In vivo 1H magnetic resonance spectroscopy in young-adult daily marijuana users

Ryan L. Muetzel, Malgorzata Marjańska, Paul F. Collins, Mary P. Becker, Romain Valabrègue, Edward J. Auerbach, Kelvin O. Lim, Monica Luciana

NeuroImage: Clinical 2 (2013) 581–589

Contents lists available at ScienceDirect

NeuroImage: Clinical

journal homepage: www.elsevier.com/locate/ynicl

In vivo ^{1}H magnetic resonance spectroscopy in young-adult daily marijuana users

Ryan L. Muetzel [a,*,1], Małgorzata Marjańska [b,1], Paul F. Collins [a], Mary P. Becker [a], Romain Valabrègue [c], Edward J. Auerbach [b], Kelvin O. Lim [d], Monica Luciana [a]

[a] Department of Psychology, University of Minnesota, Minneapolis, MN, USA
[b] Center for Magnetic Resonance Research and Department of Radiology, University of Minnesota, Minneapolis, MN, USA
[c] CRICM (CENIR), Hôpital Pitié-Salpêtrière, Paris, France
[d] Department of Psychiatry, University of Minnesota, Minneapolis, MN, USA

ARTICLE INFO

Article history:
Received 27 January 2013
Received in revised form 13 April 2013
Accepted 16 April 2013
Available online 22 April 2013

Keywords:
Cannabis
Glutamate
Basal ganglia
Adolescence

ABSTRACT

To date, there has been little work describing the neurochemical profile of young, heavy marijuana users. In this study, we examined 27 young-adult marijuana users and 26 healthy controls using single-voxel magnetic resonance spectroscopy on a 3 T scanner. The voxel was placed in the dorsal striatum, and estimated concentrations of glutamate + glutamine, *myo*-inositol, taurine + glucose, total choline and total *N*-acetylaspartate were examined between groups. There were no overall group effects, but two metabolites showed group by sex interactions. Lower levels of glutamate + glutamine (scaled to total creatine) were observed in female, but not male, marijuana users compared to controls. Higher levels of *myo*-inositol were observed in female users compared to female non-users and to males in both groups. Findings are discussed in relation to patterns of corticostriatal connectivity and function, in the context of marijuana abuse.

1. Introduction

Illicit marijuana use in the United States has been a longstanding public health concern for both adolescents and adults. As many as 44% of college-aged individuals endorse having used marijuana at some point in their life, and 21% of college-aged individuals report marijuana use in the past 30 days (Johnston et al., 2011). Marijuana intoxication is associated with motor coordination deficits, euphoria, impaired temporal estimation, and a variety of other psychological phenomena (Hall and Solowij, 1998). Marijuana use has also been associated with more specific cognitive deficits, even after acute intoxication has subsided (Pope and Yurgelun-Todd, 1996), and with the development of severe psychopathology (McGrath et al., 2010). Furthermore, chronic marijuana use has been related to adverse physiological consequences in the cardiovascular and respiratory systems (Mittleman et al., 2001; Sherrill et al., 1991). Adolescence and young adulthood represent periods of the lifespan when increased risk-taking occurs, including the use of illicit substances, such as marijuana. The combination of an innate propensity for risk-taking (e.g., driving motor vehicles recklessly, unprotected sex, etc.) and use of a judgment-altering substance is a striking example of the immediate public health concern over marijuana use in young-adults. This concern is particularly pertinent in light of recent efforts in support of marijuana's legalization in the United States. A challenge for the field is to identify which chemical systems and associated information processing networks are most affected by chronic marijuana use.

The main psychoactive component of marijuana, Δ^{9}-tetrahydrocannabinol (THC), acts as an agonist in central nervous system (CNS) cannabinoid (CB_1) receptors and in other peripheral cell types, primarily immune cells (CB_2 receptors) (Pertwee, 2008). In the CNS, CB_1 receptor density is high in the basal ganglia, particularly in the dorsal striatum (Herkenham et al., 1990). Cannabinoid receptor signaling acts on multiple neurotransmitters through a variety of biochemical cascades, including inhibition of voltage-dependent calcium channels (thereby inhibiting calcium-dependent vesicle release) and by directly inhibiting vesicle release (via a calcium independent process) (Szabo and Schlicker, 2005). Both excitatory and inhibitory neurotransmitters, including glutamate (Glu), γ-aminobutyric acid (GABA) and dopamine, are either directly or indirectly affected by CB_1 receptor activation (Schlicker and Kathmann, 2001). For marijuana and other drugs of abuse and dependence, the dorsal striatum has been hypothesized to play a key role in the transition from intermittent drug use to compulsive habit-based drug-taking via mechanisms that underlie long-term synaptic plasticity (Kalivas et al., 2009). Exogenous activation of CB_1 receptors, as occurs with marijuana intoxication, inhibits the release of glutamate as well as GABA in both the dorsal and ventral striatum

* Corresponding author at: Department of Child and Adolescent Psychiatry, Erasmus MC—Sophia Children's Hospital, P.O. Box 2040, 3000 CA Rotterdam, Rotterdam, The Netherlands. Tel.: +31 107038019.
E-mail address: r.muetzel@erasmusmc.nl (R.L. Muetzel).
[1] These authors contributed equally to this work.

2213-1582
http://dx.doi.org/10.1016/j.nicl.2013.04.011

(Gerdeman and Lovinger, 2001; Gerdeman et al., 2003; Hoffman and Lupica, 2001; Szabo et al., 1998). This inhibition facilitates the development of long-term depression (LTD) in the striatum, which is a critical component in the altered synaptic plasticity that accompanies drug addiction (Gerdeman et al., 2003). Thus, the manner in which corticostriatal functional connectivity is altered in the context of marijuana use is of interest, as is metabolic activity within the chemical systems that contribute to those alterations.

Magnetic resonance spectroscopy (MRS) is a widely used tool, allowing for *in vivo* characterizations of various brain metabolites. MRS data is acquired either from single voxel (SVS) or multiple voxels (spectroscopic imaging, MRSI: (de Graaf, 2007)). The SVS method typically benefits from high spectral resolution and signal-to-noise ratio (SNR). MRSI has better spatial resolution compared to SVS, but typically has a much more limited spectral resolution (i.e., fewer metabolites are quantifiable resulting from lower SNR and broader line-widths). The application of MRS to the study of chronic marijuana users is limited in the current literature. To the best of our knowledge, only four other studies utilizing some form of MRS to examine marijuana users have been published, and the methods of these studies are relatively heterogeneous (Chang et al., 2006; Hermann et al., 2007; Prescot et al., 2011; Silveri et al., 2011). The existing studies are summarized in Table 1. Individuals ages 16-to-42 years were studied with either SVS or MRSI. In two of the studies, only males were examined (Hermann et al., 2007; Silveri et al., 2011). In most cases, marijuana use was reported at 20 or more days per month. Lower levels of Glu, *N*-acetylaspartate (NAA), and *myo*-inositol (mIns) were observed in marijuana users compared to controls in regions known to be associated with substance use, including the basal ganglia (lower Glu, NAA and choline: Chang et al., 2006), thalamus (higher total creatine: Chang et al., 2006), cingulate cortex (lower Glu, NAA, tCr, and mIns: Prescot et al., 2011), dorsolateral prefrontal cortex (lower NAA: Hermann et al., 2007), and the striatum as well as posterior cortical regions (lower mIns: Silveri et al., 2011). The methods, ages of subjects, and extent of current marijuana use in the samples tested vary considerably across studies as summarized in Table 1.

As disruptions in glutamate activity have been implicated in the development of addiction (Koob and Volkow, 2010), we hypothesized disruptions in glutamate concentrations in marijuana users compared to controls. Several lines of evidence suggest inhibition of glutamate excitotoxicity by marijuana (Hampson et al., 1998; Marsicano et al., 2003). In addition, based on the MRS literature described above related to the basal ganglia of adult marijuana users (Chang et al., 2006) and literature describing the inhibitory effects of CB_1 receptors on glutamate release, we specifically hypothesized that young-adult MJU subjects would show lower levels of Glu + glutamine (Glu + Gln = Glx) in the basal ganglia compared to their non-using counterparts. We did not have a specific hypothesis regarding concentrations of other metabolites given that other researchers have not concentrated their assessments on the striatum. However, the limited available literature suggested the possibility of altered mIns as well as NAA levels in users versus controls.

