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Research report

Combined effects of marijuana and nicotine on memory performance and hippocampal volume

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HIGHLIGHTS

- Examined drug effects of tobacco, marijuana and combined marijuana + nicotine use.
- Hippocampal volumes were smaller in marijuana users (with or without nicotine).
- Abnormal brain-behavior relationships in combined marijuana + nicotine users.

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ABSTRACT

Combined use of marijuana (MJ) and tobacco is highly prevalent in today's population. Individual use of either substance is linked to structural brain changes and altered cognitive function, especially with consistent reports of hippocampal volume deficits and poorer memory performance. However, the combined effects of MJ and tobacco on hippocampal structure and on learning and memory processes remain unknown. In this study, we examined both the individual and combined effects of MJ and tobacco on hippocampal volumes and memory performance in four groups of adults taken from two larger studies: MJ-only users ($n = 36$), nicotine-only (Nic-only, $n = 19$), combined marijuana and nicotine users (MJ + Nic, $n = 19$) and non-using healthy controls ($n = 16$). Total bilateral hippocampal volumes and memory performance (WMS-III logical memory) were compared across groups controlling for total brain size and recent alcohol use. Results found MJ and MJ + Nic groups had smaller total hippocampal volumes compared to Nic-only and controls. No significant difference between groups was found between immediate and delayed story recall. However, the controls showed a trend for larger hippocampal volumes being associated with better memory scores, while MJ + Nic users showed a unique inversion, whereby smaller hippocampal volume was associated with better memory. Overall, results suggest abnormalities in the brain-behavior relationships underlying memory processes with combined use of marijuana and nicotine use. Further research will need to address these complex interactions between MJ and nicotine.

1. Introduction

Marijuana (MJ) and tobacco products remain two of the most widely used substances worldwide. In the U.S., combined use of both substances is upwards of 60–70% in MJ users and more than five times as likely as measured by past month use in tobacco users [1,2]. Moreover, in some countries, smoked MJ joints are almost exclusively mixed with tobacco [3]. Despite the widespread prevalence of MJ and tobacco co-use, interactive effects of marijuana and nicotine are scantly characterized in the existing literature and lacking direct comparisons of separate (MJ-only, Nicotine-only) and combined uses (MJ + Nicotine) is a limitation in most studies of marijuana use.

Individually, MJ and tobacco are associated with changes to brain structure and function. Structural neuroimaging studies in MJ users have indicated that volumes of several brain areas are smaller in heavy MJ users [4–8], especially in areas enriched with cannabinoid type I (CB1) receptors such as medial temporal lobe structures [9]. Of these structures, the hippocampus appears to be particularly sensitive to heavy marijuana use. Delta9-tetrahydrocannabinol (THC), the primary psychoactive component in marijuana, which binds to CB1 receptors, is associated with

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cell shrinkage and damage to DNA strands in THC-treated hippocampal neuron cultures [10]. The association of these alterations, such as smaller hippocampal volume with greater lifetime duration of use and cumulative amount [4,8] as well as with recent use [11], suggest that these changes are consequences of exposure to MJ. A recent study by Smith et al. [12] examined the interaction between cannabis use and schizophrenia on hippocampal morphology and found a main effect of cannabis use such that altered hippocampal shape was found in both cannabis users with and without schizophrenia. Moreover, these hippocampal differences were related to poorer episodic memory performance emphasizing the relationship between hippocampal morphology and memory. Taken together, smaller brain volumes in MJ users may reflect potential neurotoxic influence of exogenous cannabinoid exposure.

Relative to MJ, less is known about structural brain changes specific to chronic nicotine use. However, existing studies report lower gray matter densities across widespread areas (e.g., prefrontal cortex, cingulate gyrus, parietal lobe, cerebellum, thalamus, striatum and medial temporal lobe) in tobacco smokers [13–15]. Animal models of rats exposed to nicotine show reduced cell numbers, increased markers of apoptosis and alterations in synaptic activity in these regions [16,17]. These regions express dense levels of acetylcholine receptors that are primary binding targets for nicotine, which further supports the potential for nicotine-related brain changes. Thus, it is likely that similar to MJ's effects, reported morphometric changes result from nicotine-related neurotoxicity.

