] (Fig. 2*a*, Table 2) in *Concordant Exposed* siblings compared to both *Discordant Unexposed* and *Discordant Exposed* groups supporting the graded liability hypothesis. When sibling-pairs belonging to the *Discordant Exposed* group were investigated, the right entorhinal cortex thickness of the siblings exposed to cannabis was not significantly different from their *Discordant Unexposed* siblings. However, when both siblings were exposed to cannabis, as in the case of those belonging to the *Concordant Exposed* group, their entorhinal cortex was thinner compared to siblings belonging to *Discordant Exposed* groups. Together, these results supported the graded liability hypothesis suggesting that shared genetic liability or rearing environment increase the liability for both reduced thickness of the right entorhinal cortex and cannabis use or that reduced thickness of the right entorhinal cortex may increase the liability to cannabis use in a graded manner, such that the risk was greater in sibling pairs concordantly exposed to cannabis use compared to when only one of the siblings from the sibling pairs was unexposed (discordant pairs).

However, there was no evidence in support of the causal and predisposition hypotheses. Age of first use of cannabis was significantly ($\rho = 0.14$, $p = 0.022$) correlated with the right entorhinal cortex thickness in the cannabis exposed participants. Such a relationship was not observed between cannabis use parameters and cortical thickness for any of the other brain regions considered ($p > 0.05$).

Surface area

Mean surface area of the right entorhinal cortex of the *Discordant Exposed* siblings was significantly (FDR-corrected, $q < 0.05$) smaller than their *Discordant Unexposed* siblings (Fig. 2*b*), supporting the causal hypothesis that reduced right entorhinal cortex surface area was an effect of cannabis use. However, there was no evidence in support of the graded liability and predisposition hypotheses. Frequency of cannabis use was significantly ($\rho = -0.13$, $p = 0.020$) correlated with surface of the right entorhinal cortex in the cannabis exposed participants. Correlation between cannabis use parameters and cortical surface area of other brain regions tested were not significant ($p > 0.05$).

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Table 1. Characteristics of the 404 siblings.

Sample characteristics	Cannabis unexposed ($N = 190$) Mean (s.d.)	Cannabis exposed ($N = 214$) Mean (s.d.)	p value
Age (years)	29.19 (3.59)	29.14 (3.35)	0.86
Handedness	63.31 (48.56)	68.39 (41.85)	0.26
Delay discounting (AUC)	0.26 (0.21)	0.23 (0.19)	0.17
Age adjusted picture vocabulary	107.58 (14.52)	107.02 (15.30)	0.71
Total household income	5.19 (2.13)	5.10 (2.13)	0.65
NEO-FFI Openness	26.65 (5.54)	28.45 (6.35)	0.0028
NEO-FFI Contentiousness	34.86 (6.00)	34.48 (6.12)	0.53
NEO-FFI Neuroticism	16.47 (6.34)	15.79 (6.96)	0.31
NEO-FFI Extraversion	30.15 (6.40)	31.28 (5.98)	0.07
NEO-FFI Agreeableness	32.73 (5.25)	32.00 (4.76)	0.15
Childhood conduct problem (%)	36.32	42.06	0.26
Depression history (%)	15.79	22.43	0.10
Male (%)	35.26	40.65	0.30
White (%)	77.89	66.82	0.01
Twin (%)	80.00	71.5	0.06
Monozygotic twin (%)	48.21	35.51	0.01
Never smoked cigarettes (%)	75.26	33.65	$<10^{-16}$
Never used illicit drugs (%)	97.37	63.55	$<10^{-18}$
Alcoholic drinks/day >2			
(during heaviest 12 months period)	40.00	74.30	<0.0003
Age of onset (Cannabis use) <18 years	–	49.53	
Lifetime cannabis use > 100 times	–	31.31	
Cannabis dependence (SSAGA criteria)	–	16.36	

AUC, area under curve; NEO-FFI, NEO five factor inventory; SSAGA, semi-structured assessment for the genetics of alcoholism
Group differences of the first 10 variables were tested using unpaired t tests. For subsequent nine variables, we have used Fisher's exact tests