2. Materials and methods

2.1. Participants

Twenty-seven marijuana users (MJU: 16 males, 11 females) were recruited into the study through local advertisements on the University of Minnesota-Twin Cities campus. Marijuana users' ages ranged from 18-to-21 years, with a mean and standard deviation of 19.5 ± 0.6 years (Table 2). Exclusion criteria are described below. Twenty-six healthy young adult non-users (10 males, 16 females), who were participants in a large, longitudinal study of normal brain development, served as a control sample. Control participants' ages ranged from 13-to-24 years, with a mean and standard deviation of 19.3 ± 3.1 years. The recruitment strategy for the control sample has been described elsewhere (Muetzel et al., 2008; Olson et al., 2009; Porter et al., 2011). Briefly, participants younger than 18 years of age were recruited through a database of research volunteers throughout the Metro community, through post-cards mailed to University of Minnesota civil service employees, and through local advertisements. Participants over the age of 18 years were recruited using on-campus advertisements. During the controls' third longitudinal follow-up visit, MRS was added to the protocol as time allowed. Thus, the control sample described in this study has a broader age range than the MJU sample, a feature that was considered in the statistical approach described below.

A description of the study was initially given to both the MJU and control participants over the phone. Interested participants were then invited to complete a brief phone screening to ascertain study eligibility. Exclusion criteria included major physical, neurological or psychiatric illness, substance use disorders (other than marijuana and alcohol use for the user group), head injuries resulting in loss of consciousness >20 min, mental retardation, learning disabilities, current use of psychoactive medications, non-native English speaking, vision or hearing that was not normal or corrected to normal, complications at birth, current pregnancy, and MRI contraindications (e.g., metallic implants, severe claustrophobia, orthodontic braces, etc.). Inclusion criteria for MJU participants included current use of marijuana at least five times per week for at least one year, and an age of onset of use prior to the age of 17 years. Marijuana users were also excluded if they were daily cigarette smokers, or if their alcohol use exceeded four drinks for females and five drinks for males on more than two occasions per week. Marijuana users were asked to refrain from drug use for at least 12 h prior to their visit (as assessed through self report) to avoid acute intoxication during study procedures. Participants provided written informed consent (or assent when applicable; parents of participants younger than age 18 provided consent)

Table 1
Prior studies using MRS spectroscopy to investigate associations with marijuana use.

Study	Method	Field (T)	N (female)		Age (years)		MJ use (days/month)	Targeted region	Results
			MJU	Control	MJU	Control			
Chang et al. (2006)	SVS	4	24 (4)	30 (6)	36 ± 2	42 ± 2	20	Basal ganglia Thalamus	Lower Glu, NAA, Cho in MJU Higher tCr in MJU
Hermann et al. (2007)	MRSI	1.5	12 (0)	10 (0)	22 ± 2	23 ± 2	25	DLPFC	Lower NAA
Prescot et al. (2011)	SVS	3	17 (2)	17 (9)	18 ± 1	16 ± 2	*	Cingulate cortex	Lower Glu, tCr, mIns, and NAA in MJU
Silveri et al. (2011)	MRSI	4	13 (0)	10 (0)	21 ± 3	25 ± 5	22	Striatum, occipital lobes, parietal lobes	Lower mIns in MJU

Note: Ages are reported as the mean age ± standard deviation, * = days per month use not reported. Abbreviations: Cho = choline, DLPFC = dorsolateral prefrontal cortex, Glu = glutamate, mIns = myo-inositol, MJ = marijuana, MJU = marijuana users, MRSI = magnetic resonance spectroscopic imaging, NAA = N-acetylaspartate, SVS = single voxel spectroscopy, T = Tesla, tCr = total creatine.

Table 2
Sample characteristics.

	MJU (n = 27)	Controls (n = 26)	t(df)	p
Males (n)	16	10	–	–
Female (n)	11	16	–	–
Age (range, yrs)	18.4–20.9	13.3–24.5	–	–
Age (mean ± SD, yrs)	19.5 ± 0.6	19.3 ± 3.1	−0.22(51)	0.83
FSIQ (mean ± SD)	114 ± 12	117 ± 9	0.7(51)	0.49
PEI Alcohol Use (mean ± SD)	3.7 ± 1.0	1.5 ± 1.4	−6.6(51)	<0.001

Note: Sex distributions by group did not differ statistically [$X^2(1, n = 53) = 2.29$, $p = 0.17$], FSIQ = full scale intelligence quotient, estimated with the Wechsler Abbreviated Intelligence Scale.

and all study procedures were approved by the University of Minnesota's Institutional Review Board.

2.2. Assessments

2.2.1. Diagnostic assessment

After the phone interview, eligible participants were invited to the University of Minnesota's Center for Neurobehavioral Development for an in-person screening session to further ascertain eligibility and to verify information given over the phone. The Kiddie Schedule for Affective Disorders and Schizophrenia, Present and Lifetime version (K-SADS-PL) was used to assess for current or past Diagnostic and Statistical Manual, Fourth Edition (DSM-IV) axis I disorders, including childhood disorders given the relative youth of the sample (Kaufman et al., 1997). The presence or absence of DSM-IV disorders was confirmed by case consensus meetings with staff members including a license-eligible clinical psychologist. In addition, a two-subtest (Vocabulary and Matrix Reasoning) version of the Wechsler Abbreviated Scale of Intelligence was administered to yield estimated full scale IQ (Wechsler, 1999). Participants who met all inclusion criteria after the in-person interview were invited back for a comprehensive neuropsychological testing battery and an MRI scan. This report focuses on spectroscopy findings.

2.2.2. Substance use assessment

In addition to the K-SADS-PL, the Personal Experience Inventory (PEI) (Henly and Winters, 1989) was used to further assess alcohol and marijuana use in both the MJU group and in the healthy controls. Briefly, the PEI consists of two main sections, one focused on patterns and severity of substance use, and the other focused on psychosocial consequences of use. In most cases, participants endorse items from the inventory using a four-point Likert response format (e.g., *strongly disagree*, *disagree*, *agree*, *strongly agree*). Different versions of the PEI have been developed for adolescents versus adults. Participants younger than 18 years of age received the adolescent version and participants older than 18 years of age received the adult version; both versions were computer administered. All MJU participants received the adult version. Scoring was implemented to create comparable metrics across the two versions. Finally, an in-house questionnaire based on guidelines provided by the National Institute on Alcohol Abuse and Alcoholism was implemented to assess detailed daily, weekly, yearly and lifetime use patterns of alcohol and marijuana in the sample, considering frequency and amount of use.

2.3. MR data acquisition

Magnetic resonance data were acquired using a 3 Tesla (T) whole-body TIM TRIO system (Siemens, Erlangen, Germany) housed at the University of Minnesota's Center for Magnetic Resonance Research. Radiofrequency transmission was performed with a whole body coil, and signal was received with a 12-channel receive-only head coil.

A 10-second, 3-plane localizer image was first acquired for positioning of subsequent scans. A coronal T_1-weighted, magnetization prepared rapid gradient echo (Mugler and Brookeman, 1990) sequence was used to acquire a high-resolution scan for MRS voxel positioning and tissue segmentation (repetition time (T_R) = 2530 ms, echo time (T_E) = 3.65 ms, inversion time (T_I) = 1100 ms, flip angle = 7°, number of slices = 240, matrix size = 256 × 256, field of view (FOV) = 256 mm × 256 mm, slice thickness = 1 mm).

All spectra were acquired using a localization by adiabatic selective refocusing (Garwood and DelaBarre, 2001) sequence from an 8 mL (2 cm × 2 cm × 2 cm) voxel placed in the right basal ganglia (Fig. 1). After water suppression was performed with variable pulse power and optimized relaxation delays (Tkac et al., 1999), all resonances were excited by using a nonselective numerically optimized 5.12 ms adiabatic half-passage pulse. Three-dimensional localization was then performed with a pair of adiabatic full-passage pulses in each dimension. Each adiabatic full-passage pulse was an offset-independent adiabatic pulse, HS1, with a pulse length of 8 ms and a bandwidth of 2.5 kHz (Garwood and DelaBarre, 2001; Silver et al., 1984). Each free induction decay (FID) was acquired with 2048 complex points and a spectral width of 1.5 kHz. FIDs were stored separately in memory and then both frequency and phase corrected based on NAA signal before summation. The T_R was 3 s, T_E was 70.8 ms, and the number of scans (N_S) was 192. A water reference was also acquired. Each voxel measurement began with an adjustment of the first- and second-order shims using the standard Siemens shimming method. In cases where poor water line-width was observed after the standard shimming method, FAST(EST)MAP was applied (Gruetter, 1993; Gruetter and Tkac, 2000).