In addition to structural changes, MJ and tobacco have also been individually associated with declines in cognitive function. Existing studies suggest that tobacco use is associated with impaired working memory, attention, and verbal abilities [18,19] that map on to brain structures that undergo changes due to tobacco use (e.g., frontal and parietal cortices, striatum and hippocampus). In terms of MJ's effects on cognition, studies have reported widespread deficits across various domains such as memory [20], attention [21], and learning [22] that are dependent on CB1 receptor activation [23]; however, deficits in working memory appear to be the most consistent [24–27]. While individual studies provide evidence for neurocognitive consequences of MJ and nicotine, the independent drug effects may not generalize to the context of combined use. Interactions between the two substances have been described at the cellular level wherein CB1 and nicotinic acetylcholine (nACH) receptors are densely co-localized in hippocampal regions and both are involved in a diverse set of modulatory processes (for review see Viveros et al., 2006 [28]). For example, chronic nicotine treatment in rats results in altered endocannabinoid levels in the brain [29]. There is also pharmacological evidence that cannabinoids alter nicotinic-acetylecholinergic receptor response [30]. Moreover, Valjent, et al. [31] noted altered fear, withdrawal, and tolerance behaviors in rats co-treated with THC and nicotine, suggesting functional-biochemical interactions. Taken together, there is convergent evidence from human, animal and pharmacological studies supporting the potential for additional consequences on the integrity of the hippocampal structure and function with combined MJ and nicotine use. However, to date, this has not yet been directly examined. In this study, we aimed to characterize the differential and combined impact of marijuana (MJ) and nicotine (Nic) on hippocampal morphometry and memory function among marijuana-only users, nicotine-only users, and comorbid marijuana and nicotine users (MJ + Nic) with a non-using comparison control group. As a primary aim, we compared groups on hippocampal volume. To then further characterize any difference found in hippocampal volumes, we also compared groups on memory performance and examined relationships between morphometry, memory and substance use patterns. Given findings from existing literature, we anticipated that MJ and nicotine individually and in combination would be associated with smaller hippocampal volumes and poorer memory scores that are inversely related to substance use patterns [4,8,24–27].

2. Materials and methods

2.1. Participants

Participants were recruited through flyers and advertisements in the Albuquerque, New Mexico metro area. The community subsample used for this study originated from two larger studies conducted at the University of New Mexico (UNM; see [32]). Informed consent was provided by all of the participants in accordance with the Institutional Review Board (IRB) at UNM. Participants were compensated for their time. To be eligible for the study, all individuals had to meet the following criteria: (a) be between the ages of 18 and 50 years; (b) be right-handed; (c) have no magnetic resonance imaging (MRI) contraindications (e.g., no metallic implants, pregnancy, claustrophobia, etc.); (d) have no symptoms of psychosis (via SCID psychosis screen) and (e) be fluent in both oral and written English. Furthermore, individuals with fewer than 10 years of education, IQs less than 75, or illicit drug use (other than marijuana) were excluded from our sample. We were interested in differences resulting from regular, heavy marijuana and nicotine use rather than from recreational marijuana and nicotine use. To that end, the marijuana users were also required to report using marijuana (verified by urinalysis) at least 4 times per week over the past six months. Nicotine users were included if they reported nicotine use (verified by carbon monoxide breath monitor) of 10 or more times daily and had less than three months of abstinence in the past year. Controls were included if they reported no marijuana use occasions and no tobacco use occasions in the preceding three months, and did not meet criteria for any drug or alcohol abuse or dependence according to the Structured Clinical Interview for DSM-IV disorders.

For our study, participants were categorized into four groups based on substance use: MJ (marijuana users), NIC (nicotine users), MJ + NIC (marijuana and nicotine users), and non-using controls (Table 1). The combined chronic marijuana and nicotine smoking group (MJ + NIC) was derived from the two studies, with participants having to meet criteria for both chronic marijuana and frequent nicotine use to be part of this group.