Heritability analysis and genetic and environmental contributions to phenotypic measures

Total cortical surface area, average cortical thickness, right entorhinal cortex thickness and surface area were significantly heritable ($p < 10^{-6}$; Table 3). Frequency of cannabis use was also significantly heritable (0.45 ± 0.08, $p < 10^{-6}$). There was significant phenotypic ($\rho_p = -0.10$; $p = 0.02$) correlation between right entorhinal cortex thickness and frequency of cannabis use, which was a result of significant shared additive genetic ($\rho_g = -0.19 \pm 0.08$; $p = 0.02$) but not unique environmental ($\rho_e = 0.05 \pm 0.09$; $p = 0.53$) correlation. Phenotypic, genetic and individual environmental correlations between surface area of the right entorhinal

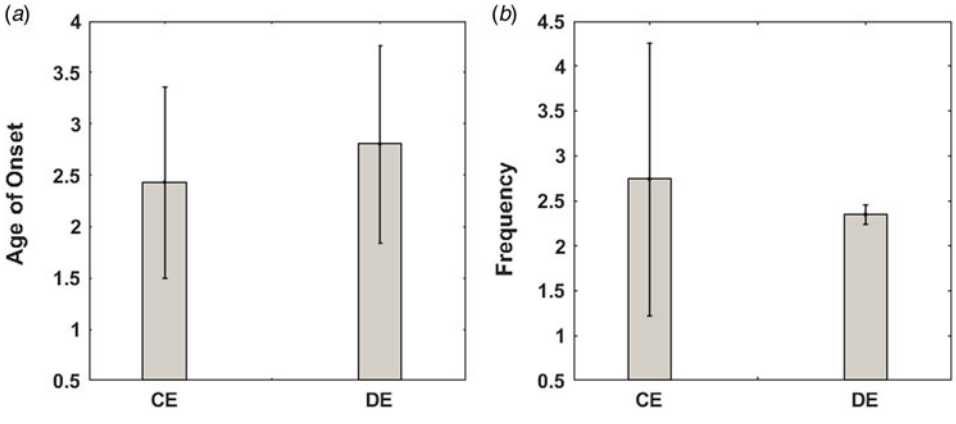


Fig. 1. Mean (s.d.) ordinal values corresponding to the age of onset of cannabis use and the frequency of lifetime cannabis use of concordant exposed (CE) and Discordant Exposed (DE) groups have been displayed in (*a*) and (*b*), respectively.

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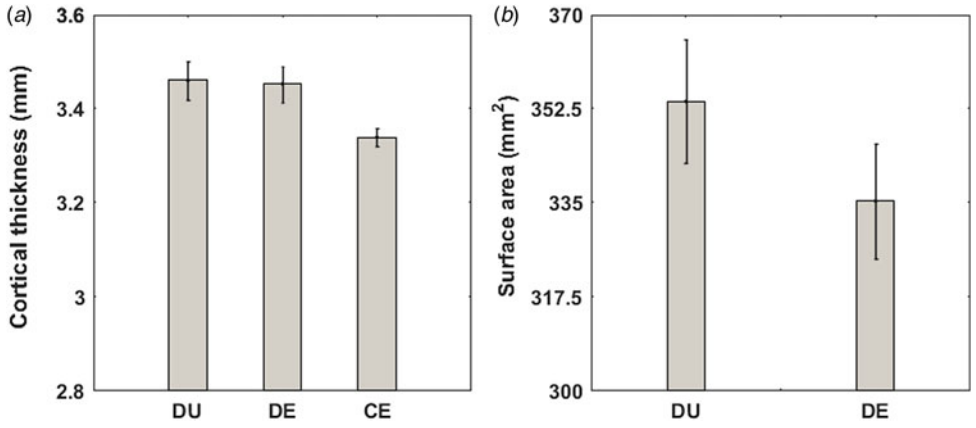


Fig. 2. Mean (S.E.) values of the cortical thickness of the right entorhinal cortex related to the graded liability hypothesis have been displayed in (*a*). Mean (S.E.) values of the surface area of the right entorhinal cortex related to the causal hypothesis have been displayed in (*b*). DU, DE and CE refer to the Discordant Unexposed, Discordant Exposed and Concordant Exposed sibling groups, respectively.

cortex and frequency of cannabis use were not significant ($p > 0.05$). Common rearing environment did not contribute significantly ($p > 0.05$) to these measures.

