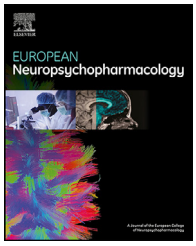
European Neuropsychopharmacology 74 (2023) 47–63



www.elsevier.com/locate/euroneuro

The long-term effects of adolescent Δ9-tetrahydrocannabinol on brain structure and function assessed through neuroimaging techniques in male and female rats

Javier Orihuel[a], Roberto Capellán[a], Marta Casquero-Veiga[b,c,e], María Luisa Soto-Montenegro[b,c,g], Manuel Desco[b,c,d,e], Marta Oteo-Vives[f], Marta Ibáñez-Moragues[f], Natalia Magro-Calvo[f], Víctor M. Luján[f], Miguel Ángel Morcillo[f], Emilio Ambrosio[a], Alejandro Higuera-Matas[a,*]

[a] *Department of Psychobiology, School of Psychology, National University for Distance Learning (UNED), C/Juan del Rosal 10, Madrid, Spain*
[b] *Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain*
[c] *CIBER de Salud Mental (CIBERSAM), Madrid, Spain*
[d] *Departamento de Bioingeniería e Ingeniería Aeroespacial, Universidad Carlos III de Madrid, Spain*
[e] *Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain*
[f] *CIEMAT - Research Centre for Energy, Environment and Technology, Medical Applications of Ionizing Radiations Unit, Madrid, Spain*
[g] *Grupo de Fisiopatología y Farmacología del Sistema Digestivo de la Universidad Rey Juan Carlos (NEUGUT), Madrid, Spain*

Received 29 December 2022; received in revised form 24 March 2023; accepted 22 May 2023

* Corresponding author.
E-mail address: ahiguera@psi.uned.es (A. Higuera-Matas).

https://doi.org/10.1016/j.euroneuro.2023.05.005

KEYWORDS
Adolescence;
MRI;
PET;
Spectroscopy;
THC

Abstract
Several studies performed on human subjects have examined the effects of adolescent cannabis consumption on brain structure or function using brain imaging techniques. However, the evidence from these studies is usually heterogenous and affected by several confounding variables. Animal models of adolescent cannabinoid exposure may help to overcome these difficulties. In this exploratory study, we aim to increase our understanding of the protracted effects of adolescent Δ^9-tetrahydrocannabinol (THC) in rats of both sexes using magnetic resonance (MR) to obtain volumetric data, assess grey and white matter microstructure with diffusion tensor imaging (DTI) and measure brain metabolites with ^{1}H-MR spectroscopy (MRS); in addition, we studied brain function using positron emission tomography (PET) with 2-deoxy-2-[^{18}F]fluoro-D-glucose as the tracer. THC-exposed rats exhibited volumetric and microstructural alterations in the striatum, globus pallidus, lateral ventricles, thalamus, and septal nuclei in a sex-specific manner. THC administration also reduced fractional anisotropy in several white matter tracts, prominently in rostral sections, while in vivo MRS identified lower levels of cortical choline compounds. THC-treated males had increased metabolism in the cerebellum and olfactory bulb and decreased metabolism in the cingulate cortex. By contrast, THC-treated females showed hypermetabolism in a cluster of voxels comprising the entorhinal piriform cortices and in the cingulate cortex. These results indicate that mild THC exposure during adolescence leaves a lingering mark on brain structure and function in a sex-dependant manner. Some of the changes found here resemble those observed in human studies and highlight the importance of studying sex-specific effects in cannabinoid research.

1. Introduction

Besides alcohol and tobacco, cannabis derivatives are the most widely used drugs, with increasing prevalence and more commonly consumed by adolescents (United Nations Office on Drugs and Crime, 2022).

Apart from the potential problems associated with acute cannabis use and intoxication, long-lasting effects after repeated use are especially worrying and potentially compromising at the social level. Cannabis use disorders remain largely undertreated and, noteworthy, present a high level of comorbidity with psychophysiological alterations and mood disorders (Hasin et al., 2016). Prolonged cannabis use has a facilitating effect on the onset of psychiatric problems, including substance use disorders (Blanco et al., 2016). Remarkably, early onset of cannabis consumption and adolescent use is associated with cognitive impairments, less evident in late-onset and adult users (Gruber et al., 2012), and with structural changes in brain areas responsible for pleasure and reward (Worley, 2019).

Imaging techniques provide an excellent non-invasive way to study the structure and function of the brain. These technologies have already been broadly used to assess reward-related behaviours and to characterise behavioural endophenotypes of drug addiction (Jupp and Dalley, 2014). Brain imaging studies have also extensively addressed the effects of cannabinoids, frequently focusing on adolescent and young adult users and/or the long-term effects of cannabis (Ganzer et al., 2016).

Regarding the long-term effects of cannabis use, white matter abnormalities are typically observed (Hampton et al., 2019). This is particularly relevant since proper white matter integrity is crucial for efficient communication between brain regions, shaping cognitive and behavioural performance (Fjell et al., 2011). A longitudinal study found that cannabis users displayed deviations from the expected fractional anisotropy (FA) signal growth during development; moreover, reduced longitudinal growth in FA and functional impairment correlated with cannabis intake (Becker et al., 2015). Another study found that earlier age of cannabis use onset was associated with lower white matter coherence (Orr et al., 2016). Notably, this latter study also found volumetric alterations within structures of the reward system linked to early age of onset in cannabis use or consumption levels. In addition, cannabis use may have a significant impact on the integrity of white matter fibre tracts in the prefrontal region (Gruber and Yurgelun-Todd, 2005). Other studies have also focused on grey matter changes, showing that cannabis may affect the density, volume, and shape of structures such as the nucleus accumbens, hypothalamus, and amygdala (Gilman et al., 2014). Additional structural and functional imaging studies also provided clear evidence of long-term changes that include structural differences in the brain after prolonged periods of abstinence (9 out of 10 studies) and functional activity (16 out of 17 studies) in the prefrontal and hippocampal areas but also, in the cerebellum (Ganzer et al., 2016). Blest-Hopley et al. (2018) performed a metanalysis of the residual effects of cannabis use in both adolescent and adult brains, excluding common effects following cannabinoid challenge and confirmed the existence of different patterns of brain activity in adult and adolescent cannabis users.

However, the wide variety of research methodologies and distinct demographic characteristics of the samples hinder reaching conclusions. For example, age differences amongst the samples and in the onset ages of cannabis use, premorbid and comorbid variables, the patterns of consumption and the heterogeneity of the periods of abstinence (when addressing non-acute effects), together with the consideration of sex-specific variables, are amongst the

most relevant sources of disparity. Given this situation, preclinical research represents a valuable tool to shed light on some aspects of the impact of adolescent cannabis use on neurobiological changes. Despite their strong translational relevance to human studies, preclinical research leveraging on imaging studies is scarce. Although, some studies have also employed imaging techniques to assess the effects of cannabinoid exposure (Miederer et al., 2017; Nguyen et al., 2012) to our knowledge only four imaging studies have been performed so far that address long-term effects of adolescent cannabinoid exposure. One recent study employed magnetic resonance imaging (MRI) techniques in mice after subjecting them to daily exposure to vaporised cannabis during adolescence to study brain structure and function. Marked sex differences were evident regarding the effects of the cannabis treatment, being females more leant to show changes in fractional anisotropy and apparent diffusion coefficient signal in the forebrain and hindbrain. At the same time, males tended to present functional coupling increases in the thalamus, hypothalamus and brainstem reticular activating system (Coleman et al., 2022). By contrast, another study found no sex differences in the effects of the chronic THC treatment during adolescence in mice, which were short-lived and disappeared after a washout period (Guma et al., 2023) The other two of them were performed by us. They used a fluorodeoxyglucose ([^{18}F]-FDG) positron emission tomography (PET) scan on two similar animal models focused on the long-term effects of periadolescent exposure to cannabinoids (Higuera-Matas et al., 2011, 2008). In these studies, clear sex-dependant outcomes were detected. Still, there are other known protracted outcomes of adolescent cannabis use in humans that have been detected in clinical settings but have not been replicated yet in animal model studies using brain imaging techniques.

Consequently, to gain a deeper understanding of the long-term sex-specific effects of adolescent THC exposure, we performed a series of imaging studies to explore the presence of alterations in adult animals induced by chronic THC treatment during adolescence. It is important to note that this study is exploratory in nature, aiming to identify potential alterations in brain structure and function induced by chronic THC exposure during adolescence in rats. The brain regions of interest were selected on previous extensive research in the field demonstrating that they are affected by cannabinoid exposure in both human and animal studies.

2. Experimental procedures

2.1. Animals

Wistar albino (from Charles-River S.A. Saint-Germain-sur-l'Arbresle, France) rat litters were sex-balanced and culled to a litter size of 10 ± 2 pups per dam between postnatal day -PND-0 and PND1. The animals were weaned at PND22 and placed in different cages of 2 or 3 sibling animals for each experimental group (sex and treatment). In the MRI experiment, male rats weighed 73.67 g on average at the beginning of THC/vehicle treatment (see below) while females weighed 68.87 g on average. In the PET experiment, the average body weight of the females was 73.04 g and the mean body weight of the males was 80.25 g. All animals were maintained at a constant temperature (20±2 °C) under a reverse 12 h/12 h light/dark cycle (lights on at 20:00 h), with free access to food and water (commercial diet for rodents A04/A03; Panlab, Barcelona, Spain), unless otherwise specified at the beginning of some of the experimental procedures. Importantly, all efforts were made to minimise the pain and discomfort of the experimental animals. All the procedures were conducted following the European Union legislation on the protection of animals used for scientific purposes (2010/63/EU Directive). They were approved by the Ethics Board of the Universidad Nacional de Educación a Distancia and the Centre for Energy, Environmental and Technological Research (CIEMAT).

2.2. Adolescent THC treatment

THC was purchased from THCPharm (Frankfurt, Germany) as resin and dissolved in pure ethanol (Merck). The THC-ethanol solutions were aliquoted into opaque vials filled with nitrogen to avoid oxidation and stored at −20 °C 1-2 days prior to the beginning of the adolescent THC treatment. Pure ethanol was similarly aliquoted and stored. On the treatment days, the final solution was prepared by adding kolliphor (PEG-35 castor oil; Merck) and saline (0.9% NaCl solution; Vitulia, Spain) in a 1:1:18 proportion. Animals were weighed and then received an intraperitoneal injection (2 mL/kg) using a calibrated syringe, which delivered a dose of 3 mg/kg THC to the treatment groups. This THC dose is considered mild and non-aversive, although it can produce neurochemical changes in synaptic plasticity in brain regions involved in reward learning (Mato et al., 2005, 2004). Ethanol concentration in the final solutions was 5%, with each animal receiving a dose of approximately 0.0789 gr/kg, which does not induce significant behavioural effects (Frye and Breese, 1981). The equivalency of this dose in human patterns of consumption would be similar to smoking one or two marihuana cigarettes assuming a THC concentration of around 8% (ElSohly et al., 2016; Walden and Earleywine, 2008). The adolescent chronic THC treatment was performed every other day from PND28 to PND44 in the MRI experiment and from PND38 to PND54 in the PET study (see below). Fig. 1 shows a summary of the experimental timelines of both experiments.

2.3. MRI

At PND80, 12 vehicle (VEH) rats (5 males and 7 females) and 16 THC rats (9 males and 7 females) underwent the MRI and spectroscopy studies at the Instituto de Investigaciones Biomédicas. The MRI experiments were performed using a Bruker Pharmascan system (Bruker Medical Gmbh, Ettlingen, Germany) using a 7.0-T horizontal-bore superconducting magnet equipped with a ^{1}H selective quadrature 40 mm coil and a Bruker gradient insert with a 90 mm diameter (maximum intensity 36 G/cm). All data were acquired using a Hewlett-Packard console running Paravision 5.1 software (Bruker Medical Gmbh) operating on a Linux platform.