2.2. Study procedures

The study took place over two separate visits. The first visit included assessments of substance use history and neuropsychological tests. The second visit was scheduled three days after the first visit and consisted of an MRI scan. Participants were required to abstain from MJ and illicit drugs between the two visits so that MRI and cognitive measures did not reflect effects of acute intoxication. This resulted in a ~72-hour abstinence period confirmed by self-report. To promote compliance with the 72-hour abstinence from marijuana, we followed a bogus pipeline by collecting a urine cannabis toxicity screen before and after abstinence (visit 1, visit 2). While the urinalysis is insensitive to 72-hour abstinence, this method has been shown to increase accuracy of self-report (17). Only those who reported 72-hour abstinence were included in the study.

Participants were also asked not to use caffeine or tobacco for two to four hours prior to their brain scan and neither were permitted during their MRI appointment. During session two, each participant had a head MRI scan and each was administered a brief

Table 1
Demographic and substance use characteristics of the sample. Nic = nicotine; MJ = marijuana; MJ + Nic = marijuana plus nicotine; BAI = Beck Anxiety Invetory; BDI = Beck Depression Invetory; ADHD CSS = Attention Deficit Hyperactivity Disorder Current Symptoms Scale; WASI = Wechsler Adult Scale of Intelligence; Pack years = cigarettes per day/number of years.