Discussion

In the present study, we investigated the nature of the relationship between cannabis use and cortical thickness and surface area of

Table 2. Results of PALM-based statistical comparisons of neuroanatomical measures between the sibling groups

	p values (cortical thickness)			
Sibling groups	Left Entorhinal	Left Parahippocampal	Right Entorhinal	Right Parahippocampal
Causal hypothesis				
DU *v.* DE	0.72	0.72	0.82	0.82
Graded liability hypothesis				
DU *v.* CE	0.02	0.82	0.0001*	0.91
DE *v.* CE	0.19	0.49	0.003*	0.35
Predisposition hypothesis				
CU *v.* CE	0.32	0.59	0.0002*	0.79
CU *v.* DE	0.89	0.64	0.80	0.84
CU *v.* DU	0.69	0.42	0.68	0.71
	p values (surface area)			
	Left Entorhinal	Left Parahippocampal	Right Entorhinal	Right Parahippocampal
Causal hypothesis				
DU *v.* DE	0.47	0.93	0.006*	0.04
Graded liability hypothesis				
DU *v.* CE	0.69	0.66	0.49	0.20
DE *v.* CE	0.62	0.38	0.93	0.79
Predisposition hypothesis				
CU *v.* CE	0.01	0.03	0.09	0.003*
CU *v.* DE	0.09	0.38	0.003*	0.02
CU *v.* DU	0.06	0.08	0.20	0.10

DU, discordant unexposed; DE, discordant exposed; CE, concordant exposed; CU, concordant unexposed
*FDR (false discovery rate)-corrected, $q < 0.05$

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Table 3. Results of SOLAR analysis

SOLAR estimates	Average cortical thickness	Total cortical surface area	Right entorhinal thickness	Right entorhinal surface area
Heritability (S.E.)	0.83 (0.03)	0.92 (0.1)	0.47(0.06)	0.53 (0.06)
p	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$	$<0^{-6}$
Phenotypic correlation	−0.15	0.06	−0.10	−0.08
p	0.002	0.20	0.02	0.10
Genetic correlation (S.E.)	−0.21 (0.07)	−0.04 (0.09)	−0.19 (0.08)	−0.11 (0.13)
p	0.004	0.66	0.02	0.41
Environmental correlation (S.E.)	0.02 (0.10)	−0.18 (0.11)	0.05 (0.09)	−0.05 (0.09)
p	0.85	0.11	0.53	0.56

the entorhinal and parahippocampal cortices using the largest sibling-pair sample investigated to date. As predicted, we found that reduced thickness and surface of the right entorhinal cortex were associated with cannabis use in this sample, which persisted even after correcting for a number of potential confounders. Such an association was not found in the entorhinal cortex on the left side or in the parahippocampal cortex bilaterally. Furthermore, contrary to our expectations, we found that the association between reduced right entorhinal cortex thickness and cannabis use reflected the effect of a shared factor (e.g. genetic or environmental) that is likely to have increased the liability to both cannabis use and reduced cortical thickness in a graded manner, such that the risk was greater in sibling pairs concordantly exposed to cannabis use compared to when one of the siblings from the sibling pairs was unexposed (discordant pairs). Whilst this may suggest that this liability factor may have resulted in reduced thickness of the right entorhinal cortex, which in turn may have increased the risk of cannabis use, it is also possible that this liability factor may have increased the risk of both cannabis use and of thinner entorhinal cortex. Additional analyses suggested that this association was likely a result of shared genetic liability as opposed to shared or unique environmental influence, the latter providing further support against a causal effect of cannabis use on right entorhinal cortical thickness.