2.4. MRS voxel placement

The MR spectroscopy voxel was positioned in the right basal ganglia using the T_1-weighted image. The caudate and putamen were the primary regions of interest. The voxel was positioned in the following way: (1) left/right—the voxel was positioned so that it was as medial as possible, without containing any portion of the lateral ventricle, (2) anterior/posterior—the voxel was positioned as anterior as possible in the caudate, without entering the anterior horn of the lateral ventricle, (3) superior/inferior—the voxel was positioned such that the inferior portion of the voxel was as close as possible to the most inferior aspect of the putamen (to avoid artifact from the vasculature inferior to this position), and such that the superior portion of the voxel was approximately 3 mm inferior to the most superior aspect of the caudate (to avoid signal contamination from the lateral ventricles). Fig. 1 illustrates the voxel placement in a typical subject. Confirmation of consistent voxel placement across subjects was achieved by segmenting and parcellating the T_1-weighted image.

2.5. MR data processing

2.5.1. Structural MRI data processing

A high-resolution structural scan was acquired to position the voxel during data acquisition and to determine the tissue composition of the voxel through segmentation. The T_1-weighted scan was processed using the standard FreeSurfer pipeline (http://surfer.nmr.mgh.harvard.edu) for tissue segmentation and anatomical parcellation (Fischl et al., 2002). Further details related to the FreeSurfer processing can be found online, and in one of our previous publications (Porter et al., 2011). In-house software was used to compute the transformation matrix from the scanner coordinates to the FreeSurfer-processed T_1-weighted image. A mask representing the spectroscopy voxel in the anatomical image space was then created using tools from the FMRIB Software Library (Smith et al., 2004), which was subsequently segmented and parcellated using the FreeSurfer anatomical information. Thus, each T_1-weighted voxel (1 mm isotropic resolution) within the spectroscopy volume (2 cm × 2 cm × 2 cm), was classified as either white matter, gray matter, cerebrospinal fluid (CSF), or non-brain, and was further

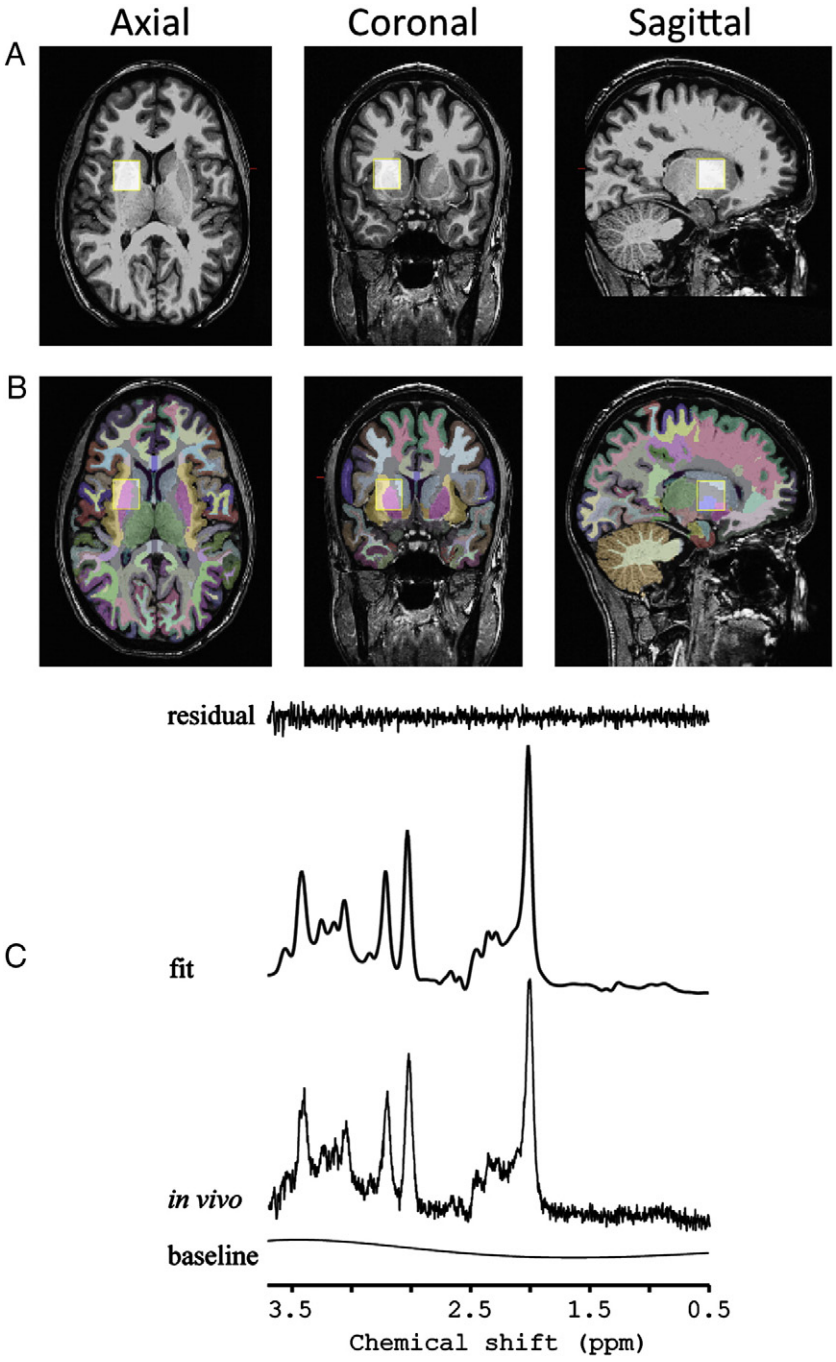

Fig. 1. Voxel placement and representative spectrum. The location and size ($2 \times 2 \times 2$ cm^3) of the voxel shown as yellow box on (A) T_1-weighted and (B) parcellated with FreeSurfer images in axial, coronal, and sagittal views. (C) *In vivo* data, LCModel fit, residual, and baseline for the representative spectrum. A close match between the LCModel fit and the *in vivo* spectrum was achieved as evidenced by the noise-dominated fit residual.

parcellated into subcortical and cortical structures. This was done to confirm a consistent voxel placement across all subjects (i.e., the majority of the spectroscopy voxel contents were within the basal ganglia in all subjects) and to determine the basic tissue composition within the voxel (i.e., gray matter, white matter, or CSF). Further details of the voxel composition can be found in the results section below.

2.5.2. Quantification

The acquired spectra were analyzed using LCModel 6.1-4A (Provencher, 1993, 2001)(Stephen Provencher, Inc., Oakville, Ontario, Canada), with the basis set generated using in-house programs based on the density matrix formalism (Henry et al., 2006) in Matlab (The MathWorks, Inc., Natick, MA, USA) using the known chemical shifts and J-couplings (Govindaraju et al., 2000; Kaiser et al., 2010). The simulated spectra of the following twenty metabolites were included in the basis set for LCModel: alanine (Ala), ascorbate (Asc), aspartate (Asp), Cr, GABA, glucose (Glc), Gln, Glu, glycerophosphorylcholine (GPC), glycine (Gly), glutathione (GSH), lactate (Lac), mIns, NAA, N-acetylaspartylglutamate (NAAG), phosphocreatine (PCr), phosphorylcholine (PCho), phosphorylethanolamine (PE), scyllo-inositol (sIns), and taurine (Tau). Experimentally measured metabolite-nulled macromolecular spectra from 41 subjects were also included in the basis set ($T_I = 827$ ms, $N_S = 64$). No baseline correction,

zero-filling or line broadening were applied to the *in vivo* data prior to the analysis. The LCModel fitting was performed over the spectral range from 0.5 to 4.2 ppm.