	Controls		Nic		MJ		MJ + Nic	
	(n = 16)		(n = 19)		(n = 36)		(n = 19)	
Variable	% or M	SD	% or M	SD	% or M	SD	% or M	SD
Age	26.88	6.89	29.63	11.31	24.92	8.78	23.26	7.32
Years of education	14.38	1.20	14.18	2.31	13.79	2.18	13.34	2.09
BAI	3.75	4.583	7.16	7.381	6.42	7.688	6.63	7.335
BDI	4.72	5.013	8.76	9.329	6.69	6.583	5.71	6.174
CSS ADHD t score	42.17	5.759	48.96	8.625	44.74	6.846	47.46	9.610
WASI IQ	113.50	5.87	112.84	12.19	108.43	10.26	107.68	10.52
Gender (% male)	31%		53%		58%		74%	
Drinks per drinking day (past 90d)*	2.11	2.01	3.31	2.20	3.57	2.76	4.61	2.63
Smoking days (past 90d)	N/A		89.79	0.92	0.97	0.92	86.58	8.93
Cigarettes/day (past 90d)	N/A		12.59	3.88	0.14	0.43	10.25	5.39
Pack-years	N/A		8.03	10.06	N/A		4.84	7.61
MJ smoking Days (past 90d)	N/A		N/A		80.58	14.18	82.16	11.49
Lifetime MJ dependence Sx	N/A		N/A		2.37	2.00	2.68	2.87
Lifetime MJ use (episodes)	N/A		N/A		5.565	8.566	6.704	9.450
Post-hoc t-tests compared to Control group:								
Age	–	–	$t(30.3) = -0.85$	$p = 0.40$	$t(50) = 0.79$	$p = 0.43$	$t(33) = 1.49$	$p = 0.15$
Years of education	–	–	$t(28.03) = 0.31$	$p = 0.76$	$t(47.436) = 1.24$	$p = 0.22$	$t(33) = 1.75$	$p = 0.09$
BAI	–	–	$t(33) = -1.6$	$p = 0.118$	$t(45.49) = -1.551$	$p = 0.128$	$t(33) = -1.362$	$p = 0.18$
BDI	–	–	$t(28.45) = -1.63$	$p = 0.11$	$t(50) = -1.07$	$p = 0.290$	$t(33) = -0.52$	$p = 0.61$
CSS ADHD t score	–	–	$t(32) = -2.66$	$p = 0.01$	$t(49) = -1.30$	$p = 0.12$	$t(32) = -1.914$	$p = 0.07$
WASI IQ	–	–	$t(33) = 0.2$	$p = 0.85$	$t(46.32) = 2.23$	$p = 0.03$	$t(33) = 1.81$	$p = 0.08$
Gender (% male)	–	–	$t(33) = 1.27$	$p = 0.2$	$t(50) = 1.83$	$p = 0.07$	$t(33) = 2.69$	$p = 0.01$
Drinks per drinking day (past 90d)*	–	–	$t(33) = -1.67$	$p = 0.1$	$t(50) = -1.90$	$p = 0.06$	$t(33) = -3.12$	$p = 0.004$
Smoking days (past 90d)	–	–	$t(33) = -390.45$	$p < 0.001$	$t(35) = -1.97$	$p = 0.06$	$t(18) = -42.27$	$p < 0.001$
Cigarettes/day (past 90d)	–	–	$t(18) = -14.15$	$p < 0.001$	$t(35) = -2.236$	$p = 0.03$	$t(18) = -8.28$	$p < 0.001$
Pack-years	–	–	$t(18) = -3.48$	$p = 0.003$	$t(35) = -1.86$	$p = 0.07$	$t(18) = -2.77$	$p = 0.01$
MJ smoking Days (past 90d)	–	–	$t(33) = 0.18$	$p = 0.86$	$t(42.716) = -23.67$	$p < 0.001$	$t(32.950) = -22.78$	$p < 0.001$
Lifetime MJ dependence Sx	–	–	$t(33) = -1.71$	$p = 0.1$	$t(48.49) = -5.04$	$p < 0.001$	$t(25.53) = -4.19$	$p < 0.001$
Lifetime MJ use (episodes)	–	–	N/A	N/A	N/A			
Post-hoc t-tests compared to Nicotine group:								
Age	–	–	–	–	$t(29.74) = 1.58$	$p = 0.12$	$t(30.822) = 2.06$	$p = 0.05$
Years of education	–	–	–	–	$t(53) = 0.62$	$p = 0.54$	$t(36) = 1.18$	$p = 0.25$
BAI	–	–	–	–	$t(53) = 0.345$	$p = 0.73$	$t(36) = -0.220$	$p = 0.827$
BDI	–	–	–	–	$t(53) = 0.96$	$p = 0.34$	$t(36) = 1.189$	$p = 0.242$
CSS ADHD t score	–	–	–	–	$t(51) = 1.94$	$p = 0.06$	$t(34) = 0.49$	$p = 0.63$
WASI IQ	–	–	–	–	$t(52) = 1.41$	$p = 0.16$	$t(36) = 1.34$	$p = 0.19$
Gender (% male)	–	–	–	–	$t(53) = 0.4$	$p = 0.62$	$t(35.45) = 1.34$	$p = 0.19$
Drinks per drinking day (past 90d)*	–	–	–	–	$t(53) = -0.36$	$p = 0.72$	$t(53) = -1.36$	$p = 0.18$
Smoking days (past 90d)	–	–	–	–	$t(53) = 74.71$	$p < 0.001$	$t(18.38) = 1.56$	$p = 0.14$
Cigarettes/day (past 90d)	–	–	–	–	$t(53) = 19.14$	$p < 0.001$	$t(36) = 1.54$	$p = 0.13$
Pack-years	–	–	–	–	$t(53) = 4.79$	$p < 0.001$	$t(36) = 1.10$	$p = 0.28$
MJ smoking Days (past 90d)	–	–	–	–	$t(53) = -22.94$	$p < 0.001$	$t(28.14) = -26.65$	$p < 0.001$
Lifetime MJ dependence Sx	–	–	–	–	$t(52) = -1.88$	$p = 0.06$	$t(36) = -1.96$	$p = 0.06$
Lifetime MJ use (episodes)	–	–	–	–	N/A	N/A		
Post-hoc t-tests between marijuana and combined marijuana + nicotine groups								
Age	–	–	–	–	–	–	$t(53) = 0.7$	$p = 0.49$
Years of education	–	–	–	–	–	–	$t(53) = 0.74$	$p = 0.46$
BAI	–	–	–	–	–	–	$t(53) = -0.100$	$p = 0.921$
BDI	–	–	–	–	–	–	$t(53) = 0.538$	$p = 0.593$
CSS ADHD t score	–	–	–	–	–	–	$t(51) = -1.190$	$p = 0.239$
WASI IQ	–	–	–	–	–	–	$t(52) = 0.24$	$p = 0.81$
Gender (% male)	–	–	–	–	–	–	$t(53) = 1.12$	$p = 0.27$
Drinks per drinking day (past 90d)*	–	–	–	–	–	–	$t(53) = -1.36$	$p = 0.18$
Smoking days (past 90d)	–	–	–	–	–	–	$t(24.30) = -38.32$	$p < 0.001$
Cigarettes/day (past 90d)	–	–	–	–	–	–	$t(18.13) = -8.14$	$p < 0.001$
Pack-years	–	–	–	–	–	–	$t(18.02) = -2.74$	$p = 0.01$
MJ smoking Days (past 90d)	–	–	–	–	–	–	$t(53) = -0.41$	$p = 0.68$
Lifetime MJ dependence Sx	–	–	–	–	–	–	$t(52) = -0.52$	$p = 0.6$
Lifetime MJ use (episodes)	–	–	–	–	–	–	$t(44) = -0.41$	$p = 0.69$