These results are consistent with previous evidence of association between entorhinal cortex thickness and cannabis use (Jacobus *et al.* 2014; French *et al.* 2015; Jacobus *et al.* 2015) but extend it by suggesting that shared genetic liability may underlie the association between cannabis exposure and thinner right entorhinal cortex. However, whether genetic liability results in thinner right entorhinal cortex which in turn increases the risk of cannabis use behaviour remains to be tested. How thinner entorhinal cortex may increase the liability of cannabis use is unclear and may reflect the role of stress/ emotional dysregulation in drug use behaviour (Koob & Volkow, 2016; Volkow *et al.* 2017), given its connectivity with regions implicated in emotional processing and reward-related learning (Oades & Halliday, 1987; Sugase-Miyamoto & Richmond, 2007; Schultz *et al.* 2015; Tomas Pereira *et al.* 2016). Furthermore, evidence from preclinical research suggests that the entorhinal cortex is critical for learning the motivational significance of stimuli (Liu *et al.* 2000), as well as for using memory of previous rewards to estimate the value of future rewards (Clark *et al.* 2012) and reciprocal connections of the entorhinal cortex with the orbital prefrontal cortex play a critical role in evaluating and estimating the expected value of rewards suggesting a role in modulating behaviour in response to expected reward value (Clark *et al.* 2013). Therefore, one may speculate that thinner entorhinal cortex may increase the liability of cannabis use in man by interfering with its critical role in helping modulate of behaviour in light of expected reward value through impairment in the estimation and learning of the motivational significance of drugs and evaluation of drug use in light of past adverse experience. However, the present data does not allow us to test these hypotheses. It is also worth noting in this context that, orbitofrontal cortex sulcogyral pattern, a structural variation that is established early on in life and thought to remain relatively stable thereafter, has been linked to a predisposition for greater levels of cannabis use over lifetime, in the absence of a contribution to the risk of becoming a cannabis user (Chye *et al.* 2017).

These results are to be considered in light of certain limitations of the present sample as well as the study design. Firstly, accurate characterisation of temporal sequence of exposure and cause/ effect through multi-point brain imaging and exposure data collection within prospective longitudinal designs are necessary to definitively disentangle cause and effect relationships. This was not possible with the present data and need to be carried out in future studies. Limited by the very nature of the present cohort, information on cannabis use was not as detailed as would have been necessary to systematically examine dose-response relationships, also important for evaluating the nature of such associations. In particular, we were not able to differentiate between cannabis users who quit from those who continued to use. Future studies need to examine these groups separately to investigate whether the neural correlates of liability to use cannabis may differ between those who continue to use compared to those who stop. It is also worth noting that we focused on specific brain regions of interest, namely the entorhinal and parahippocampal cortices rather than employ a whole brain analysis approach or consider other brain regions high in cannabinoid receptors. Instead, we employed a hypothesis-driven approach, focusing on brain areas that subserve a cognitive function (i.e. memory) for which there is robust evidence of impairment associated with cannabis use both in experimental and observational studies. This was done to ensure that sample size of the groups tested based on their sibship information, cannabis use and gender leant them adequate power to detect significant differences with confidence, which would not have been feasible had we

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investigated multiple other brain regions. Finally, the present study was also limited by the number of twin pairs with and without exposure of interest and in particular the number of discordant monozygotic twin pairs for whom data was available.

Nevertheless, these results suggest that thinner right entorhinal cortex may be associated with genetically mediated liability to cannabis use behaviour and underscore the importance of longitudinal studies to confirm the nature of this association as well as investigate potential cognitive mechanisms that may underlie it. Independent replication of these results as well as characterisation of the cognitive mechanisms that may underlie the association between entorhinal thinning and cannabis use behaviour is necessary for a clear understanding of the precise clinical significance of these results.

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Ethical standard. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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