Within 5 days of counterbalancing the groups, all the rats were tested. Animals were anaesthetised with a 2% isoflurane-oxygen mixture in an induction chamber, and the flow of anaesthetic gas was constantly regulated to maintain a heart rate of 50 ± 20 bpm. The animals were placed into the centre of the volume radio frequency coil and positioned in the magnet under continuous inhalation anaesthesia via a nose cone. A respiratory sensor connected to a monitoring system (SA Instruments, Stony Brook, NY) was placed under the abdomen to monitor the rate and depth of respiration.

2.3.1. Volumetry

T2-weighted (T2-W) spin-echo anatomical images were acquired with a rapid acquisition with relaxation enhancement (RARE) sequence in axial and coronal orientations applying the following parameters: TR, 3000 ms; TE, 44 ms; RARE factor, 8; *Av*, 3; FOV, 3.5 cm; acquisition matrix, 256 × 256 corresponding to an in-plane

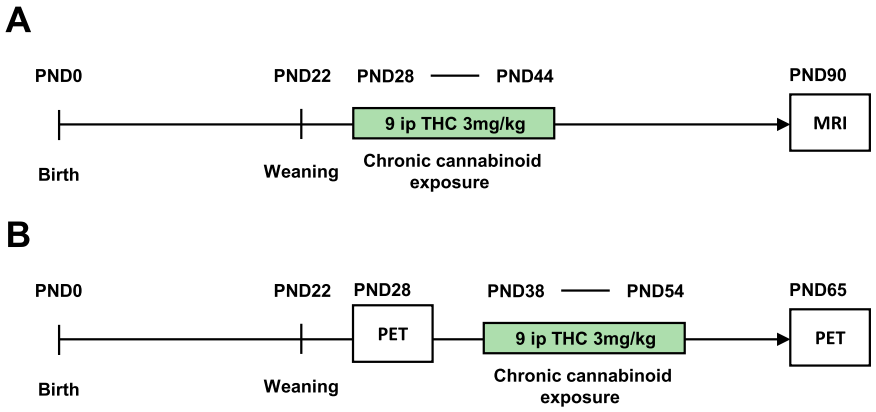


Fig. 1 Experimental timelines. Timelines depicting the experimental manipulations and measurement timepoints in the MRI and PET experiments.

resolution of 136 × 136 μm^2; slice thickness, 1.50 mm which produced a total of 18 slices for axial and 8 for coronal images. Volumetric analyses were made by manually selecting the region of interest (ROI) of each anatomical image and then calculating the area with Image J software. All measurements were obtained blind to the animal's experimental group to avoid bias. For the quantitative analysis, total brain volume and the relative regional area or volume were calculated for the dorsal striatum, nucleus accumbens, hippocampus, cerebral cortex, globus pallidus, thalamus, amygdala, septal nuclei, and cerebellum, using ImageJ. In addition, the volume occupied by each ventricle and the total ventricular volume, relative to the total and regional brain volume, respectively, were also calculated.

2.3.2. DTI

Diffusion-weighted images were acquired with a spin-echo single-shot echo-planar imaging (EPI) pulse sequence using the following parameters: Repetition Time and Echo Time (TR/TE) 3500/40 ms; averages 1; diffusion gradient duration 3.5 ms; diffusion gradient separation 20 ms; gradient directions 7; two b values (100 and 1400s/mm^2); slices thickness 1.5 mm without a gap. All the EPI data were acquired with a single-shot EPI sequence, a 96 × 96 matrix and a zero-filled in k space to construct a 128 × 128 image matrix corresponding to an in-plane resolution of 273 × 273 μm^2. FA, mean diffusivity (MD), trace, eigenvalues, and eigenvector maps were calculated with an in-house software application written in Matlab (R2007a). The values of these indices were extracted using the Image J software in the maps obtained by manually selecting ROIs in each slice and using the corresponding T2 anatomical image and the Paxinos-Watson brain atlas as a reference. Grey matter values of FA and MD were obtained from the cingulate cortex, dorsal striatum, nucleus accumbens, hippocampus, globus pallidus, thalamus and septal nuclei, and the white matter FA signal was obtained from the corpus callosum, internal capsule and hippocampal commissure tracts.

2.3.3. ^{1}H-MRS

After obtaining the T2 images, a ^{1}H MR in vivo spectroscopy study of the cerebral cortex and striatum was performed. The spectroscopy protocol used a Point-Resolved Spatially Spectroscopy, combined with VAriable Power radiofrequency pulses with Optimised Relaxation delays (VAPOUR) water suppression, applying the following parameters: TR 3000 ms; TE 35 ms; *Av* 128; voxel volume 3 mm^3. First and Second-order shims were automatically adjusted using the FASTMP application in a 4 mm^3 voxel. All ^{1}H spectra were automatically analysed using LCModel version 6.2-OR (Stephen Provencher, Oakville, ON; Canada). Statistical analysis was performed (see below) with the concentration values of each metabolite relative to creatine (Cr) + phosphocreatine (PCr) for those with a standard deviation under 20%.

2.4. PET

A total of 24 rats (12 males (VH $n = 6$; THC $n = 6$) and 12 females (VH $n = 6$; THC $n = 6$)) underwent two different PET scans, before and after the chronic THC exposure, at PND28 and again at PND65. One male rat was lost during the imaging procedure resulting in a final group size of 5 VH males. PET-CT studies were performed at the Centre for Energy, Environmental and Technological Research (CIEMAT) in Madrid (Spain), using a small-animal PET-CT apparatus (Argus, SEDECAL, Madrid, Spain). Static PET images were obtained for 45 min 30 min post intravenous administration with 176 ± 37 MBq/kg body weight of 2-deoxy-2-[18F] fluoro-D-glucose ([^{18}F]-FDG). Animals fasted 16 h prior to the injection of the tracer.

Static PET images were obtained for 45 min 30 min post intravenous administration with 176 ± 37 MBq/kg body weight of [^{18}F]-FDG. Briefly, the rats were anaesthetised with 2-3% isoflurane in medical oxygen (1 L/min), and their temperature was maintained at 37 °C using a heating pad during PET acquisition. The PET data obtained was reconstructed using a 2D-OSEM algorithm (16 subsets and 3 iterations) with random and scatter corrections. The PET images underwent pre-processing using a protocol described previously (Casquero-Veiga et al., 2019). Briefly, each PET image was spatially co-registered to a common reference CT scan for each sex by an automatic method based on mutual information (Pascau et al., 2009), then subjected to 9-point scaling in the three spatial directions. The PET intensity values were then normalised to the average uptake in the septum (a structure invariant across treatments). Ten brain ROIs were segmented: cerebral cortex, nucleus accumbens, dorsal striatum, hippocampus, hypothalamus, thalamus, midbrain and cerebellum.

2.5. Statistical analysis

After checking the assumptions of homoscedastic variances and normality, we performed analyses of variance (ANOVAs) to detect statistically significant differences. For MRI data, two-way ANOVAs were performed, and simple effects analysis was used to follow up significant interactions. For PET data, the statistical analysis was a three-way mixed ANOVA, with Sex (male or female) and Treatment (THC or vehicle) as between-subject factors and Time (first PET,

Table 1 Summary of the main results.

	THC Male vs VEH Male		THC Female vs VEH Female
Brain Ventricle Volumetry		↓ lateral ventricles	
	↓ aqueduct		
Grey Matter Volumetry		↓ right globus pallidus ↓globus pallidus ($p = 0.057$)	
			↓dorsal striatum
Grey Matter MD			↓rostral septal nuclei
Grey Matter FA		↓globus pallidus ↓caudal septal nuclei	
	↓rostral dorsal striatum ↑thalamus		
White Matter FA		↓corpus callosum ↓anterior capsule ↓hippocampal commisure ($p = 0.051$)	
^{1}H MR Spectroscopy		↓GPC+PCh in cortex	
PET	↓cingulate cortex ↑olfactory bulb ↑thalamus ↑somatosensory cortex ↑cerebellum		↑cingulate cortex ↑entorhinal-piriform cortex

second PET) as the within-subject factor. Statistical analyses were performed in IBM Statistics 24.0; a p-value of less than 0.05 was considered statistically significant. Effect size statistics (partial eta squared) are also provided where appropriate.

For the PND65 PET scans, we also performed a voxel-based 2-sample *t*-test ($p<0.05$ uncorrected) for each sex with Statistical Parametric Mapping (SPM) software (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/). The PET images were smoothed with a Gaussian kernel 2.5 times the voxel size at full width at a half maximum (FWHM) and masked to exclude extracerebral voxels from the analyses. Only clusters more extensive than 50 adjacent voxels were considered to minimise the effect of type I errors.

3. Results

3.1. MRI

The main differences in the adult rat brain after adolescent THC exposure are summarised in Table 1. Full statistical details are provided in the supplementary tables (Tables S1 to S7).

3.1.1. Volumetry

Brain ventricle volumetry revealed a global reduction in the ventricular space in adult animals treated with THC ($F_{1,23}$=8.961; $p = 0.006$; ηp2=0.280). Upon closer inspection, this global Treatment effect was evident in the lateral ventricles ($F_{1,24}$=6.341; $p = 0.019$; ηp2=0.192). Exposure to THC during adolescence did not significantly impact the third ventricle volume, but there was a smaller aqueduct of Silvius volume in THC males compared to control males. The fourth ventricle presented a clear and significant effect of the Sex in a male bigger than female ($m>f$) pattern ($F_{1,25}$=9.053; $p = 0.006$; ηp2=0.266), but there was no significant Treatment effect (see Fig. 2 and Table S1).

The MRI data showed that exposure to THC during adolescence inducesstructural alterations evident in adult animals. Specifically, there was a significant effect of the Sex*Treatment interaction that resulted in a reduction in the volume of the dorsal striatum in adult females exposed to chronic THC during adolescence compared to control females ($F_{1,25}$=5.783; $p = 0.024$; ηp2=0.19). When the globus pallidus of both hemispheres were analysed globally, they showed a trend towards displaying a smaller size in THC-exposed animals ($F_{1,23}$=4.022; $p = 0.057$; ηp2=0.15), although the effect of Treatment was only significant in the right hemisphere ($F_{1,22}$=4.494; $p = 0.046$; ηp2=0.17) (see Fig. 3). A significant effect of Sex was patent in the total brain ($F_{1,25}$=43.27; $p = 0.000$; ηp2=0.63), cerebral cortex ($F_{1,25}$=5.248; $p = 0.031$; ηp2=0.17), cerebellum ($F_{1,25}$=6.896; $p = 0.015$; ηp2=0.22) and left hippocampus ($F_{1,25}$=5.389; $p = 0.029$; ηp2=0.18) volumes, with a $m>f$ pattern in each of those areas. No differences were detected between males and females in the total areas of the brain slices corrected for body weight or as an effect of Treatment (see Table S2).