cognitive battery including standardized tests of new learning and memory (detailed below).

2.2.1. Brain imaging

Imaging was conducted at the UNM Mind Research Network with a 3T TIM TRIO scanner (Siemens, Erlangen, Germany) and a multi-echo magnetization prepared rapid gradient echo (MPRAGE) sequence with the following parameters: TR/TE/TI = 2300/2.74/900 ms, flip angle = 8°, FOV = 256 × 256 mm, slab thickness = 176 mm, voxel size = 1 × 1 × 1mm3, number of echoes = 4, pixel bandwidth = 650 Hz, total scan time = 6 min. Before volumetric analysis, images were inspected for motion quality control and obvious pathology.

Table 2
Brain and behavior measures. The WMS-III Logical Memory subtests assessed learning and memory of narrative material. ss = raw scores from immediate recall trials and recall converted to scaled scores normalized to age. Group comparisons controlled for IQ, gender, number of drinks per occasion, ADHD symptoms and age.

	Controls		Nic		MJ		MJ + Nic		*F*
Measure	(*n* = 16)		(*n* = 19)		(*n* = 36)		(*n* = 19)		
Story memory-Immediate (ss)	10.63	2.06	11.00	2.83	9.59	2.55	8.61	3.07	$F(3,74) = 0.542$, $p = 0.655$
Story memory-Delayed (ss)	11.06	2.57	11.78	3.02	10.36	2.63	9.11	3.05	$F(3,73) = 0.737$, $p = 0.533$
Total brain volume (cm^3)	1,206	106	1,259	109	1,254	148	1,318	145	$F(3,77) = 0.420$, $p = 0.739$
Left hippocampus (cm^3)	4.32	0.44	4.37	0.42	4.18	0.49	4.3	0.46	$F(3,77) = 1.576$, $p = 0.202$
Right hippocampus (cm^3)	4.4	0.42	4.44	0.4	4.15	0.49	4.29	0.43	$F(3,77) = 4.362$, $p = 0.007$

Posthoc pairwise comparisons against the Control group

	Controls		Nic		MJ		MJ + Nic	
Measure	(*n* = 16)		(*n* = 19)		(*n* = 36)		(*n* = 19)	
Story memory-Immediate (ss)	–	–	$F(1,26) = 0.06$	$p = 0.81$	$F(1,42) = 0.001$	$p = 0.98$	$F(1,26) = 0.01$	$p = 0.91$
Story memory-Delayed (ss)	–	–	$F(1,26) = 0.66$	$p = 0.43$	$F(1,41) = 0.26$	$p = 0.61$	$F(1,26) = 0.07$	$p = 0.79$
Total brain volume (cm^3)	–	–	$F(1,27) = 1.51$	$p = 0.23$	$F(1,43) = 1.01$	$p = 0.32$	$F(1,27) = 1.02$	$p = 0.32$
Left hippocampus (cm^3)	–	–	$F(1,27) = 0.39$	$p = 0.54$	$F(1,43) = 2.39$	$p = 0.13$	$F(1,27) = 0.55$	$p = 0.47$
Right hippocampus (cm^3)	–	–	$F(1,27) = 0.25$	$p = 0.62$	$F(1,43) = 9.23$	$p = 0.004$	$F(1,27) = 2.96$	$p = 0.09$