Subjects were excluded from the analysis if their NAA line-width was greater than 8 Hz. Further, criteria for selecting reliable metabolite concentrations were based on Cramér-Rao lower bounds (CRLB), which are estimates of the %SD of the fit for each metabolite (Provencher, 1993). Only results with a CRLB ≤30% were included in the analysis. Concentrations with CRLB >30% were classified as not detected. Only metabolites that had a CRLB below 30% in more than 75% of the spectra were included in the neurochemical profile. If the covariance between two metabolites was consistently high (inverse correlation coefficient <−0.5), such as in the case of Cr and PCr, their sum (total creatine, tCr) was reported rather than their individual values. The tCr concentration was quantified using the water reference. Since no difference was observed in tCr between groups, all subsequent metabolite concentrations were quantified using the tCr concentration (assumed to be 8 mM).

2.6. Statistical approach

Data were analyzed with the Statistical Package for the Social Sciences, version 19 (SPSS Inc., Chicago, IL, USA, www.spss.com). Data were examined for normality in order to ensure appropriateness of parametric statistics. Univariate analyses of covariance (ANCOVA) were used to test group effects between the MJU individuals and the controls, with age and alcohol use entered as covariates. Group and sex were both entered as between-subjects variables. Alcohol use frequency over the past 12-month period summarized by the PEI was used in the above model as the alcohol use covariate. Two-way interaction effects between group and sex, when present, were examined further by running the model separately in males and females (to quantify the effect of group within sex), or by examining sex effects within MJU individuals and controls (to examine the effect of sex within group). Finally, significant effects were re-evaluated by matching the MJU and control samples by age to verify that patterns remained significant with more stringent control over developmental differences that might otherwise impact the findings.

3. Results

3.1. Sample demographic characteristics

The MJU and control groups were well matched in terms of sex (chi squared test, $X^2(1, n = 53) = 2.29$, $p = 0.130$), and mean age ($F(1,51) = 0.047$, $p = 0.83$), despite the larger range in age in the controls. The groups were matched in estimated two-scale IQ ($F(1,51) = 0.49$, $p = 0.49$) with mean IQs in the high average range. There was no group by sex interaction for age or for IQ. Marijuana users were college students of middle to high-middle socioeconomic backgrounds and most were free of a non-substance DSM-IV Axis I diagnosis (Table 3). None were psychotic. Nearly all met DSM-IV diagnostic criteria for marijuana abuse or dependence. Use of other recreational drugs within the MJU group was limited, with no participants meeting DSM-IV criteria for abuse or dependence. One subject met diagnostic criteria for current alcohol dependence, and a small proportion met criteria for alcohol abuse (30%). Compared to controls, alcohol use over the past twelve months use was found to be significantly higher in the MJU group, $F(1,51) = 43.93$, $p < 0.001$. Marijuana users on average had a PEI score of 3.7, which corresponds to endorsing use of alcohol between 21 and 100 times in the previous 12 months. Controls on average had a PEI score of 1.5, which corresponds to endorsing use of alcohol between 1 and 20 times in the previous 12 months. When the sample is restricted to include only individuals aged 17 and higher, the difference in alcohol use remains significant but the mean value for control participants is slightly higher at 1.9. Thus, the amount of alcohol use endorsed over the past twelve months was entered as a covariate in analyses comparing metabolite concentrations between groups.

Table 3
Substance use characteristics in male versus female marijuana users.

Variable	Males	Females	F	P
Age of marijuana use onset	15.3 (0.95)	15.1 (1.4)	0.25	.63
Current marijuana use occasions past month	25.4 (4.5)	24.5 (3.5)	1.42	.25
Current marijuana use hits per day (past month)	11.3 (7.6)	7.1 (3.7)	2.85	.11
Maximum hits in 24 h (past year)	38.6 (24.9)	24.5 (19.7)	2.19	.15
Number of symptoms of marijuana dependence	4.1 (1.5)	3.5 (2.2)	0.98	.33
Number of symptoms of alcohol dependence	1.6 (1.6)	0.45 (0.52)	4.89	.05
Proportion with presence of non-marijuana and non-alcohol related DSM-IV psychopathology (current or past)	0.06	0.36		.13[a]

[a] Note: Fisher's Exact Test was used due to insufficient cell counts for the Chi-Square test.

The marijuana users reported that their age of first use of marijuana was 15.2 ± 1.2 years, and also reported smoking 9.8 (±8.7) hits per day during the past year. In addition, supplemental analyses were conducted to verify that female users did not differ from male users in their self-reported patterns of use, age of use onset, use of alcohol, or symptoms of psychopathology (based on K-SADS screening items and supplement items when applicable). Findings are presented in Table 3. The only group difference to emerge was that female users reported fewer symptoms overall of alcohol abuse/dependence than did males. Otherwise, they did not significantly differ in variables that would suggest an increased frequency or duration of marijuana use, use of other substances, or presence of concomitant psychopathology.

3.2. Voxel tissue composition

The spectroscopy voxel was consistently placed in the same anatomical location, centered in striatum, in both marijuana users and controls (Table 4). The voxel was primarily composed of gray matter, as determined by the FreeSurfer parcellation procedure. The majority of the voxel composition (roughly 98% of the volume) was statistically similar between groups, with the exception of the pars opercularis, which accounted for less than 1% of the total voxel composition. The remaining 2% of the voxel composition was relatively variable (i.e., the parcellations not included in Table 4). Moreover, these additional regions always represented very small amounts of tissue (<1% of the total voxel volume in all cases and <0.5% in most cases), and were not represented in all subjects.

3.3. Data quality

Independent samples t-tests were used to examine measures of data quality, and no differences were found between marijuana users and controls in line-width ($t = 0.91(51)$, $p = 0.37$) or SNR ($t = -1.05(51)$, $p = 0.30$), Table 5.

3.4. Group comparisons of metabolite concentrations

Table 5 shows study sample sizes for each metabolite after quality control criteria (CRLB, line-width, minimum SNR) were applied to the data. When these criteria were applied, there were sufficient cases to examine, NAA + NAAG (tNAA), tCr, total choline (tCho), Glx, mIns, and Tau + Glc. In total, two MJU subjects and three controls were

Table 4
Subcortical volumes represented within the MRS voxel.

	Volume proportion		*p*
FreeSurfer Label	MJU	Controls	
Putamen	0.451	0.445	0.635
Insula (white)	0.169	0.160	0.339
Unsegmented (white)	0.150	0.144	0.609
Pallidum	0.108	0.122	0.094
Insula	0.058	0.055	0.787
Caudate	0.036	0.035	0.922
Pars Opercularis	**0.008**	**0.004**	**0.062**
Vessel (other)	0.005	0.005	0.570
Sum of Labels	0.982	0.977	0.358

Note: The labels listed in Table 3 are those that are represented in both controls and marijuana users, and make up roughly 98% of the tissue within the spectroscopy voxel for both groups. The remaining tissue classifications within the voxel (i.e., 2% of the tissue) were not represented in both groups (i.e., meaningful statistics could not be computed). Structures are classified as gray matter unless otherwise noted in parentheses next to the FreeSurfer label and bold indicates there was a group difference in volume.

excluded from analyses based on line-width (Table 5). The additional two MJU subjects and one control subject were excluded from analyses of Tau + Glc due to high CRLB. Table 5 also shows descriptive statistics for each metabolite, including means and CRLB for marijuana users and controls. As described above, metabolite concentrations were examined after scaling to tCr since no difference was observed in tCr between groups when using water as the reference ($p > 0.05$).

Univariate ANCOVAs with age and alcohol use entered as covariates, were used to compare metabolite concentrations in the MJU and control groups, and to examine sex differences. Findings are presented in Table 6. No main effects of group, sex, age or alcohol use were observed for tNAA or Tau + Glc.

A main effect of sex was observed in the tCho, independent of group, age, and alcohol use (Table 6). Males demonstrated higher levels. There was no significant effect of group nor was the group by sex interaction significant.

When mIns was examined, there were no main effects of group or sex, but there was a significant group by sex interaction (Table 6). When the sexes were examined separately, there was no main effect of group within males ($p = 0.37$), but there was a significant group difference in females, $F(1,20) = 6.48$, $p = 0.02$, $\acute{\eta}_p^2 = 0.25$ with higher values in MJUs versus controls. Within users, females also demonstrated higher values than males, $F(1,21) = 5.39$, $p = 0.03$, $\acute{\eta}_p^2 = 0.20$.