3.2. DTI

The DTI analysis revealed a significant Sex*Treatment interaction ($F_{1,17}$=6.364; $p = 0.022$; ηp2=0.272), and simple effect analyses confirmed a reduced FA in the rostral part of the dorsal striatum of male-THC rats compared to male-VEH ($F_{1,20}$=7.057; $p = 0.015$; ηp2=0.261). The thalamic FA signal showed a significant Sex*Treatment interaction ($F_{1,17}$=6.364; $p = 0.022$; ηp2=0.272) in the opposite direction, increased thalamic FA in THC males relative to their controls ($F_{1,20}$=8.144; $p = 0.010$; ηp2=0.289), and compared to THC females ($F_{1,20}$=8.346; $p = 0.009$; ηp2=0.294). The significant Treatment effects suggested a reduced FA for both sexes in the globus pallidus ($F_{1,21}$=4.309; $p = 0.050$; ηp2=0.170) and caudal

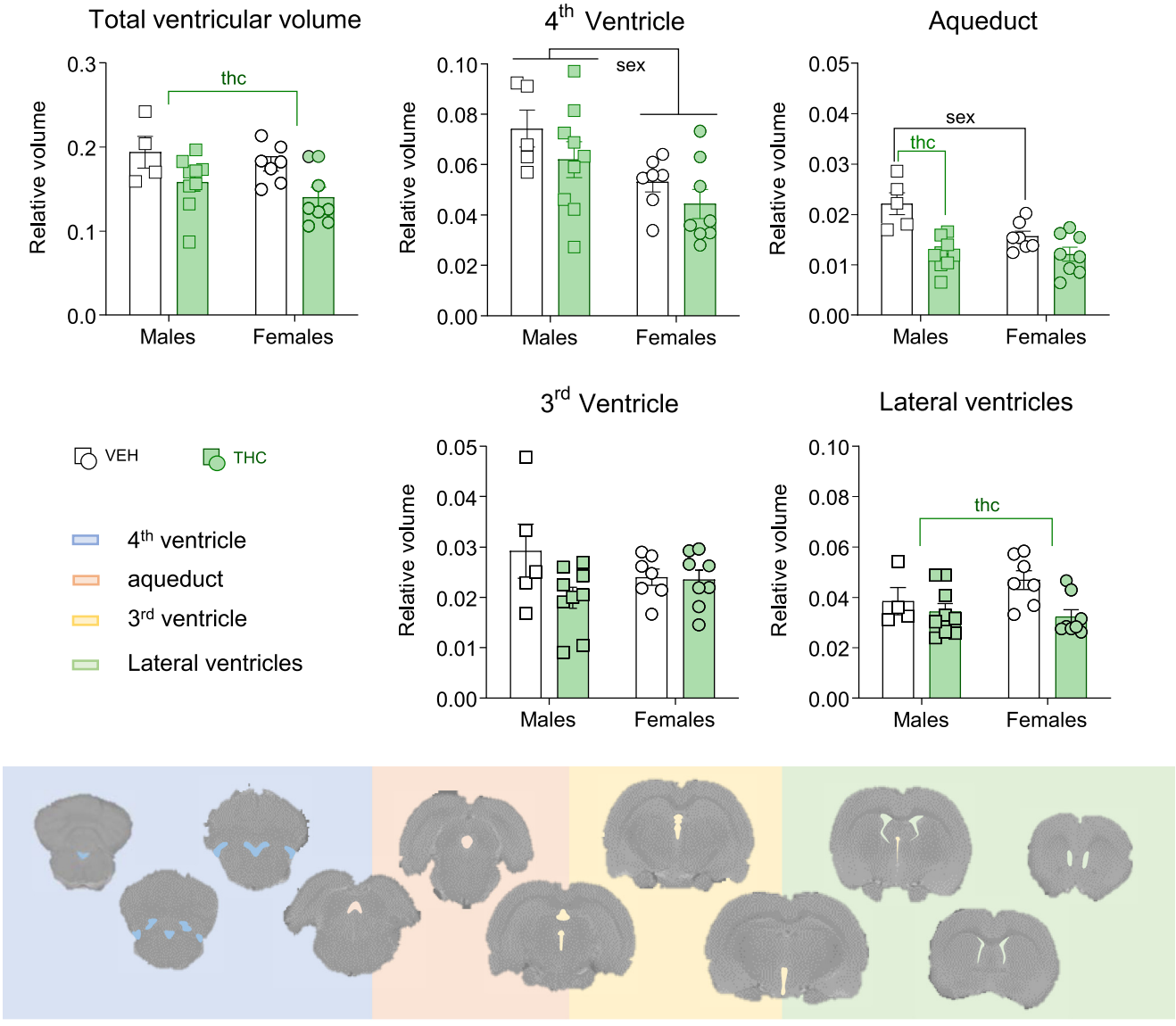


Fig. 2 Brain ventricle volumetry. The graphs show the individual values (dots) and Mean ± SEM (bars). A) The whole ventricular volume decreased in THC-treated animals (F1,23=8.961; $p = 0.006$; ηp2=0.28). In order to explore the source of this effect, we analysed the different sections of the brain ventricular system. B) In the fourth ventricle we only observed a male>female sexual dimorphism (F1,25=9.053; $p = 0.006$; ηp2=0.26). C) A significant Sex x Adolescent Treatment interaction (F1,25= 5.575; $p = 0.026$; ηp2=0.18) appeared in the brain aqueduct, and our follow-up analysis showed a male>female sexual dimorphism in VEH animals (F1,25= 9.598; $p = 0.005$; ηp2=0.27) and significant differences between within the males. More specifically, THC-exposed male rats had a smaller volume (F1,25= 24.51; $p<0.000$; ηp2= 0.49). D) In the third ventricle there was a trend towards a smaller volume in THC animals (F1,25= 3.408; $p = 0.077$; ηp2= 0,12). E) In the lateral ventricles the volume was smaller in THC animals (F1,25= 6.341; $p = 0.019$; ηp2= 0.19). F) The different fill colours represent the ventricle area used to obtain the values represented in each corresponding graph. From caudal (left) to rostral (right): IV ventricle, aqueduct, III ventricle and lateral ventricles. The full results can be seen in Table S1.

septal nuclei ($F_{1,21}$=6.999; $p = 0.015$; ηp2=0.250). Relative to grey matter MD analysis, we detected a significant Sex*Treatment interaction ($H = 9.284$; $p = 0.026$; ηp2=0.299) due to a reduced MD in the rostral section of the septal nuclei in THC-treated females relative to VEH-treated females ($U = 1$; $p = 0.012$; ηp2=0.391); additionally, VEH treated females showed a higher MD compared to VEH treated males ($U = 1$; $p = 0.028$; ηp2=0.345). There were no other significant effects regarding grey matter MD in other areas (See Fig. 3 for a graphical representation and Tables S3 and S4 for additional analyses).

White matter FA analysis showed a significant effect of Treatment in rostral sections, particularly in the corpus callosum ($F_{1,19}$=5.297; $p = 0.034$; ηp2=0.238) and anterior commissure ($F_{1,17}$=5.322; $p = 0.034$; ηp2=0.238), being FA signal weaker in animals of both sexes exposed to THC as adolescents. By contrast, no significant effects were detected in the posterior sections of these tracts or

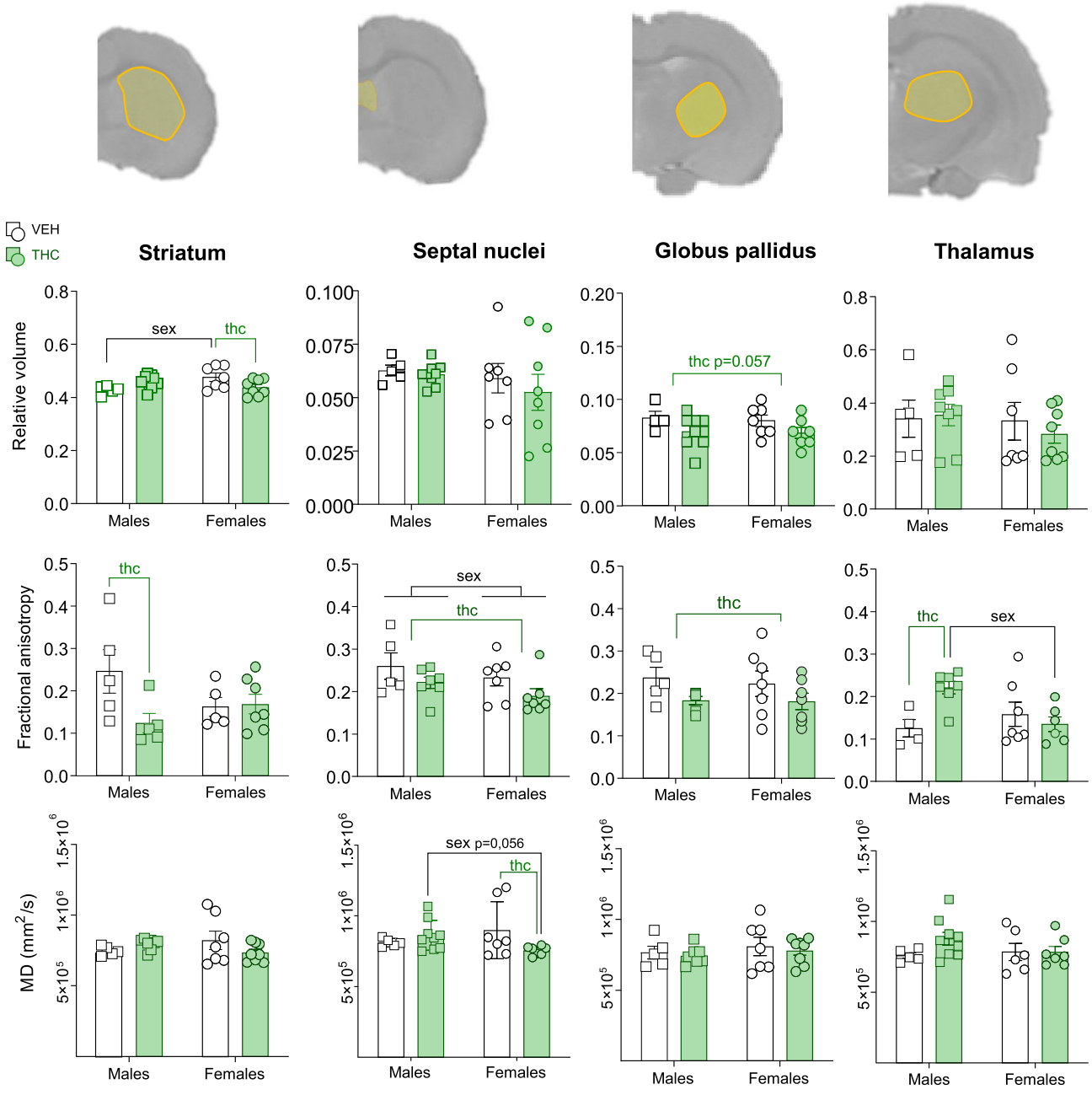


Fig. 3 MRI Grey matter analysis. The most representative effects are depicted. Graphs represent the individual values (dots) and the mean ± SEM (bars). Within each graph, the green lines and "thc" represent a significant effect of the THC treatment (Adolescent Treatment), while the black lines and "sex" represent statistically significant effects of the factor Sex. The columns from left to right represent the volumetric analysis, calculated as the relative volume of the structure within the sections it was contained in, the DTI values obtained for mean diffusivity (MD) and fractional anisotropy in each of the four different structures depicted in each row; from top to bottom: dorsal striatum, septal nuclei, globus pallidus and thalamus. See Tables S2-S4 for more details concerning statistical test results. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the internal capsule. Moreover, the FA signal in the hippocampal commissure showed a significant Sex*Treatment interaction ($F_{1,18}$=5.693; p = 0.03; ηp2=0.235), and subsequent simple effect analysis revealed a dampened FA signal in THC-treated females compared to VEH-treated females ($F_{1,18}$=5.693; p = 0.028; ηp2=0.24) (see Fig. 4 and Table S5).