Posthoc pairwise comparisons against the Nicotine group

	Controls		Nic		MJ		MJ + Nic	
Measure	(*n* = 16)		(*n* = 19)		(*n* = 36)		(*n* = 19)	
Story memory-Immediate (ss)	–	–	–	–	$F(1,43) = 0.54$	$p = 0.47$	$F(1,27) = 1.32$	$p = 0.26$
Story memory-Delayed (ss)	–	–	–	–	$F(1,42) = 0.43$	$p = 0.51$	$F(1,27) = 2.36$	$p = 0.14$
Total brain volume (cm^3)	–	–	–	–	$F(1,45) = 1.3$	$p = 0.26$	$F(1,29) = 0.16$	$p = 0.7$
Left hippocampus (cm^3)	–	–	–	–	$F(1,45) = 3.7$	$p = 0.06$	$F(1,29) = 1.323$	$p = 0.26$
Right hippocampus (cm^3)	–	–	–	–	$F(1,45) = 5.79$	$p = 0.02$	$F(1,29) = 2.75$	$p = 0.11$

Posthoc pairwise comparisons between MJ users and MJ+Nic

	Controls		Nic		MJ		MJ + Nic	
Measure	(*n* = 16)		(*n* = 19)		(*n* = 36)		(*n* = 19)	
Story memory-Immediate (ss)	–	–	–	–	–	–	$F(1,43) = 0.62$	$p = 0.44$
Story memory-Delayed (ss)	–	–	–	–	–	–	$F(1,42) = 0.81$	$p = 0.37$
Total brain volume (cm^3)	–	–	–	–	–	–	$F(1,45) = 0.34$	$p = 0.57$
Left hippocampus (cm^3)	–	–	–	–	–	–	$F(1,45) = 0.03$	$p = 0.87$
Right hippocampus (cm^3)	–	–	–	–	–	–	$F(1,45) = 0.01$	$p = 0.93$

2.3. Measures

2.3.1. Sample characteristics

Age, gender, education level and other background information were obtained using a standard demographics questionnaire. Clinical symptom inventories assessed potential psychological confounds associated with both marijuana and nicotine use (Breslaw, Kilbey, & Andreski, 1991) such as the Beck Depression Inventory [37] and the Beck Anxiety Inventory [38]. Barkley's Current Symptoms Scale [40] provided age-normed scores of self-report current ADHD symptoms.

2.3.2. Substance use

The Substance Use Disorder modules of the Structured Clinical Interview for DSM-IV (SCID) were administered by a trained research assistant to assess for lifetime and current symptoms of abuse and dependence for alcohol, nicotine, marijuana and other substances [41]. A Time Line Follow-Back (TLFB) approach was used to quantify alcohol, nicotine, and marijuana use patterns for 90 days prior to study participation [42].

2.3.3. Neurocognitive assessments

The two-subtest administration of the Wechsler Abbreviated Scale of Intelligence provided estimates of intellect [33]. The WMS-III Logical Memory subtests [43] assessed learning and memory of narrative material. Raw scores from immediate recall trials and recall following a 30-minute delay were converted to scaled scores normalized to age.

2.4. Data Processing and Analysis

2.4.1. Brain volumes

High resolution MPRAGE anatomical scans from each participant were spatially normalized, field-bias corrected and parcellated using FreeSurfer v4.5 (http://surfer.nmr.mgh.harvard.edu; [44]). Total brain volumes (TBV) and hippocampal volumes were extracted for analysis in SPSS. Volumes were visually inspected for accuracy and manually edited as necessary by TM. Hippocampal volume was expressed as a TBV ratio (hippocampus/TBV) to control for individual differences in head size.

2.4.2. Statistical analyses

Statistical analyses were conducted in SPSS 18.0. ANOVAs and chi-square tests compared groups on background and demographic variables that may also relate to brain structure (See Table 1). Similar group comparisons were performed on substance use variables and intracranial volumes for descriptive purposes. Because demographics, background variables, and alcohol use are related to brain structure, those factors that differed by group were included as nuisance covariates in subsequent analyses. Other variables with known links to brain structure were explored in follow-up analyses regardless of whether groups were different (e.g., gender) to assess for potential brain-behavior relationship moderators. Interpreta-