Similarly, for the analysis of Glx, there were no main effects of group or sex, controlling for age and alcohol use, but there was a significant group by sex interaction (Table 6). When the sexes were examined separately, there was a main effect of group within females, $F(1,23) = 4.99$, $p = 0.04$, $\acute{\eta}_p^2 = 0.20$, while male MJ users did not differ statistically from male controls ($p = 0.92$, $\acute{\eta}_p^2 = 0.00$). Further analyses indicated that within controls, males and females did not differ in their values ($p = 0.30$, $\acute{\eta}_p^2 = 0.06$). Within users, female MJ users showed lower Glx than male MJ users, $F(1,24) = 8.02$, $p = 0.01$, $\acute{\eta}_p^2 = 0.28$.

Although age was not statistically different between the MJU group and the control group, it was verified that in the above Glx and mIns analyses, age was not a significant contributor to either model. In addition, further ANCOVA analyses were conducted to restrict the overall age range in the control group. For this analysis, the six control subjects who were younger than 17 years of age were removed, and the ANCOVAs, with group and sex as between subjects factors, and age and alcohol use as covariates, were re-run. This analysis yielded similar effects to those described above. For Glx, the two-way interaction effect between group and sex was even stronger than what was observed in the full sample. The group by sex interaction was reduced to a trend level ($p = 0.09$) for mIns but with a similar effect size ($\acute{\eta}_p^2 = 0.08$) suggesting that a reduction in statistical power accounted for the loss of significance.

Lastly, while not a significant predictor in our models, alcohol use was further explored for interaction effects in Glx and mIns given the group-by-sex interaction found for these metabolites, and because alcohol use was less prominent in females in the MJU group. When a group-by-alcohol use (summarized by the PEI) interaction term was added to the model, the results remained unchanged in relation to the group-by-sex interaction, and the new interaction term was not significant. The addition of a sex-by-alcohol use interaction term was not significant, though it did result in slightly larger p-values for the mIns group-by-sex interaction, and also the Glx group-by-sex interaction. However, in both metabolites, the group-by-sex interaction remained with a trend-level p-value. It does not seem, then, that the extent of alcohol use within the MJU group drives the group-by-sex interaction that was observed for Glx and mIns. The study is not adequately powered to be able to reliably detect a three-way group-by-sex-by-alcohol use interaction.

4. Discussion

This study examined a cohort of college-aged heavy marijuana users and a control group of non-using young-adults. Using MR-spectroscopy, it was shown that females, but not males, who used marijuana heavily starting in mid-adolescence and persisting for several years have lower levels of glutamate and glutamine (scaled to tCr) in the dorsal striatum when compared to controls, even after accounting for age and alcohol use. Similarly, female but not male users differ from controls in their estimated concentrations of *myo*-inositol, demonstrating higher levels than controls. These patterns are interpreted as pathological in the female users given that male users had comparable levels to controls of both sexes. Female users did not differ from male users in their overall rates of self-reported marijuana use, in their concomitant level of alcohol use (though they did report fewer alcohol-related symptoms), in their numbers of symptoms of marijuana dependence, or presence of other conditions that might impact brain metabolism.

Table 5
MRS quality measures and metabolite summary.

	Cumulative	MJU			Control		
Measure	% of sample	N	Mean ± SD	CRLB ± SD	N	Mean ± SD	CRLB ± SD
Line-width$_{NAA}$(Hz)	100	27	6.25 ± 1.40	–	26	6.65 ± 1.78	–
SNR$_{NAA}$	100	27	25.5 ± 3.6	–	26	24.5 ± 3.2	–
tNAA	90	25	11.1 ± 0.52	1.12 ± 0.33	23	10.9 ± 0.71	1.22 ± 0.42
tCho	90	25	1.84 ± 0.19	3.12 ± 0.67	23	1.74 ± 0.19	3.30 ± 0.56
Glx	90	25	10.1 ± 1.37	6.84 ± 1.28	23	10.7 ± 0.94	6.57 ± 0.99
mIns	90	25	3.57 ± 0.74	10.6 ± 4.06	23	3.55 ± 0.80	10.5 ± 4.02
Tau + Glc	85	23	1.70 ± 0.48	17.1 ± 4.69	22	1.70 ± 0.46	18.1 ± 6.26
tCr	90	25	8.00 ± 0.00	1.76 ± 0.44	23	8.00 ± 0.00	1.96 ± 0.21

Note: *NAA signal was used to estimate linewidth and SNR in LCModel.*

Table 6
ANCOVA results comparing metabolite concentrations in mju and control groups by sex.

	MJU		Control										
	Male	Female	Male	Female	*Group*			*Sex*			*Group by sex*		
Metabolite	M (SEM)	M (SEM)	M (SEM)	M (SEM)	*F*	p	η^2_p	*F*	p	η^2_p	*F*	p	η^2_p
tNAA	11.29 (.23)	11.05 (.19)	10.68 (.26)	10.92 (.19)	1.58	.22	.04	.00	.98	.00	1.28	.26	.03
tCho	1.93 (.07)	1.77 (.05)	1.85 (.07)	1.62 (.05)	2.06	.16	.05	14.68	.00	.26	0.30	.59	.01
Glx	10.87 (.42)	9.47 (.35)	10.12 (.47)	10.82 (.35)	.33	.57	.01	1.12	.30	.03	7.98	.01	.16
mIns	3.40 (.28)	3.89 (.23)	3.81 (.31)	3.26 (.24)	.10	.76	.00	.02	.90	.00	4.23	.05	.09
Tau + Glc	1.58 (.20)	1.75 (.15)	1.94 (.20)	1.58 (.16)	.16	.69	.00	.40	.53	.01	2.68	.11	.06

Mean values represent estimated marginal means ± 1 SEM, controlling for the effects of age and alcohol use frequency. Statistics are presented on tests of the estimated marginal means.

These findings have broad parallels in the extant literature, both in relation to the overall patterns observed but also in relation to sex differences. Decreased glutamate/glutamine concentrations have been reported in two other MRS studies of marijuana users, one that focused on the basal ganglia (Chang et al., 2006) and one that targeted the anterior cingulate cortex (Prescot et al., 2011). First, in an older cohort of marijuana users than is described in the current study, Chang et al. (2006) reported lower glutamate levels in the basal ganglia, suggesting that heavy marijuana use during young adulthood as well as later in life is associated with disruptions in glutamate signaling as has been shown for other drugs of abuse (Kalivas et al., 2009). Recently, Prescot et al. (2011) reported lower glutamate concentrations in the anterior cingulate cortex, which was nonetheless strongest when females were eliminated from the analysis. Interpretation of the current findings is complicated by poor resolution of the glutamate versus glutamine signal. Glutamate is present in all cell types with the largest pools evident in glutamatergic neurons; smaller pools are evident in GABA-ergic neurons and astroglia. Upon release, astroglia convert glutamate to glutamine, which in turn is transferred back to the neuron for conversion once again to glutamate (Albrecht et al., 2010; Daikhin and Yudkoff, 2000). Glutamine is primarily located in astroglia. Thus, low glutamate levels would be difficult to ascribe to a particular neuronal process. In contrast, if glutamine levels are low, then glial dysfunction may be present, a finding that would be consistent with white matter aberrations in marijuana users (Matochik et al., 2005; Zalesky et al., 2012).

Others have not reported specific metabolic disruptions in female marijuana users; indeed, within young samples, marijuana is more commonly used in males (Johnston et al., 2011). Although it has been recognized that females are at an increased risk for some behavioral consequences of drug use such as sexual risk-taking (Hallfors et al., 2005) and an increased risk of depression and anxiety following a pattern of daily marijuana use (Patton et al., 2002), there are relatively few human studies of brain-based sex differences associated with marijuana. Women have shown slightly more severe neurocogntive deficits related to marijuana use compared to men (Pope et al., 1997). McQueeny et al. (2011) showed adolescent girls had larger amygdalae and increased internalizing symptoms when compared to both control and marijuana using boys. Moreover, certain behavioral problems have also been linked to prenatal marijuana exposure in girls, but not in boys (El Marroun et al., 2011). Recent neuroimaging work suggests that young female users may be vulnerable to marijuana-induced alterations in brain volume, given suggestions of greater prefrontal cortex volumes and relatively poorer levels of executive function (Medina et al., 2009). Alcohol is similarly disruptive to females' cognitive function and regional brain morphology (Medina et al., 2008; Squeglia et al., 2009b), and it has long been recognized that females are more vulnerable to psychomotor sensitization with psychostimulant exposure (Camp and Robinson, 1988).