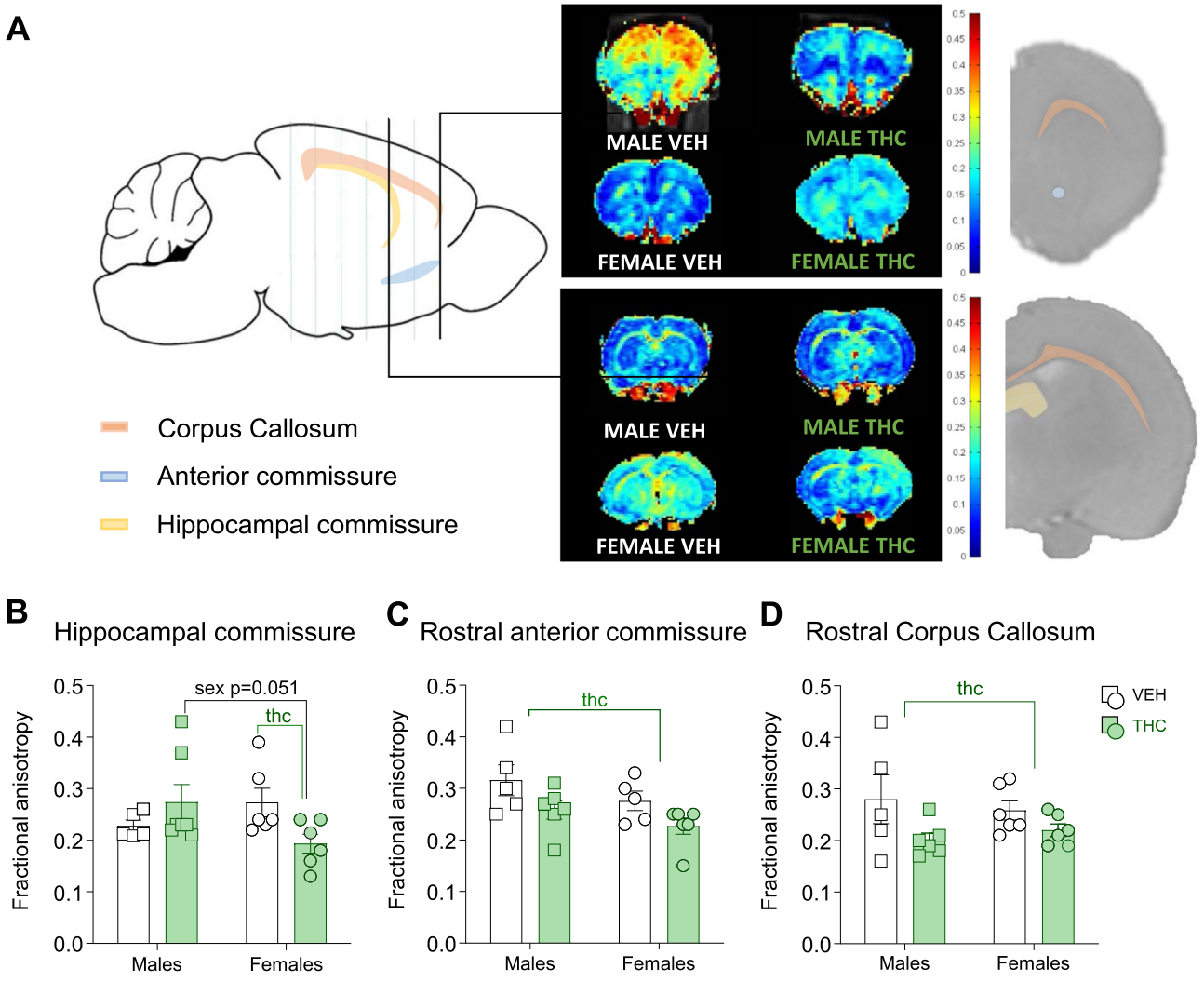


Fig. 4 DTI FA Analysis of white matter tracts. The graphs show the individual values (dots) and the mean ± SEM (bars). Within each graph, the green lines and "thc" represent a significant effect of the factor Treatment. The black lines and "sex" represent statistically significant effects of the factor Sex. A) Representation of the three major white matter tracts and the corresponding DTI FA maps where we detected significant changes in the signal. Graphs of the FA values obtained in the tracts mentioned above: B) The FA signal in the hippocampal commissure, C) anterior commissure and D) corpus callosum. No significant effects of the Adolescent THC Treatment were observed in the internal capsule (data not shown). See Table S5 for more details concerning statistical tests results. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. ^{1}H-MRS

A smaller choline compounds (GPCP+Ch) peak was detected in adult subjects treated with THC in the voxel used for obtaining the cortical measurements (significant effect of Treatment: $F_{1,25}$=4.629; p = 0.041; ηp2=0.156). No other metabolite changes were found in the cortex or in the striatumvoxel employed (see Fig. 5 and Table S6).

3.4. PET

We detected some generalized maturational effects consisting in lower metabolism present in the adult stage compared to the juvenile phase (Table S7) which, in the case of the thalamus, depended on the sex and treatment of the animals. Thus, we observed a significant Treatment*Sex*Time interaction in this structure ($F_{1,19}$=5.182; p = 0.03; ηp2=0.21), which, upon further inspection, showed that while males exposed to VH did not significantly modify their FDG uptake over time, males exposed to THC did show a decreased metabolism at PND 68 as compared to PND 28. In the cortex, there was a trend for the same effect (in fact, the main interaction was significant, but the follow-up analyses were not) (see Table S7). The SPM analysis performed at PND65 after the chronic THC treatment and washout showed some subtle differences (see Fig. 6). THC-exposed males showed, as compared to males treated with vehicle, increased activation of the olfactory bulb, the thalamus, a small cluster in the somatosensory cortex, and the cerebellum and a decreased brain metabolic activity in the cingulate cortex (see Fig. 6A and 6B). In the females, we only

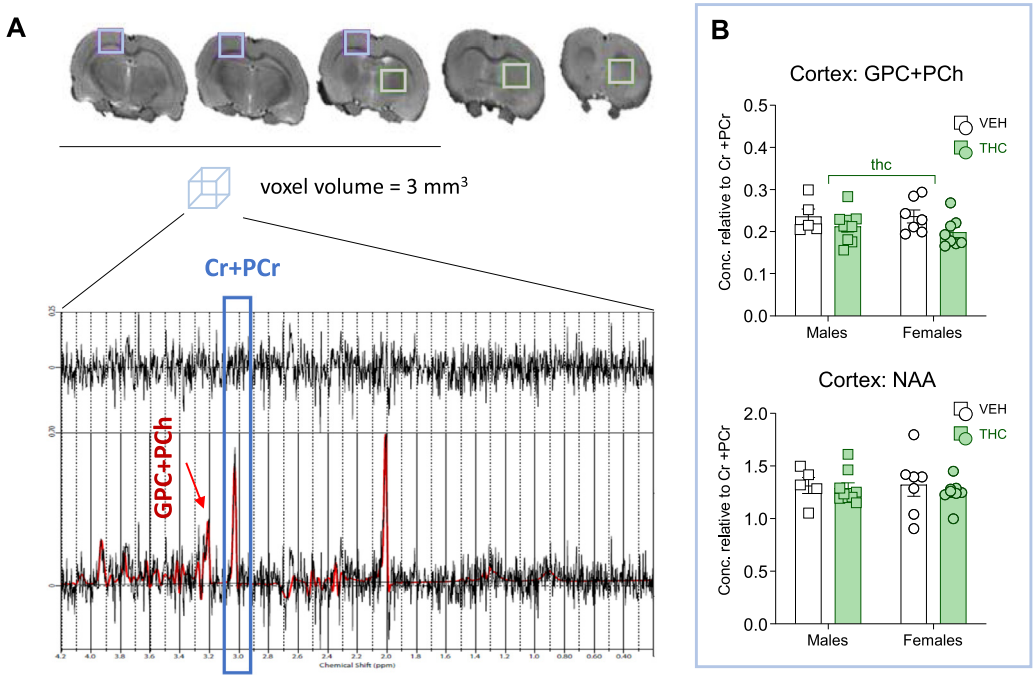


Fig. 5 ^{1}H MR Spectroscopy. A) The 3 mm3 voxel located in the cerebral cortex (in blue) or the striatum (in grey) used to obtain the spectra. B) Cortical GPC+PCh signal (glycerophosphorylcholine, phosphorylcholine, choline) and NAA+NAAG values. The graphs represent the individual values (dots) and the mean ± SEM (bars). Green lines and "thc" represent a significant effect of the factor Treatment. In the striatum, neither the GPC+PCh nor NAA were altered by THC treatment. There were no sex-specific differences detected. See Table S6 for further details. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

observed some small clusters of increased activity in the cingulate cortex and entorhinal-piriform cortices in rats exposed to THC compared to vehicles (Fig. 6A and 6B).

4. Discussion

The present study confirmed the existence of volumetric, microstructural and functional alterations in a rodent model aimed to assess the protracted effects of THC exposure during adolescence (Table 1). We detected reduced volume of the lateral ventricles and the globus pallidus (right hemisphere) in animals treated with THC and a decreased dorsal striatal volume in THC-treated females but not males. THC administration reduced FA in several white matter tracts, especially in the corpus callosum and anterior commissure rostral sections. Lower levels of choline compounds were also found in the cortical regions of animals treated with THC. Metabolic changes evidenced by a PET study suggest an increased metabolism in the somatosensory, cerebellum, thalamus, and olfactory bulb and decreased metabolism in the cingulate cortex in THC-treated males. By contrast, THC-treated females showed hypermetabolism in a cluster of voxels comprising the entorhinal and piriform cortices and the cingulate cortex. The following sections will contextualise these results within the available epidemiological, clinical, and preclinical evidence.

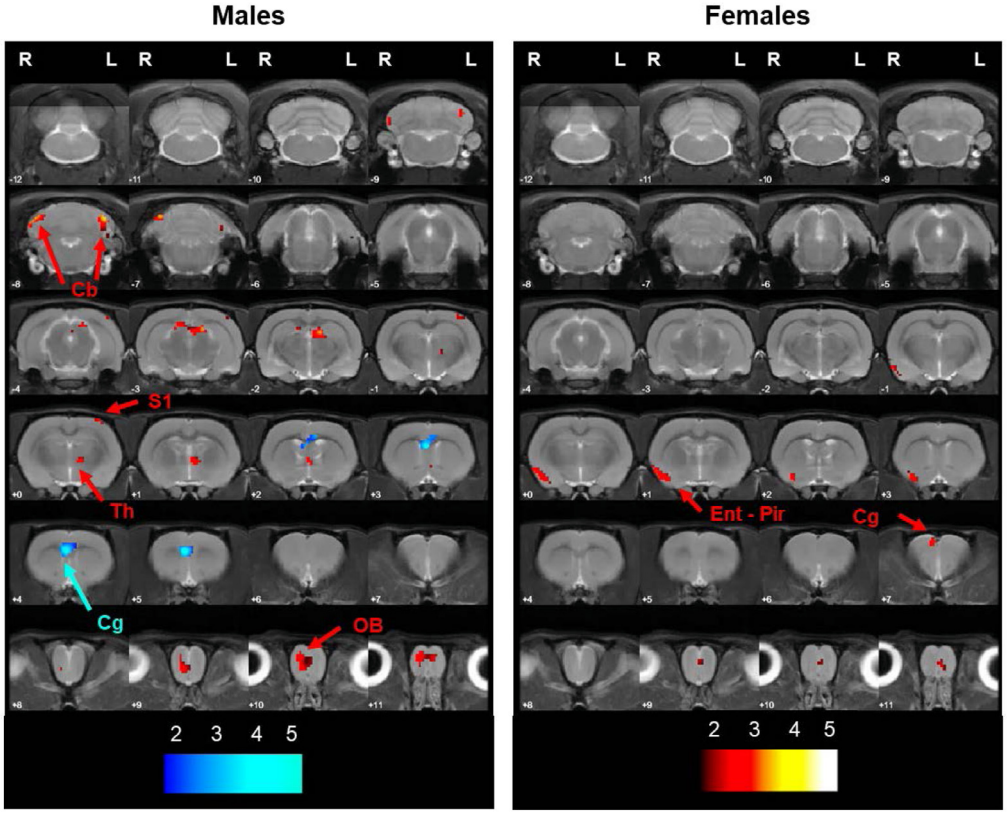
4.1. MRI

4.1.1. Decreased ventricular volume in adults after adolescent cannabinoid exposure

In line with the decrease in ventricular volume found in our animal model, a lower ventricular cerebrospinal fluid (CSF) volume was also reported in young adult humans that frequently used cannabis (Block et al., 2000). Human MRI studies into the relationship of cannabis use with psychosis and schizophrenia represent a frequent source of ventricular alterations associated with cannabis (Rapp et al., 2012). However, these studies draw a complex picture where cannabis use may prevent ventricular enlargement, frequent in patients with schizophrenia (Welch et al., 2011), or drive a pronounced enlargement as the pathology and the consumption patterns evolve over the years (Rais et al., 2008). Interestingly, CSF endocannabinoid content, which may mediate autoinflammatory processes, have distinct features in low or high cannabis users with and without reported psychiatric comorbidities (Leweke et al., 2007; Morgan et al., 2013, 2013). Notably, the subjects in these studies were current users, so the stability of this feature in humans remains unclear. Endocannabinoid ligand CSF content in preclinical adolescent cannabinoid exposure studies has not been explored yet.

Besides CSF endocannabinoid content, the aetiology of our results might be influenced in part by THC-induced developmental changes in features that determine the volume and production of CSF. The lateral ventricular volume de-

A



B

THC effects on brain metabolism

ROI	Side	T	k	↓/↑	p_{unc} peak level	p_{unc} cluster level	p_{FWE} cluster level
A) Males							
Cg	R & L	4.66	185	↓	0.001	0.218	0.957
Cb	R	4.44	118	↑	0.001	0.324	0.991
Cb	L	3.84	151	↑	0.002	0.265	0.978
Th	L	3.65	326	↑	0.003	0.109	0.791
OB	R & L	3.14	197	↑	0.006	0.205	0.948
S1	L	2.33	92	↑	0.010	0.385	0.996
B) Females							
Cg	R	3.49	139	↑	0.003	0.409	0.982
Ent - Pir	R	3.20	202	↑	0.005	0.318	0.956

Cb: cerebellum, Cg: cingulate cortex, Ent: entorhinal cortex, OB: olfactory bulb, Pir: piriform cortex, S1: primary somatosensory cortex, Th: thalamus.
ROI: Region of interest. Side: Right (R) and Left (L). T: t value, k: cluster size. Glucose metabolism: Increase (↑) and Decrease (↓). p_{unc}: p value uncorrected, FWE: Family wise error correction.

Fig. 6 Positron emission tomography at PND65. A) The effects of THC on brain glucose metabolism in males and females are represented as statistical parametric T-maps overlaid on a T2 image as a template. T-maps were obtained as results from 2-sample *t*-test analyses, applying a cluster size threshold of 50 adjacent voxels and a p-value of 0.05 (uncorrected). The colour bars represent the t-values corresponding to reductions (cold colours) or increases (hot colours) in brain metabolism. The intensity of the colour negatively correlates with the t-value of the difference in the cluster represented. B) Detailed SPM data per sex.

creases irrespective of sex, with a similar pattern of evolution during adolescence in both male and female rats. We would expect an increasing slope with few sex-related differences from at least PND35 to 56 (Piontkewitz et al., 2011). Thus, the decrease in volume in adult animals could be due to the interruption of this expected growth due to THC interactions with eCB signalling. In this sense, data from other animal studies indicate that THC has an inhibitory effect on CSF production and flux, presumably affecting ventricle volume. Indeed, this phenomenon was proposed to influence choroidal synaptosomal neurotransmitters (Mancall et al., 1985).

4.1.2. Volumetric alterations within the basal ganglia

Regarding other volumetric alterations, we found an evident reduction in two basal ganglia structures after exposure to THC during adolescence: globus pallidus and dorsal striatum (decreased in THC females but not males). It is well-documented in the literature that human cannabis use reduces the volume of grey matter in CB1-rich regions. However, this is usually linked to current cannabis use rather than associated with disruptions of normal ontogenic maturation (Battistella et al., 2014). CB_1 receptors are expressed strongly in the basal ganglia, especially in the globus pallidus of both humans and rodents (Glass et al., 1997; Herkenham et al., 1991). Several studies in human cannabis users have reported alterations within the basal ganglia and their functional connectivity with other brain areas (Filbey et al., 2016). However, besides morphological alterations, fewer and more variable volumetric abnormalities have been reported (Orr et al., 2016). Most of the volumetric studies assessing the dorsal striatum (caudate and putamen nuclei) or other basal ganglia structures failed to find ostensible differences even after short periods of abstinence (Ganzer et al., 2016), although there is evidence of possible alterations. One study did find a reduction in the right ventral striatum due to cannabis exposure (Pagliaccio et al., 2015), and more recently, another report found an increased basal ganglia grey matter volume in heavy cannabis users (Moreno-Alcázar et al., 2018). Nonetheless, as this later study involved very short periods of abstinence (just 24 h), a plausible explanation, in the light of the absence of studies reporting these same alterations, is that these results could be influenced by residual cannabis effects and/or cannabis withdrawal itself.

Given the specificity of the change in the striatum of THC females, it cannot be solely attributed to a CB_1 receptor-mediated mechanism or a disruption of the developmental trajectory of this nucleus. Adolescent THC exposure induces sex-specific changes that involve long-lasting changes in basal ganglia structures that modulate crucial neurotransmitter systems, including dopamine (DA) signalling, that potentially interact to produce this outcome (Higuera-Matas et al., 2015; Orihuel et al., 2021; Stringfield and Torregrossa, 2021). It was recently proposed that dysregulated DAergic activity may modulate volumetric changes in specific areas (Chang et al., 2020). In this regard, conditions associated with dampened DAergic signalling (e.g. depression, anhedonia, substance use disorders) have been repeatedly associated with a reduction in the volume of basal ganglia structures (Barrós-Loscertales et al., 2011; Belujon and Grace, 2017; Harvey et al., 2007). Conversely, individuals with hyperdopaminergic pathologies (e.g. attention-deficit/hyperactivity disorder, psychopathy) display an increase in the volume of basal ganglia structures (Glenn et al., 2010; Onnink et al., 2016). Remarkably, the increase in DAergic activity associated with specific behaviours (such as sports or video games) can also influence striatal volume (Becker et al., 2016; Erickson et al., 2010).

Interestingly, the globus pallidus is an output region of the dorsal striatum; thus, changes in such structures could affect the downstream areas. In this regard, interactive changes in the volume of these two structures have been associated with the scores of participants in an autism spectrum disorder scale (O'Dwyer et al., 2016). In addition, volumetric changes in the globus pallidus and dorsal striatum might be associated with disrupted DAergic signalling that has already been documented in cannabis users (Bloomfield et al., 2016; Volkow et al., 2014) and in preclinical models with sex-specific variations (for an in-depth overview see (Higuera-Matas et al., 2015; Stringfield and Torregrossa, 2021) Moreover, we have reported in the past some evidence of decreased DA levels (i.e. increased DA transporter expression), specifically in the adult female striatum after adolescent cannabinoid exposure (Higuera-Matas et al., 2010).

4.1.3. DTI and grey matter measurements

Evidence for subcortical microstructural alterations. From PDN21 to 90, the myelinisation of white matter bundles within the dorsal striatum of Wistar males is still developing and increasing, as inferred by the FA signal, and during the same period, cell density decreases, as reflected by the slow decline in the MD signal over time (Mengler et al., 2014). In males, adolescent exposure to THC seems to disrupt and delay myelinisation in the dorsal striatum, as suggested by the lower FA value. The MD values only showed a trend towards an interaction in the dorsal striatum (male-VEH$<$ male-THC and female-VEH$>$female-THC: $p = 0.055$ in whole dorsal striatum, and $p = 0.052$ in the Bregma -0.5 mm slice, respectively). Thus, this could be a secondary effect that will require further confirmation. Nonetheless, adolescent THC might have also interacted with some of the sex-specific developmental differences that arise within the striatum and its connections to other areas during adolescence (Lei et al., 2016).

FA has also been associated with changes in the myelinisation of DA-rich areas and tracts. DAergic alterations resulting from methamphetamine abuse (Volkow et al., 2001) can blunt the FA signal in the dorsal striatum (Alicata et al., 2009) and increased FA in subcortical DArgic tracts has been found in the circuits underlying symptom generation in schizophrenia (Alba-Ferrara and de Erausquin, 2013). In addition, a relationship between MD and DA synthesis capacity was detected in the posterior caudate and putamen, suggesting that DA synthesis may be related to the density of DArgic neural fibres (Kawaguchi et al., 2014). In the case of THC females, the lower MD (trend) could parallel the loss of volume observed, further suggesting a DAergic aetiology of volumetric differences already commented.

Conversely, the FA signal increased in the THA of male rats that received THC relative to both VEH males and THC females. From a neurodevelopmental perspective, there is evidence of a progressive weakening from adolescence to

adulthood of some thalamocortical connections (Fair et al., 2010), and thus, the elevation in FA signal in this region could be due to an aberrant axonal pruning provoked by adolescent THC. In this regard, there is evidence of the early influence of eCB signalling in shaping the thalamocortical projections (Itami et al., 2016) and that CB_1 agonists may prevent pruning at cortical glutamatergic synapses during adolescence (Rubino et al., 2015). Moreover, an increased FA relative to control or non-pathological baseline conditions has been interpreted as a compensatory mechanism that could be associated or compatible with a loss of FA or other alterations in different regions (Mole et al., 2016), changes that may reflect how aberrant structural connectivity (Hoeft et al., 2007) compromises the diffusion of the signal (Alba-Ferrara and de Erausquin, 2013).

Notably, we observed a reduced MD in the rostral septal nuclei of THC females. Septal nuclei have strong reciprocal connections with the thalamus via the stria medullaris thalami (Felten et al., 2016), as well as with other common areas altered by cannabis like the hippocampus (via the fornix), the amygdala (via the stria terminalis) and the ventral tegmental area (via the medial forebrain bundle) amongst others (Willis and Haines, 2018). All these connections make the septal nuclei an important hub capable of modulating memory formation (Khakpai et al., 2013), reward (and avoidance) related learning and even drug-related behaviours (Luo et al., 2011) that are frequently altered in human and animals exposed to THC (Higuera-Matas et al., 2015; Stringfield and Torregrossa, 2021). Moreover, septal nuclei activity might also be relevant for the sex-specific differences in response to cannabis, which also communicates with the hypothalamus and may regulate neuroendocrine and autonomic responses (Risold, 2004).

Adolescent treatment with thc reduces the cortical GPC+PCh metabolite signal. Choline compounds (found to be downregulated in the cortex of adult animals exposed to THC as adolescents) are a marker of cell membrane turnover, cell density and membrane integrity, and they are increased in conditions of membrane breakdown and inflammation; however, an increase in choline compounds has also been associated with myelinisation (Dager et al., 2008) and there is evidence for reduced GPC+PCh after exposure to demyelinating agents (Yan et al., 2015), indicating that a reduction of this parameter may reflect impaired myelinisation. As regards this, the voxel employed for the cortical measurements included a partial segment of the corpus callosum that might influence the signal, supporting the association of this reduced GPC+PCh with non-efficient myelination, which will be discussed in the next section.

4.1.4. White matter alterations inferred by DTI

White matter alterations associated with axon myelinisation are amongst the main effects of cannabinoid agonists like THC (Lubman et al., 2015) frequently associated with cannabis consumption in human MRI studies (Becker et al., 2015; Gruber et al., 2011; Orr et al., 2016). CB_1 receptors are present in the main white matter structures, including the corpus callosum, anterior and hippocampal commissures, stria terminalis and stria medullaris, presumably modulating their development from the early perinatal stage (Romero et al., 1997). After birth, under normal circumstances, white matter bundles gradually increase, and the amount of myelin and myelinated axons they contain grows as adolescence progresses. In rats, the number of myelinated axons in the corpus callosum suffers a dramatic increase from PND15 to 25, and exponential growth continues from PND25 to 60 (Juraska and Willing, 2017). The present adolescent THC treatment coincided with the beginning of one of the most sensitive time windows for white matter development and reproduced similar white matter alterations seen in clinical settings.

An unequivocal causal role in cognitive functions is hard to establish, but the loss of white matter integrity is related to a variety of functional implications depending on the location of the axons affected, such that motor, sensory and/or cognitive functions may be altered (Crawford et al., 2009). In this sense, the loss of white matter integrity in frontolimbic areas in regular cannabis users was associated with apathy and depressive-like symptoms (Shollenbarger et al., 2015) that may ultimately affect reward processing (Admon and Pizzagalli, 2015). Thus, it is highly probable that the FA changes detected here may be associated with different behavioural outcomes seen in animal models that mimic features reported by human studies (See also (Higuera-Matas et al., 2015; Stringfield and Torregrossa, 2021) for a review of behavioural outcomes).