Preclinical data are somewhat stronger and indicate that female adolescents are particularly vulnerable to the effects of long-term THC administration on the CB_1 receptor system in multiple brain regions, including the prefrontal cortex, striatum, and periaqueductal gray (Burston et al., 2010). A recent study of THC in mid-adolescent rats during the period of drug administration and following abstinence indicated greater sensitization of THC-induced locomotor depression in females versus males. Moreover, high doses resulted in increased anxiety-like behaviors during THC administration, particularly in females (Harte-Hargrove and Dow-Edwards, 2012), although a general tendency is for females to experience greater anxiolytic effects of the drug. Glutamate is critically important in the neuroplasticity that accompanies the transition from drug use to abuse (Kalivas and Volkow, 2005). Under conditions of extreme trauma or stress, its release is associated with neurotoxicity and cell death (Wang and Qin, 2010). Endocannabinoids block glutamate release under such conditions (Gerdeman and Lovinger, 2001), which could lead to neuroprotection. However, the concomitant observation of high mIns levels argues against this interpretation. Given that mIns is considered to be a glial marker, high levels would be associated with gliosis as well as white matter injury as occurs in the context of neural injury. High mIns concentrations have been observed in early dementia, in frank Alzheimer's disease, as well as in abstinent methamphetamine users, although this latter observation was in the frontal lobes (Ernst et al., 2000; Huang et al., 1999; Jack, 2012). This pattern is intriguing given that deficits in learning and memory represent one of the robust areas of reported cognitive dysfunction in marijuana users (Solowij and Battisti, 2008). Although our data analyses do not suggest that female marijuana users in this sample are more vulnerable to cognitive impairments (Becker et al., under review), this is a relatively young and high functioning sample. It may be that frank behavioral deficits will emerge more strongly in females over time as chronicity of use progresses. We hypothesize, too, that we may have observed altered NAA levels had we also measured frontal concentrations of each metabolite.

Even though our statistical analyses do not show any significant effect of alcohol, it is important to consider the possibility of an underlying biological interaction between the two substances. Male marijuana users in this study had the highest levels of alcohol use, but did not show significant neurochemical alterations relative to controls. Females showed the greatest apparent impact of marijuana use on Glx and mIns, but in the context of lower levels of alcohol use. These findings could suggest a neuroprotective effect in individuals who use both marijuana and alochol, as described by others (Squeglia et al., 2009a). Alternatively, previous work has shown greater levels of Glx in the anterior cingulate of chronic alcohol users relative to controls (Yeo et al., 2013). Considering this, taken together with the findings of the present study, it is possible use of the two substances together may drive metabolite concentrations to “normal” levels via opposing processes, as has also been suggested by others in the context of brain morphology (Squeglia et al., 2009a). Differences in metabolic function in heavier versus lighter alcohol users can also impact the conversion of acetate into glutamate (Jiang et al., 2013). It is possible, then, that the male marijuana users in this study who were heavier alcohol users as compared to females, demonstrated differences in glutamate metabolism, contributing to the observed sex difference. However this

assertion is only speculative. While our data do not fully support these conclusions, the issue of alcohol use in the context of marijuana use requires careful examination in future studies.

Sex but not group-related effects were also observed in total choline estimated concentrations (scaled to total creatine). Independent of marijuana use, males showed higher estimated concentrations of tCho compared to females. Numerous choline-containing compounds contribute to the tCho signal measured in this study, complicating the interpretation of this sex difference. For example, phosphatidylcholine plays an important role in the phospholipid bilayer in cell membranes, and choline is essential in the formation of the neurotransmitter acetylcholine. Generally speaking, increases in choline signal in the brain have been demonstrated in cases with pathology (Govindaraju et al., 2000).

5. Limitations of the study

While this study has numerous strengths, it is not without limitations. Given time constraints on the scanning protocol, glutamate and glutamine could not be resolved separately from the acquired spectra. Even though this is a common problem, especially at lower field strengths (i.e., 3 T and lower), it poses limitations on the interpretation of the data because of the different biochemical functions of these metabolites. After release of glutamate into the synapse, cycling between glutamate and glutamine occurs in glial support cells in order to maintain high SNR in glutamatergic neurons, and to protect against adverse excitotoxic effects (Daikhin and Yudkoff, 2000). Resolution of the glutamate versus glutamine signals would allow stronger interpretations to be offered regarding the meaning of the low levels observed in female users. Given that more extensive spectroscopy scanning is time-intensive and requires higher field strengths to be conducted most efficiently, these findings together with other recent studies (Chang et al., 2006; Prescot et al., 2011; Silveri et al., 2011) suggest that a more in-depth examination of neurochemical metabolism within frontostriatal circuits in heavy marijuana users is warranted. Another limitation of the study is the constrained spatial resolution of the spectra. It would be beneficial to examine additional brain structures, however spectral resolution was chosen over spatial resolution for the current study. Moreover, while the sample sizes are small in relation to the reported group by sex interactions, numerous reports exist which demonstrate a similar a pattern of sex-effects, where females who use or are exposed to illicit substances (including marijuana) are differentially affected (El Marroun et al., 2011; McQueeny et al., 2011; Squeglia et al., 2011). Finally, we did not measure urine or hair concentrations of THC, so it is possible that participants in the study used less marijuana than they reported. We find this to be unlikely given the level of detail that was provided about habits surrounding use in our direct interviews, participants' consistent reporting regarding their symptoms of DSM-IV marijuana dependence, and concomitant evidence of neurocognitive impairment consistent with marijuana exposure (Becker et al., under review). Further, the majority of previous studies that collected urine/hair data and quantified cannabinoid concentrations did not show significant associations between these concentrations and brain metabolite data, suggesting such data are perhaps not necessary for this type of analysis in the presence of detailed clinical assessments. Nonetheless, the study would be strengthened by the ability to compare brain metabolic data with cannabinoid levels as obtained by blood, hair or urine analysis.

6. Conclusion

Marijuana use is becoming more prevalent on college campuses and its legalization is being increasingly discussed and advocated (Caulkins et al., 2012; SAMHSA, 2011). The sample studied here is representative of relatively high functioning college students (and thus typical of users on college campuses) in terms of their higher-than-average IQs, middle income status, and low risk for other forms of psychopathology. Alcohol use, which was more extreme in the drug user sample, was controlled in the data analysis. Thus, the patterns observed here can be more readily linked to marijuana exposure and are not likely due to the presence of other confounds that have been raised in other studies such as comorbid depression and concomitant psychoactive medication use (Prescot et al., 2011), as well as limitations of analyses restricted to only one sex (Hermann et al., 2007; Silveri et al., 2011), Moreover, like Silveri et al. (2011), this study focuses on individuals in the midst of active use versus abstinence or withdrawal and thus represents a snapshot of how the brain is metabolically functioning during daily life.

Moving forward, it will be important to collect additional data, which has both higher spectral and spatial resolution. Having higher spectral resolution will allow for the distinction between glutamate and glutamine to be made, and will also allow for additional metabolites to be quantified. Better spatial resolution will allow for researchers to decipher whether the effects described in this report are localized to the basal ganglia, or if they are distributed throughout cortical and subcortical regions. Moreover, the sex differences reported here suggest intriguing avenues through which hormonal state might interact with neurochemistry in the basal ganglia to impact that region's integrity of function in the context of drug use. In addition, the question of whether marijuana use leads to tissue damage, or whether the neurochemical imbalances observed here represent characteristics inherent to those who use the substance on a regular basis, remains unclear. Prospective longitudinal studies are needed to follow individuals over time, prior to and after the initiation of substance use, to gain a better understanding of the exact interplay between substance use and the underlying neurophysiology.