4.2. PET

To the best of our knowledge, no long-term PET studies with [^{18}F]-FDG in humans have ascertained the long-term functional effects of cannabis use during the adolescent period after a long period of withdrawal. The only study we found similar to our design was a work by Sevy and coworkers where they imaged abstinent cannabis users with an age of onset between 11 and 13 years of age. Participants had been consuming cannabis for 5 years on average (2–5 year range) and had remained abstinent for at least 12 weeks (range: 12–25 weeks). Compared to controls, subjects with cannabis dependence had lower glucose metabolism in the orbitofrontal cortex, putamen and precuneus (Sevy et al., 2008). At the preclinical level, hyperactivations have previously been reported. For example, a previous report in rats by our group suggested that adolescent exposure to the synthetic cannabinoid CP 55,940 was associated with increased brain metabolism in the frontal cortex (although we also detected a hypoactivation in the amygdalo-entorhinal cortex, -interestingly, only in the females- (Higuera-Matas et al., 2008)). The brain responses to a cocaine injection were also found to be different in animals exposed to CP 55,940 during adolescence (Higuera-Matas et al., 2011), similar to what is seen in current (female) cannabis user challenged with the DArgic agent methylphenidate (Wiers et al., 2016). However, a new study in rats has shown that in adult animals, a chronic THC regime decreased thalamic glucose metabolism in rats (Miederer et al., 2023), in a similar way as the long-term effects that we observe in our developmental ROI data. Interestingly, the results of the present work show that males seem to be more affected than females in terms of the number of areas affected by exposure to THC during adolescence, as opposed to our previous work with the synthetic cannabinoid. Of note, some of the long-lasting alterations reported here are similar to

what is found in current cannabis abusers; others show opposite patterns. For example, the decreased metabolism observed in the cingulate cortex in the males is also reported in the report of Wiers and colleagues. Still, in the females, we observed hyperactivation. This hyperactivation in the cingulate cortex in the females and the remaining areas in both males and females is different from what was reported previously (Bloomfield et al., 2019). These sex-dependant effects are typically reported in the literature on the long-term effects of cannabinoids (Higuera-Matas et al., 2015) and could reflect the basal differences and regulation of the endocannabinoid system between males and females (Simone et al., 2022; Tirado-Muñoz et al., 2020). We would like to highlight that our results in the cerebellum (one of the areas with a higher concentration of cannabinoid type 1 receptors) represent the first preclinical PET evidence indicative of a long-lasting alteration in this structure, which are, on the other hand, frequently reported in clinical settings (Ganzer et al., 2016). The mechanisms underlying these alterations are beginning to be unveiled, and they are likely to involve microglial activation. For example, in mice, sub-chronic administration of THC activated cerebellar microglia and increased the expression of neuroinflammatory markers, including IL-1β. Moreover, this neuroinflammatory phenotype was correlated with deficits in cerebellar conditioned learning and fine motor coordination (Cutando et al., 2013). In general, our PET data suggest that the long-term effects of cannabis depend on the sex of the animals and that sensorimotor and cognitive areas (thalamus and cortex, amongst others) are affected in the males, while a more restricted network of regions involved in cognitive processes seems to be impacted in the females.

5. Caveats and conclusions

An important caveat to consider in this work is that we have opted for a passive i.p. administration route. We chose this route of administration to ensure a homogenous and comparable exposure to THC across subjects and comparisons with the majority of preclinical work so far. However, this limitation should be kept in mind when considering the general translatability of our results.

For similar reasons, we set the developmental exposure window according to previous work in the field. The rapid changes that take place during the periadolescent period entail that other exposure periods may render slightly different outcomes for some of the measurements reported. Likewise, we cannot expect MRI and PET results to be present in all the animals since THC exposure and manipulations were not equal for the animals in the two experiments. In addition, we only have one measurement timepoint at adulthood, which makes it impossible to assess the potential reversibility of the changes observed. Future studies should be performed to study if the changes here reported persist into the senescent period.

Regarding technical limitations, the resolution in the MRI study did not permit the reliable identification of the specific thalamic or septal nuclei altered. Thus, a more exhaustive analysis of the changes within each nucleus might be an interesting approach for future studies using or not imaging techniques. Moreover, for obvious reasons, we had to perform the imaging studies under anaesthesia, which in the case of PET could be a limitation. However, given that tracer injection and uptake took place before anaesthesia, it is unlikely that our data are not significantly impacted by this (Han et al., 2021). Lastly, in the SPM analysis of adult brain PET scans analysis, we have not corrected for multiple comparisons since individual analysis methods (SPM) provide some correction, being a common practice in exploratory works (Althouse, 2016). In addition, Bonferroni correction assumes independence of the voxels, which is not the case in brain imaging studies and would underestimate the actual effects.

It is important to note that we did not take into consideration the potential impact of the oestrous cycle on volumetric measurements and other brain physiological variables (Rocks et al., 2022). Even if previous brain imaging studies have not considered oestrous cycle information in the analysis of sex differences, future studies should assess the impact of this variable on brain structure and function after adolescent THC exposure.

With this multiparametric imaging work, the first to be performed in an animal model, we have corroborated that THC exposure during adolescence has protracted effects on brain structure and microstructural elements, along with functional features. Noteworthy, the results are coherent with the most consistent clinical findings, such as decreased myelinisation and cortical alterations. Interestingly, the subcortical changes in brain areas are more elusive in clinical settings. The outcomes observed may represent a particular specificity of animal models of adolescent cannabinoid exposure that should be considered for translational purposes. However, these changes were associated with key learning and motivational features that are frequently altered in similar preclinical models and present in human studies.

Moreover, some of our results promise further insights. Changes in the thalamic and septal areas, for example, are exciting since they have been poorly explored but may be central to the development of the adolescent cannabinoid exposure phenotype and the patent sexual dimorphism. Lastly, these results again highlight the importance of addressing sexual differences and the highly sex-dimorphic nature of developmental cannabis exposure effects.

Contributors

- Alejandro Higuera-Matas designed the study and wrote the protocol.
- Javier Orihuel and Roberto Capellán performed the research and statistical analyses.
- Marta Casquero-Veiga, María Luisa Soto-Montenegro, Manuel Desco, Marta Oteo Vives, Marta Ibáñez Moragues, Natalia Magro Calvo, Víctor M. Luján, Miguel Ángel Morcillo undertook the neuroimaging experiments and performed the corresponding analysis.
- Emilio Ambrosio and Alejandro Higuera-Matas secured funding and supervised the project.
- Javier Orihuel wrote the first draft of the manuscript with the help of Alejandro Higuera-Matas.
- All authors contributed to and have approved the final manuscript.

Role of the funding source

This work has been funded by the Spanish Ministry of Economy and Competitiveness (Project n°: PSI2016-80,541-P to EA and A H-M); Ministry of Science (PID2019-104523RB-I00 to A-HM and PID2019-111594RB-100 to EA), Spanish Ministry of Health, Social Services and Equality (Network of Addictive Disorders - Project n°: RTA-RD16/020/0022 of the Institute of Health Carlos III and National Plan on Drugs, Project n°: 2016I073 to EA and 2017I042, 2012I039 to A H-M); The BBVA Foundation (Leonardo Grants) to AH-M; The European Union (Project n°: JUST- 2017- AG- DRUG-806,996-JUSTSO) to EA; and the UNED (Plan for the Promotion of Research) to EA and AH-M.

MLS was supported by the Ministerio de Ciencia e Innovación (PID2021-128862OB-100 funded/AEI /10.13039/501100011033/FEDER, UE), Instituto de Salud Carlos III (project PI17/01,766), co-financed by European Regional Development Fund (ERDF), "A way of making Europe", CIBERSAM, Delegación del Gobierno para el Plan Nacional sobre Drogas (2017/085 and 2022/008917) and Fundación Alicia Koplowitz. Fundación Tatiana Pérez de Guzmán el Bueno supported MCV. The CNIC was supported by the Spanish Ministerio de Ciencia, Innovación y Universidades (MCIU) and the Pro-CNIC Foundation and is a Severo Ochoa center of Excellence.

These funding agencies had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Conflict of Interest

The authors declare that they have no conflict of interest to disclose.

Acknowledgements

We would like to thank Rosa Ferrado, Gonzalo Moreno and Alberto Marcos for their excellent technical assistance.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2023.05.005.

References

Admon, R., Pizzagalli, D.A., 2015. Dysfunctional reward processing in depression. Curr. Opin. Psychol. doi:10.1016/j.copsyc.2014.12.011.

Alba-Ferrara, L.M., de Erausquin, G.A., 2013. What does anisotropy measure? Insights from increased and decreased anisotropy in selective fiber tracts in schizophrenia. Front. Integr. Neurosci. doi:10.3389/fnint.2013.00009.

Alicata, D., Chang, L., Cloak, C., Abe, K., Ernst, T., 2009. Higher diffusion in striatum and lower fractional anisotropy in white matter of methamphetamine users. Psychiatry Res. Neuroimaging 174, 1–8. doi:10.1016/j.pscychresns.2009.03.011.

Althouse, A.D., 2016. Adjust for multiple comparisons? It's Not that simple. Ann. Thorac. Surg. 101, 1644–1645. doi:10.1016/J.ATHORACSUR.2015.11.024.

Barrós-Loscertales, A., Garavan, H., Bustamante, J.C., Ventura-Campos, N., Llopis, J.J., Belloch, V., Parcet, M.A., Ávila, C., 2011. Reduced striatal volume in cocaine-dependent patients. Neuroimage 56, 1021–1026. doi:10.1016/j.neuroimage.2011.02.035.

Battistella, G., Fornari, E., Annoni, J.M., Chtioui, H., Dao, K., Fabritius, M., Favrat, B., Mall, J.F., Maeder, P., Giroud, C., 2014. Long-term effects of cannabis on brain structure. Neuropsychopharmacology 39, 2041–2048. doi:10.1038/npp.2014.67.

Becker, L., Kutz, D.F., Voelcker-Rehage, C., 2016. Exercise-induced changes in basal ganglia volume and their relation to cognitive performance. J. Neurol. Neuromed. 1, 19–24.

Becker, M.P., Collins, P.F., Lim, K.O., Muetzel, R.L., Luciana, M., 2015. Longitudinal changes in white matter microstructure after heavy cannabis use. Dev. Cogn. Neurosci. 16, 23–35. doi:10.1016/J.DCN.2015.10.004.

Belujon, P., Grace, A.A., 2017. Dopamine system dysregulation in major depressive disorders. Int. J. Neuropsychopharmacolog. doi:10.1093/ijnp/pyx056.

Blanco, C., Hasin, D.S., Wall, M.M., Flórez-Salamanca, L., Hoertel, N., Wang, S., Kerridge, B.T., Olfson, M., 2016. Cannabis use and risk of psychiatric disorders: prospective evidence from a US national longitudinal study. JAMA Psychiatry 73, 388–395. doi:10.1001/JAMAPSYCHIATRY.2015.3229.

Blest-Hopley, G., Giampietro, V., Bhattacharyya, S., 2018. Residual effects of cannabis use in adolescent and adult brains — A meta-analysis of fMRI studies. Neurosci. Biobehav. Rev. doi:10.1016/j.neubiorev.2018.03.008.

Block, R.I., O'Leary, D.S., Ehrhardt, J.C., Augustinack, J.C., Ghoneim, M.M., Arndt, S., Hall, J.A., 2000. Effects of frequent marijuana use on brain tissue volume and composition. Neuroreport 11, 491–498. doi:10.1097/00001756-200002280-00013.

Bloomfield, M.A.P., Ashok, A.H., Volkow, N.D., Howes, O.D., 2016. The effects of δ9-tetrahydrocannabinol on the dopamine system. Nature doi:10.1038/nature20153.

Bloomfield, M.A.P., Hindocha, C., Green, S.F., Wall, M.B., Lees, R., Petrilli, K., Costello, H., Ogunbiyi, M.O., Bossong, M.G., Freeman, T.P., 2019. The neuropsychopharmacology of cannabis: a review of human imaging studies. Pharmacol. Ther. 195, 132–161. doi:10.1016/J.PHARMTHERA.2018.10.006.

Casquero-Veiga, M., García-García, D., MacDowell, K.S., Pérez-Caballero, L., Torres-Sánchez, S., Fraguas, D., Berrocoso, E., Leza, J.C., Arango, C., Desco, M., Soto-Montenegro, M.L., 2019. Risperidone administered during adolescence induced metabolic, anatomical and inflammatory/oxidative changes in adult brain: a PET and MRI study in the maternal immune stimulation animal model. Eur. Neuropsychopharmacol. 29, 880–896. doi:10.1016/j.euroneuro.2019.05.002.

Chang, W.H., Chen, K.C., Tseng, H.H., Chiu, N.T., Lee, I.H., Chen, P.S., Yang, Y.K., 2020. Bridging the associations between dopamine, brain volumetric variation and IQ in drug-naïve schizophrenia. Schizophr. Res. 220, 248–253. doi:10.1016/j.schres.2020.03.005.

Coleman, J.R., Madularu, D., Ortiz, R.J., Athanassiou, M., Knudsen, A., Alkislar, I., Cai, X., Kulkarni, P.P., Cushing, B.S., Ferris, C.F., 2022. Changes in brain structure and function following chronic exposure to inhaled vaporised cannabis during periadolescence in female and male mice: a multimodal MRI study. Addict. Biol. 27, e13169. doi:10.1111/ADB.13169.

Crawford, D.K., Mangiardi, M., Tiwari-Woodruff, S.K., 2009. Assaying the functional effects of demyelination and remyelination: revisiting field potential recordings. J. Neurosci. Methods 182, 25–33. doi:10.1016/j.jneumeth.2009.05.013.

Cutando, L., Busquets-Garcia, A., Puighermanal, E., Gomis-González, M., Delgado-García, J.M., Gruart, A., Maldonado, R.,

Ozaita, A., 2013. Microglial activation underlies cerebellar deficits produced by repeated cannabis exposure. J. Clin. Invest. 123, 2816–2831. doi:10.1172/JCI67569.

Dager, S.R., Corrigan, N.M., Richards, T.L., Posse, S., 2008. Research applications of magnetic resonance spectroscopy to investigate psychiatric disorders. Top. Magn. Reson. Imaging. 19, 81–96. doi:10.1097/RMR.0b013e318181e0be.

ElSohly, M., Z, M., S, F., C, G., S, C., JC, C., 2016. Changes in cannabis potency over the last 2 decades (1995-2014): analysis of current data in the United States. Biol. Psychiatry 79, 613–619. doi:10.1016/J.BIOPSYCH.2016.01.004.

Erickson, K.I., Boot, W.R., Basak, C., Neider, M.B., Prakash, R.S., Voss, M.W., Graybiel, A.M., Simons, D.J., Fabiani, M., Gratton, G., Kramer, A.F., 2010. Striatal volume predicts level of video game skill acquisition. Cereb. Cortex 20, 2522–2530. doi:10.1093/cercor/bhp293.

Fair, D.A., Bathula, D., Mills, K.L., Costa Dias, T.G., Blythe, M.S., Zhang, D., Snyder, A.Z., Raichle, M.E., Stevens, A.A., Nigg, J.T., Nagel, B.J., Bathula, K.L.D., Ta, M., 2010. Maturing thalamocortical functional connectivity across development. Front. Syst. Neurosci. 4. doi:10.3389/fnsys.2010.00010.

Felten, D.L., Summo Maida, M., O'Banion, M.K., 2016. Netter's Atlas of Neuroscience | ScienceDirect [WWW Document]. https://www.sciencedirect.com/book/9780323265119/netters-atlas-of-neuroscience (accessed 3.7.21).

Filbey, F.M., Dunlop, J., Ketcherside, A., Baine, J., Rhinehardt, T., Kuhn, B., DeWitt, S., Alvi, T., 2016. fMRI study of neural sensitization to hedonic stimuli in long-term, daily cannabis users. Hum. Brain Mapp. 37, 3431–3443. doi:10.1002/hbm.23250.

Fjell, Anders.M., Westlye, Lars.T., Amlien, I.K., Walhovd, K.B., 2011. Reduced white matter integrity is related to cognitive instability. J. Neurosci. 31, 18060–18072. doi:10.1523/JNEUROSCI.4735-11.2011.

Frye, G.D., Breese, G.R., 1981. An evaluation of the locomotor stimulating action of ethanol in rats and mice. Psychopharmacology (Berl.) 75, 372–379. doi:10.1007/BF00435856.

Ganzer, F., Bröning, S., Kraft, S., Sack, P.M., Thomasius, R., 2016. Weighing the evidence: a systematic review on long-term neurocognitive effects of cannabis use in abstinent adolescents and adults. Neuropsychol. Rev. doi:10.1007/s11065-016-9316-2.

Gilman, J.M., Kuster, J.K., Lee, S., Lee, M.J., Kim, B.W., Makris, N., van der Kouwe, A., Blood, A.J., Breiter, H.C., 2014. Cannabis use is quantitatively associated with nucleus accumbens and amygdala abnormalities in young adult recreational users. J. Neurosci. 34, 5529–5538. doi:10.1523/JNEUROSCI.4745-13.2014.

Glass, M., Dragunow, M., Faull, R.L.M., 1997. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. Neuroscience 77, 299–318. doi:10.1016/S0306-4522(96)00428-9.

Glenn, A.L., Raine, A., Yaralian, P.S., Yang, Y., 2010. Increased volume of the striatum in psychopathic individuals. Biol. Psychiatry 67, 52–58. doi:10.1016/j.biopsych.2009.06.018.

Gruber, S., Sagar, K., Dahlgren, M., Racine, M., Lukas, S., 2012. Age of onset of marijuana use and executive function. Psychol. Addict. Behav. 26, 496–506. doi:10.1037/A0026269.

Gruber, S.A., Silveri, M.M., Dahlgren, M.K., Yurgelun-Todd, D., 2011. Why so impulsive? White matter alterations are associated with impulsivity in chronic marijuana smokers. Exp. Clin. Psychopharmacol. 19, 231–242. doi:10.1037/a0023034.

Gruber, S.A., Yurgelun-Todd, D.A., 2005. Neuroimaging of marijuana smokers during inhibitory processing: a pilot investigation. Cogn. Brain Res. 23, 107–118. doi:10.1016/J.COGBRAINRES.2005.02.016.

Guma, E., Cupo, L., Ma, W., Gallino, D., Moquin, L., Gratton, A., Devenyi, G.A., Chakravarty, M.M., 2023. Investigating the "two-hit hypothesis": effects of prenatal maternal immune activation and adolescent cannabis use on neurodevelopment in mice. Prog. Neuropsychopharmacol. Biol. Psychiatry 120, 110642. doi:10.1016/J.PNPBP.2022.110642.

Hampton, W.H., Hanik, I.M., Olson, I.R., 2019. Substance abuse and white matter: findings, limitations, and future of diffusion tensor imaging research. Drug Alcohol. Depend. doi:10.1016/j.drugalcdep.2019.02.005.

Han, X., Ren, H., Nandi, A., Fan, X., Koehler, R.C., 2021. Analysis of glucose metabolism by 18F-FDG-PET imaging and glucose transporter expression in a mouse model of intracerebral hemorrhage. Sci. Rep. 11. doi:10.1038/S41598-021-90216-4.

Harvey, P.O., Pruessner, J., Czechowska, Y., Lepage, M., 2007. Individual differences in trait anhedonia: a structural and functional magnetic resonance imaging study in non-clinical subjects. Mol. Psychiatry 12, 767–775. doi:10.1038/sj.mp.4002021.

Hasin, D.S., Kerridge, B.T., Saha, T.D., Huang, B., Pickering, R., Smith, S.M., Jung, J., Zhang, H., Grant, B.F., 2016. Prevalence and correlates of DSM-5 cannabis use disorder, 2012-2013: findings from the national epidemiologic survey on alcohol and related conditions-III. Am. J. Psychiatry doi:10.1176/appi.ajp.2015.15070907.

Herkenham, M., Lynn, A.B., Johnson, M.R., Melvin, L.S., de Costa, B.R., Rice, K.C., 1991. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J. Neurosci. 11, 563–583. doi:10.1523/jneurosci.11-02-00563.1991.

Higuera-Matas, A., Botreau, F., del Olmo, N., Miguéns, M., Olías, O., Montoya, G.L., García-Lecumberri, C., Ambrosio, E., 2010. Periadolescent exposure to cannabinoids alters the striatal and hippocampal dopaminergic system in the adult rat brain. Eur. Neuropsychopharmacol. 20, 895–906.

Higuera-Matas, A., Luisa Soto-Montenegro, M., del Olmo, N., Miguéns, M., Torres, I., José Vaquero, J., Sánchez, J., García-Lecumberri, C., Desco, M., Ambrosio, E., 2008. Augmented acquisition of cocaine self-administration and altered brain glucose metabolism in adult female but not male rats exposed to a cannabinoid agonist during adolescence. Neuropsychopharmacology 33, 806–813. doi:10.1038/sj.npp.1301467.

Higuera-Matas, A., Soto-Montenegro, M.L., Montoya, G.L., García-Vázquez, V., Pascau, J., Miguéns, M., del Olmo, N., Vaquero, J.J., García-Lecumberri, C., Desco, M., Ambrosio, E., 2011. Chronic cannabinoid administration to periadolescent rats modulates the metabolic response to acute cocaine in the adult brain. Mol. Imaging Biol. 13, 411–415. doi:10.1007/s11307-010-0388-8.

Higuera-Matas, A., Ucha, M., Ambrosio, E., 2015. Long-term consequences of perinatal and adolescent cannabinoid exposure on neural and psychological processes. Neurosci. Biobehav. Rev. 55, 119–146. doi:10.1016/j.neubiorev.2015.04.020.

Hoeft, F., Barnea-Goraly, N., Haas, B.W., Golarai, G., Ng, D., Mills, D., Korenberg, J., Bellugi, U., Galaburda, A., Reiss, A.L., 2007. More is not always better: increased fractional anisotropy of superior longitudinal fasciculus associated with poor visuospatial abilities in Williams syndrome. J. Neurosci. 27, 11960–11965. doi:10.1523/JNEUROSCI.3591-07.2007.

Itami, C., Huang, J.Y., Yamasaki, M., Watanabe, M., Lu, H.C., Kimura, F., 2016. Developmental switch in spike timing-dependent plasticity and cannabinoid-dependent reorganization of the thalamocortical projection in the barrel cortex. J. Neurosci. 36, 7039–7054. doi:10.1523/JNEUROSCI.4280-15.2016.

Jupp, B., Dalley, J.W., 2014. Behavioral endophenotypes of drug addiction: etiological insights from neuroimaging studies. Neuropharmacology doi:10.1016/j.neuropharm.2013.05.041.

Juraska, J.M., Willing, J., 2017. Pubertal onset as a critical transition for neural development and cognition. Brain Res. 1654, 87–94. doi:10.1016/j.brainres.2016.04.012.

Kawaguchi, H., Obata, T., Takano, H., Nogami, T., Suhara, T., Ito, H., 2014. Relation between dopamine synthesis capacity and cell-level structure in human striatum: a multi-modal study

with positron emission tomography and diffusion tensor imaging. PLoS One 9. doi:10.1371/journal.pone.0087886.

Khakpai, F., Nasehi, M., Haeri-Rohani, A., Eidi, A., Zarrindast, M.R., 2013. Septo-hippocampo-septal loop and memory formation. Basic Clin. Neurosci. 4, 5–23.

Lei, X., Han, Z., Chen, C., Bai, L., Xue, G., Dong, Q., 2016. Sex differences in fiber connection between the striatum and subcortical and cortical regions. Front. Comput. Neurosci. 10 (100). doi:10.3389/fncom.2016.00100.

Leweke, F.M., Giuffrida, A., Koethe, D., Schreiber, D., Nolden, B.M., Kranaster, L., Neatby, M.A., Schneider, M., Gerth, C.W., Hellmich, M., Klosterkötter, J., Piomelli, D., 2007. Anandamide levels in cerebrospinal fluid of first-episode schizophrenic patients: impact of cannabis use. Schizophr. Res. 94, 29–36. doi:10.1016/j.schres.2007.04.025.