Acknowledgments

This study was supported by grant R01DA017843 awarded to M. Luciana by the National Institute on Drug Abuse, by grant R01AA020033 awarded to M. Luciana by the National Institute on Alcohol Abuse and Alcoholism, by the University of Minnesota's Center for Neurobehavioral Development, by grants P41 RR008079 (NCRR), P41 EB015894 (NIBIB) and P30 NS057091 (NINDS) awarded to the University of Minnesota's Center for Magnetic Resonance Research, and by the Minnesota Supercomputing Institute. We are grateful for the contributions of the research staff who helped with subject recruitment and data acquisition, including Zach Grice-Patil, Daniel Johnson, James Porter, Ann Schissel, Brittany Schmaling, and Sasha Sommerfeldt. We would also like to thank the participants who partook in this research.

References

Albrecht, J., Sidoryk-Wegrzynowicz, M., Zielinska, M., Aschner, M., 2010. Roles of glutamine in neurotransmission. Neuron Glia Biology 6, 263–276.
Becker, M.P., Collins, P.F., Luciana, M. 2013. Neurocognition in college-aged daily marijuana users (Manuscript under review).
Burston, J.J., Wiley, J.L., Craig, A.A., Selley, D.E., Sim-Selley, L.J., 2010. Regional enhancement of cannabinoid CB1 receptor desensitization in female adolescent rats following repeated Delta-tetrahydrocannabinol exposure. British Journal of Pharmacology 161, 103–112.
Camp, D.M., Robinson, T.E., 1988. Susceptibility to sensitization. I. Sex differences in the enduring effects of chronic D-amphetamine treatment on locomotion, stereotyped behavior and brain monoamines. Behavioural Brain Research 30, 55–68.
Caulkins, J.P., Kilmer, B., MacCoun, R.J., Pacula, R.L., Reuter, P., 2012. Design considerations for legalizing cannabis: lessons inspired by analysis of California's Proposition 19. Addiction 107, 865–871.
Chang, L., Cloak, C., Yakupov, R., Ernst, T., 2006. Combined and independent effects of chronic marijuana use and HIV on brain metabolites. Journal of Neuroimmune Pharmacology 1, 65–76.
Daikhin, Y., Yudkoff, M., 2000. Compartmentation of brain glutamate metabolism in neurons and glia. Journal of Nutrition 130, 1026S–1031S.
de Graaf, R., 2007. In Vivo NMR Spectroscopy, 2nd ed. Wiley-Interscience, Chichester, England.
El Marroun, H., Hudziak, J.J., Tiemeier, H., Creemers, H., Steegers, E.A., Jaddoe, V.W., Hofman, A., Verhulst, F.C., van den Brink, W., Huizink, A.C., 2011. Intrauterine cannabis exposure leads to more aggressive behavior and attention problems in 18-month-old girls. Drug and Alcohol Dependence 118, 470–474.