Lubman, D.I., Cheetham, A., Y??cel, M., Yücel, M., Y??cel, M., 2015. Cannabis and adolescent brain development. Pharmacol. Ther. 148, 1–16. doi:10.1016/j.pharmthera.2014.11.009.

Luo, A.H., Tahsili-Fahadan, P., Wise, R.A., Lupica, C.R., Aston-Jones, G., 2011. Linking context with reward: a functional circuit from hippocampal CA3 to ventral tegmental area. Science 333, 353–357. doi:10.1126/science.1204622, (1979).

Mancall, A.C., DiGregorio, G.J., Brill, C.B., Ruch, E., 1985. The effect of △-9-tetrahydrocannabinol on rat cerebrospinal fluid. Arch. Neurol. 42, 1069–1071. doi:10.1001/archneur.1985.04060100051021.

Mato, S., Chevaleyre, V., Robbe, D., Pazos, A., Castillo, P.E., Manzoni, O.J., 2004. A single in-vivo exposure to △9THC blocks endocannabinoid-mediated synaptic plasticity. Nat. Neurosci. 7, 585–586. doi:10.1038/nn1251.

Mato, S., Robbe, D., Puente, N., Grandes, P., Manzoni, O.J., 2005. Presynaptic homeostatic plasticity rescues long-term depression after chronic △9-tetrahydrocannabinol exposure. J. Neurosci. 25, 11619–11627. doi:10.1523/JNEUROSCI.2294-05.2005.

Mengler, L., Khmelinskii, A., Diedenhofen, M., Po, C., Staring, M., Lelieveldt, B.P.F., Hoehn, M., 2014. Brain maturation of the adolescent rat cortex and striatum: changes in volume and myelination. Neuroimage 84, 35–44. doi:10.1016/J.NEUROIMAGE.2013.08.034.

Miederer, I., Schmitt, U., Bausbacher, N., Röhrich, J., Mildenberger, P., Lutz, B., Tüscher, O., Schreckenberger, M., 2023. Chronic administration of △9-tetrahydrocannabinol alters brain glucose uptake and improves waiting impulsivity in the rat. Cannabis Cannabinoid Res. doi:10.1089/CAN.2022.0268.

Miederer, I., Uebbing, K., Röhrich, J., Maus, S., Bausbacher, N., Krauter, K., Weyer-Elberich, V., Lutz, B., Schreckenberger, M., Urban, R., 2017. Effects of tetrahydrocannabinol on glucose uptake in the rat brain. NeuropharmacologyNeuropharmacology 117, 273–281. doi:10.1016/J.NEUROPHARM.2017.02.011.

Mole, J.P., Subramanian, L., Bracht, T., Morris, H., Metzler-Baddeley, C., Linden, D.E.J., 2016. Increased fractional anisotropy in the motor tracts of Parkinson's disease suggests compensatory neuroplasticity or selective neurodegeneration. Eur. Radiol. 26, 3327–3335. doi:10.1007/s00330-015-4178-1.

Moreno-Alcázar, A., Gonzalvo, B., Canales-Rodríguez, E.J., Blanco, L., Bachiller, D., Romaguera, A., Monté-Rubio, G.C., Roncero, C., McKenna, P.J., Pomarol-Clotet, E., 2018. Larger gray matter volume in the basal ganglia of heavy cannabis users detected by voxel-based morphometry and subcortical volumetric analysis. Front. Psychiatry 9 (175). doi:10.3389/fpsyt.2018.00175.

Morgan, C.J.A., Page, E., Schaefer, C., Chatten, K., Manocha, A., Gulati, S., Curran, H.V., Brandner, B., Leweke, F.M., 2013. Cerebrospinal fluid anandamide levels, cannabis use and psychotic-like symptoms. Br. J. Psychiatry 202, 381–382. doi:10.1192/bjp.bp.112.121178.

Nguyen, V.H., Verdurand, M., Dedeurwaerdere, S., Wang, H., Zahra, D., Gregoire, M.C., Zavitsanou, K., 2012. Increased brain metabolism after acute administration of the synthetic cannabinoid HU210: a small animal PET imaging study with 18F-FDG. Brain Res. Bull. 87, 172–179. doi:10.1016/j.brainresbull.2011.11.011.

O'Dwyer, L., Tanner, C., van Dongen, E.v., Greven, C.U., Bralten, J., Zwiers, M.P., Franke, B., Heslenfeld, D., Oosterlaan, J., Hoekstra, P.J., Hartman, C.A., Groen, W., Rommelse, N., Buitelaar, J.K., 2016. Decreased left caudate volume is associated with increased severity of autistic-like symptoms in a cohort of ADHD patients and their unaffected siblings. PLoS One 11, 165620. doi:10.1371/journal.pone.0165620.

Onnink, A.M.H., Franke, B., van Hulzen, K., Zwiers, M.P., Mostert, J.C., Schene, A.H., Heslenfeld, D.J., Oosterlaan, J., Hoekstra, P.J., Hartman, C.A., Vasquez, A.A., Kan, C.C., Buitelaar, J., Hoogman, M., 2016. Enlarged striatal volume in adults with ADHD carrying the 9-6 haplotype of the dopamine transporter gene DAT1. J. Neural Transm. 123, 905–915. doi:10.1007/s00702-016-1521-x.

Orihuel, J., Capellán, R., Roura-Martínez, D., Ucha, M., Ambrosio, E., Higuera-Matas, A., 2021. △ 9-tetrahydrocannabinol during adolescence reprograms the nucleus accumbens transcriptome, affecting reward processing, impulsivity, and specific aspects of cocaine addiction-like behavior in a sex-dependent manner. Int. J. Neuropsychopharmacol. 24, 920–933. doi:10.1093/IJNP/PYAB058.

Orr, J.M., Paschall, C.J., Banich, M.T., 2016. Recreational marijuana use impacts white matter integrity and subcortical (but not cortical) morphometry. Neuroimage Clin. 12, 47–56. doi:10.1016/j.nicl.2016.06.006.

Pagliaccio, D., Barch, D.M., Bogdan, R., Wood, P.K., Lynskey, M.T., Heath, A.C., Agrawal, A., 2015. Shared predisposition in the association between cannabis use and subcortical brain structure. JAMA Psychiatry 72, 994–1001. doi:10.1001/jamapsychiatry.2015.1054.

Pascau, J., Gispert, J.D., Michaelides, M., Thanos, P.K., Volkow, N.D., Vaquero, J.J., Soto-Montenegro, M.L., Desco, M., 2009. Automated method for small-animal PET image registration with intrinsic validation. Mol. Imaging Biol. 11, 107–113. doi:10.1007/s11307-008-0166-z.

Piontkewitz, Y., Arad, M., Weiner, I., 2011. Abnormal trajectories of neurodevelopment and behavior following in utero insult in the rat. Biol. Psychiatry 70, 842–851. doi:10.1016/J.BIOPSYCH.2011.06.007.

Rais, M., Cahn, W., van Haren, N., Schnack, H., Caspers, E., Pol, H.H., Kahn, R., 2008. Excessive brain volume loss over time in cannabis-using first-episode schizophrenia patients. Am. J. Psychiatry 165, 490–496. doi:10.1176/appi.ajp.2007.07071110.

Rapp, C., Bugra, H., Riecher-Rossler, A., Tamagni, C., Borgwardt, S., 2012. Effects of cannabis use on human brain structure in psychosis: a systematic review combining in vivo structural neuroimaging and post mortem studies. Curr. Pharm. Des. 18, 5070–5080. doi:10.2174/138161212802884861.

Risold, P.Y., 2004. The septal region, in: the rat nervous system. Elsevier Inc. 605–632. doi:10.1016/B978-012547638-6/50021-3.

Rocks, D., Cham, H., Kundakovic, M., 2022. Why the estrous cycle matters for neuroscience. Biol. Sex Differ. 13. doi:10.1186/S13293-022-00466-8.

Romero, J., Garcia-Palomero, E., Berrendero, F., Garcia-Gil, L., Hernandez, M.L., Ramos, J.A., Fernandez-Ruiz, J.J., 1997. Atypical location of cannabinoid receptors in white matter areas during rat brain development. Synapse 26, 317–323. doi:10.1002/(SICI)1098-2396(199707)26:3⟨317::AID-SYN12⟩3.0.CO;2-S.

Rubino, T., Prini, P., Piscitelli, F., Zamberletti, E., Trusel, M., Melis, M., Sagheddu, C., Ligresti, A., Tonini, R., di Marzo, V., Parolaro, D., 2015. Adolescent exposure to THC in female rats disrupts developmental changes in the prefrontal cortex. Neurobiol. Dis. 73, 60–69. doi:10.1016/j.nbd.2014.09.015.

Shollenbarger, S.G., Price, J., Wieser, J., Lisdahl, K., 2015. Poorer frontolimbic white matter integrity is associated with chronic cannabis use, FAAH genotype, and increased depressive and apathy symptoms in adolescents and young adults. Neuroimage Clin. 8, 117–125. doi:10.1016/j.nicl.2015.03.024.

Simone, J.J., Green, M.R., McCormick, C.M., 2022. Endocannabinoid system contributions to sex-specific adolescent neurodevelopment. Prog. Neuropsychopharmacol. Biol. Psychiatry 113. doi:10.1016/J.PNPBP.2021.110438.

Stringfield, S.J., Torregrossa, M.M., 2021. Disentangling the lasting effects of adolescent cannabinoid exposure. Prog. Neuropsychopharmacol. Biol. Psychiatry doi:10.1016/j.pnpbp.2020.110067.

Tirado-Muñoz, J., Lopez-Rodriguez, A.B., Fonseca, F., Farré, M., Torrens, M., Viveros, M.P., 2020. Effects of cannabis exposure in the prenatal and adolescent periods: preclinical and clinical studies in both sexes. Front. Neuroendocrinol. 57. doi:10.1016/J.YFRNE.2020.100841.

United Nations Office on drugs and crime, 2022. World Drug Report.

Volkow, N.D., Chang, L., Wang, G.J., Fowler, J.S., Franceschi, D., Sedler, M., Gatley, S.J., Miller, E., Hitzemann, R., Ding, Y.S., Logan, J., 2001. Loss of dopamine transporters in methamphetamine abusers recovers with protracted abstinence. J. Neurosci. 21, 9414–9418. doi:10.1523/jneurosci.21-23-09414.2001.

Volkow, N.D., Wang, G.J., Telang, F., Fowler, J.S., Alexoff, D., Logan, J., Jayne, M., Wong, C., Tomasi, D., 2014. Decreased dopamine brain reactivity in marijuana abusers is associated with negative emotionality and addiction severity. Proc. Natl. Acad. Sci. U. S. A. 111. doi:10.1073/pnas.1411228111.

Walden, N., Earleywine, M., 2008. How high: quantity as a predictor of cannabis-related problems. Harm Reduct. J. 5. doi:10.1186/1477-7517-5-20.

Welch, K.A., McIntosh, A.M., Job, D.E., Whalley, H.C., Moorhead, T.W., Hall, J., Owens, D.G.C., Lawrie, S.M., Johnstone, E.C., 2011. The impact of substance use on brain structure in people at high risk of developing schizophrenia. Schizophr. Bull. 37, 1066–1076. doi:10.1093/schbul/sbq013.

Willis, M.A., Haines, D.E., 2018. The limbic system, in: fundamental neuroscience for basic and clinical applications: fifth edition. Elsevier Inc. 457–467. doi:10.1016/B978-0-323-39632-5.00031-1, e1.

Worley, J., 2019. Teenagers and cannabis use: why it's a problem and what can be done about it. J. Psychosoc. Nurs. Ment. Health Serv. 57, 11–15. doi:10.3928/02793695-20190218-03.

Yan, G., Xuan, Y., Dai, Z., Shen, Z., Zhang, G., Xu, H., Wu, R., 2015. Brain metabolite changes in subcortical regions after exposure to cuprizone for 6 weeks: potential implications for schizophrenia. Neurochem. Res. 40, 49–58. doi:10.1007/s11064-014-1464-2.