Ernst, T., Chang, L., Leonido-Yee, M., Speck, O., 2000. Evidence for long-term neurotoxicity associated with methamphetamine abuse: a 1H MRS study. Neurology 54, 1344–1349.
Fischl, B., Salat, D.H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., van der Kouwe, A., Killiany, R., Kennedy, D., Klaveness, S., Montillo, A., Makris, N., Rosen, B., Dale, A.M., 2002. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 33, 341–355.
Garwood, M., DelaBarre, L., 2001. The return of the frequency sweep: designing adiabatic pulses for contemporary NMR. Journal of Magnetic Resonance 153, 155–177.
Gerdeman, G., Lovinger, D.M., 2001. CB1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. Journal of Neurophysiology 85, 468–471.
Gerdeman, G.L., Partridge, J.G., Lupica, C.R., Lovinger, D.M., 2003. It could be habit forming: drugs of abuse and striatal synaptic plasticity. Trends in Neurosciences 26, 184–192.
Govindaraju, V., Young, K., Maudsley, A.A., 2000. Proton NMR chemical shifts and coupling constants for brain metabolites. NMR in Biomedicine 13, 129–153.
Gruetter, R., 1993. Automatic, localized in vivo adjustment of all first- and second-order shim coils. Magnetic Resonance in Medicine 29, 804–811.
Gruetter, R., Tkac, I., 2000. Field mapping without reference scan using asymmetric echo-planar techniques. Magnetic Resonance in Medicine 43, 319–323.
Hall, W., Solowij, N., 1998. Adverse effects of cannabis. Lancet 352, 1611–1616.
Hallfors, D.D., Waller, M.W., Bauer, D., Ford, C.A., Halpern, C.T., 2005. Which comes first in adolescence—sex and drugs or depression? American Journal of Preventive Medicine 29, 163–170.
Hampson, A.J., Grimaldi, M., Axelrod, J., Wink, D., 1998. Cannabidiol and (−)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. Proceedings of the National Academy of Sciences of the United States of America 95, 8268–8273.
Harte-Hargrove, L.C., Dow-Edwards, D.L., 2012. Withdrawal from THC during adolescence: sex differences in locomotor activity and anxiety. Behavioural Brain Research 231, 48–59.
Henly, G.A., Winters, K.C., 1989. Development of psychosocial scales for the assessment of adolescents involved with alcohol and drugs. International Journal of the Addictions 24, 973–1001.
Henry, P.G., Marjanska, M., Walls, J.D., Valette, J., Gruetter, R., Ugurbil, K., 2006. Proton-observed carbon-edited NMR spectroscopy in strongly coupled second-order spin systems. Magnetic Resonance in Medicine 55, 250–257.
Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., de Costa, B.R., Rice, K.C., 1990. Cannabinoid receptor localization in brain. Proceedings of the National Academy of Sciences of the United States of America 87, 1932–1936.
Hermann, D., Sartorius, A., Welzel, H., Walter, S., Skopp, G., Ende, G., Mann, K., 2007. Dorsolateral prefrontal cortex N-acetylaspartate/total creatine (NAA/tCr) loss in male recreational cannabis users. Biological Psychiatry 61, 1281–1289.
Hoffman, A.F., Lupica, C.R., 2001. Direct actions of cannabinoids on synaptic transmission in the nucleus accumbens: a comparison with opioids. Journal of Neurophysiology 85, 72–83.
Huang, W., Alexander, G.E., Daly, E.M., Shetty, H.U., Krasuski, J.S., Rapoport, S.I., Schapiro, M.B., 1999. High brain myo-inositol levels in the predementia phase of Alzheimer's disease in adults with Down's syndrome: a 1H MRS study. The American Journal of Psychiatry 156, 1879–1886.
Jack Jr., C.R., 2012. Alzheimer disease: new concepts on its neurobiology and the clinical role imaging will play. Radiology 263, 344–361.
Jiang, L., Gulanski, B.I., De Feyter, H.M., Weinzimer, S.A., Pittman, B., Guidone, E., Koretski, J., Harman, S., Petrakis, I.L., Krystal, J.H., Mason, G.F., 2013. Increased brain uptake and oxidation of acetate in heavy drinkers. The Journal of Clinical Investigation 123, 1605–1614.
Johnston, L.D., O'Malley, P.M., Bachman, J.G., Schulenberg, J.E., 2011. Monitoring the future national survey results on drug use, 1975–2010. Institute for Social Research.The University of Michigan, Ann Arbor.
Kaiser, L.G., Marjanska, M., Matson, G.B., Iltis, I., Bush, S.D., Soher, B.J., Mueller, S., Young, K., 2010. (1)H MRS detection of glycine residue of reduced glutathione in vivo. Journal of Magnetic Resonance 202, 259–266.
Kalivas, P.W., Volkow, N.D., 2005. The neural basis of addiction: a pathology of motivation and choice. The American Journal of Psychiatry 162, 1403–1413.
Kalivas, P.W., Lalumiere, R.T., Knackstedt, L., Shen, H., 2009. Glutamate transmission in addiction. Neuropharmacology 56 (Suppl. 1), 169–173.
Kaufman, J., Birmaher, B., Brent, D., Rao, U., Flynn, C., Moreci, P., Williamson, D., Ryan, N., 1997. Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. Journal of the American Academy of Child and Adolescent Psychiatry 36, 980–988.
Koob, G.F., Volkow, N.D., 2010. Neurocircuitry of addiction. Neuropsychopharmacology 35, 217–238.
Marsicano, G., Goodenough, S., Monory, K., Hermann, H., Eder, M., Cannich, A., Azad, S.C., Cascio, M.G., Gutierrez, S.O., van der Stelt, M., Lopez-Rodriguez, M.L., Casanova, E., Schutz, G., Zieglgansberger, W., Di Marzo, V., Behl, C., Lutz, B., 2003. CB1 cannabinoid receptors and on-demand defense against excitotoxicity. Science 302, 84–88.
Matochik, J.A., Eldreth, D.A., Cadet, J.L., Bolla, K.I., 2005. Altered brain tissue composition in heavy marijuana users. Drug and Alcohol Dependence 77, 23–30.
McGrath, J., Welham, J., Scott, J., Varghese, D., Degenhardt, L., Hayatbakhsh, M.R., Alati, R., Williams, G.M., Bor, W., Najman, J.M., 2010. Association between cannabis use and psychosis-related outcomes using sibling pair analysis in a cohort of young adults. Archives of General Psychiatry 67, 440–447.
McQueeny, T., Padula, C.B., Price, J., Medina, K.L., Logan, P., Tapert, S.F., 2011. Gender effects on amygdala morphometry in adolescent marijuana users. Behavioural Brain Research 224, 128–134.
Medina, K.L., McQueeny, T., Nagel, B.J., Hanson, K.L., Schweinsburg, A.D., Tapert, S.F., 2008. Prefrontal cortex volumes in adolescents with alcohol use disorders: unique gender effects. Alcoholism, Clinical and Experimental Research 32, 386–394.
Medina, K.L., McQueeny, T., Nagel, B.J., Hanson, K.L., Yang, T.T., Tapert, S.F., 2009. Prefrontal cortex morphometry in abstinent adolescent marijuana users: subtle gender effects. Addiction Biology 14, 457–468.
Mittleman, M.A., Lewis, R.A., Maclure, M., Sherwood, J.B., Muller, J.E., 2001. Triggering myocardial infarction by marijuana. Circulation 103, 2805–2809.
Muetzel, R.L., Collins, P.F., Mueller, B.A.A.M.S., Lim, K.O., Luciana, M., 2008. The development of corpus callosum microstructure and associations with bimanual task performance in healthy adolescents. NeuroImage 39, 1918–1925.
Mugler III, J.P., Brookeman, J.R., 1990. Three-dimensional magnetization-prepared rapid gradient-echo imaging (3D MP RAGE). Magnetic Resonance in Medicine: Official Journal of the Society of Magnetic Resonance in Medicine/Society of Magnetic Resonance in Medicine 15, 152–157.
Olson, E.A., Collins, P.F., Hooper, C.J., Muetzel, R., Lim, K.O., Luciana, M., 2009. White matter integrity predicts delay discounting behavior in 9- to 23-year-olds: a diffusion tensor imaging study. Journal of Cognitive Neuroscience 21, 1406–1421.
Patton, G.C., Coffey, C., Carlin, J.B., Degenhardt, L., Lynskey, M., Hall, W., 2002. Cannabis use and mental health in young people: cohort study. BMJ 325, 1195–1198.
Pertwee, R.G., 2008. Ligands that target cannabinoid receptors in the brain: from THC to anandamide and beyond. Addiction Biology 13, 147–159.
Pope Jr., H.G., Yurgelun-Todd, D., 1996. The residual cognitive effects of heavy marijuana use in college students. Journal of the American Medical Association 275, 521–527.
Pope Jr., H.G., Jacobs, A., Mialet, J.P., Yurgelun-Todd, D., Gruber, S., 1997. Evidence for a sex-specific residual effect of cannabis on visuospatial memory. Psychotherapy and Psychosomatics 66, 179–184.
Porter, J.N., Collins, P.F., Muetzel, R.L., Lim, K.O., Luciana, M., 2011. Associations between cortical thickness and verbal fluency in childhood, adolescence, and young adulthood. NeuroImage 55, 1865–1877.
Prescot, A.P., Locatelli, A.E., Renshaw, P.F., Yurgelun-Todd, D.A., 2011. Neurochemical alterations in adolescent chronic marijuana smokers: a proton MRS study. NeuroImage 57, 69–75.
Provencher, S.W., 1993. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magnetic Resonance in Medicine 30, 672–679.
Provencher, S.W., 2001. Automatic quantitation of localized in vivo 1H spectra with LCmodel. NMR in Biomedicine 14, 260–264.
SAMHSA, 2011. Substance Abuse and Mental Health Services Administration. Results from the 2010 National Survey on Drug Use and Health: Summary of National Findings.Substance Abuse and Mental Health Services Administration, Rockville, MD.
Schlicker, E., Kathmann, M., 2001. Modulation of transmitter release via presynaptic cannabinoid receptors. Trends in Pharmacological Sciences 22, 565–572.
Sherrill, D.L., Krzyzanowski, M., Bloom, J.W., Lebowitz, M.D., 1991. Respiratory effects of non-tobacco cigarettes: a longitudinal study in general population. International Journal of Epidemiology 20, 132–137.
Silver, M.S., Joseph, R.I., Hoult, D.I., 1984. Highly selective Pi/2 and Pi-pulse generation. Journal of Magnetic Resonance 59, 347–351.
Silveri, M.M., Jensen, J.E., Rosso, I.M., Sneider, J.T., Yurgelun-Todd, D.A., 2011. Preliminary evidence for white matter metabolite differences in marijuana-dependent young men using 2D J-resolved magnetic resonance spectroscopic imaging at 4 Tesla. Psychiatry Research 191, 201–211.
Smith, S.M., Jenkinson, M., Woolrich, M.W., Beckmann, C.F., Behrens, T.E., Johansen-Berg, H., Bannister, P.R., De Luca, M., Drobnjak, I., Flitney, D.E., Niazy, R.K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J.M., Matthews, P.M., 2004. Advances in functional and structural MR image analysis and implementation as FSL. NeuroImage 23 (Suppl. 1), S208–S219.
Solowij, N., Battisti, R., 2008. The chronic effects of cannabis on memory in humans: a review. Current Drug Abuse Reviews 1, 81–98.
Squeglia, L.M., Jacobus, J., Tapert, S.F., 2009a. The influence of substance use on adolescent brain development. Clinical Eeg and Neuroscience: Official Journal of the Eeg and Clinical Neuroscience Society 40, 31–38.
Squeglia, L.M., Spadoni, A.D., Infante, M.A., Myers, M.G., Tapert, S.F., 2009b. Initiating moderate to heavy alcohol use predicts changes in neuropsychological functioning for adolescent girls and boys. Psychology of Addictive Behaviors: Journal of the Society of Psychologists in Addictive Behaviors 23, 715–722.
Squeglia, L.M., Schweinsburg, A.D., Pulido, C., Tapert, S.F., 2011. Adolescent binge drinking linked to abnormal spatial working memory brain activation: differential gender effects. Alcoholism, Clinical and Experimental Research 35, 1831–1841.
Szabo, B., Schlicker, E., 2005. Effects of cannabinoids on neurotransmission. Handbook of Experimental Pharmacology 327–365.
Szabo, B., Dorner, L., Pfreundtner, C., Norenberg, W., Starke, K., 1998. Inhibition of GABAergic inhibitory postsynaptic currents by cannabinoids in rat corpus striatum. Neuroscience 85, 395–403.
Tkac, I., Starcuk, Z., Choi, I.Y., Gruetter, R., 1999. In vivo 1H NMR spectroscopy of rat brain at 1 ms echo time. Magnetic Resonance in Medicine 41, 649–656.
Wang, Y., Qin, Z.H., 2010. Molecular and cellular mechanisms of excitotoxic neuronal death. Apoptosis: An International Journal on Programmed Cell Death 15, 1382–1402.
Wechsler, D., 1999. Manual for the Wechsler Abbreviated Scale of Intelligence. The Psychological Corporation, San Antonio, TX.
Yeo, R.A., Thoma, R.J., Gasparovic, C., Monnig, M., Harlaar, N., Calhoun, V.D., Kalyanam, R., Mayer, A.R., Durazzo, T.C., Hutchison, K.E., 2013. Neurometabolite concentration and clinical features of chronic alcohol use: a proton magnetic resonance spectroscopy study. Psychiatry Research 211, 141–147.
Zalesky, A., Solowij, N., Yucel, M., Lubman, D.I., Takagi, M., Harding, I.H., Lorenzetti, V., Wang, R., Searle, K., Pantelis, C., Seal, M., 2012. Effect of long-term cannabis use on axonal fibre connectivity. Brain: A Journal of Neurology 135, 2245–2255.