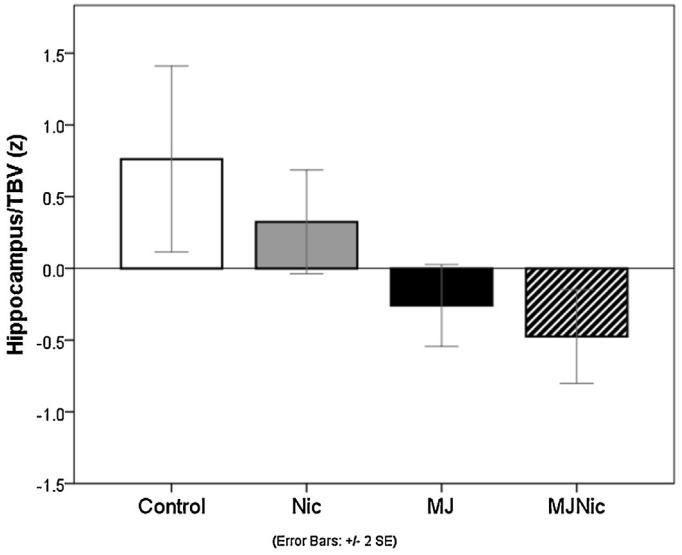

Fig. 1. Hippocampal volumes normalized as a ratio to total brain volume (TBV) expressed as a z-score by group (error bars are +2 standard error).

tions of statistical significance were made at $p < 0.05$. ANCOVA was used to determine whether TBV-adjusted hippocampal volumes and memory performance differed by group after controlling for potential confounds. We conducted Pearson correlations to evaluate the relationships between neural and cognitive measures. Fisher's *Z* tests compared correlations for significant group differences. We conducted a multiple regression to evaluate whether hippocampal volume and nicotine use severity predicted memory performance.

3. Results

3.1. Sample characteristics

One-way ANOVA and post hoc paired comparisons examined whether participant characteristics differed across the groups (see Table 1). The groups differed in age, IQ, gender, frequency of heavy drinking, and number of ADHD symptoms, therefore, subsequent analyses co-varied for these variables. Symptom ratings on mood and anxiety did not differ by group.

3.2. Substance use

As expected, tobacco smokers and marijuana users reported more nicotine and cannabis use as well as heavier recent alcohol involvement than controls (drinks per occasion, $p = 0.02$). In light of group differences in recent alcohol use, we covaried for number of drinks per drinking day from the 90-day timeline followback in subsequent statistical tests. Tobacco smokers (Nic-only vs. MJ + Nic) did not differ on average smoking days or cigarettes per day (p's > 0.05). Marijuana users (MJ-only vs. MJ + Nic) also did not differ on total marijuana use episodes (number of days used from the 90-day timeline followback) or lifetime dependence symptoms.

3.3. Hippocampal volumes

After controlling for recent number of drinks per occasion (past 90 days), IQ, gender and age, the groups differed in right hippocampal volume [$F(3,77) = 4.36$, $p = 0.007$] (Table 2). Post-hoc pairwise comparisons revealed that all marijuana users (MJ and MJ + Nic) had smaller hippocampal volumes compared to controls and Nic-only groups (Control, Nic > MJ-only, MJ + Nic; See Fig. 1).

Overall TBVs were not significantly different across controls, nicotine users and marijuana users.

3.4. Memory performance

Although MJ users' memory scores were intermediate to the Nic and MJ ± Nic groups, group differences in the WMS-III Logical Memory subtests did not reach significant thresholds (Table 2).

3.5. Memory and hippocampal volumes

Partial correlations controlling for recent alcohol use examined relationships between memory scores and hippocampal volumes separately for each group. Controls (rp = 0.20; rp = 0.37), nicotine-only (rp = 0.02; rp = −0.23), and marijuana-only (rp = −0.05; rp = −0.08) groups did not exhibit significant correlations between brain volume and immediate or delayed recall scores, respectively (p's ≥ 0.05). The MJ + Nic users showed consistent inverse relationships, whereby worse memory scores were associated with larger hippocampal volumes (immediate recall: rp = −0.49, $p = 0.05$; delayed recall: rp = −0.52, $p = 0.04$; see Fig. 2 for scatterplots by group). Further, Fisher's *z*-tests determined that the brain-behavior links among the MJ–Nic users significantly differed from controls (immediate recall: $z = 1.95$, $p = 0.05$; delayed recall: $z = 2.04$, $p = 0.04$). Whereas controls showed a positive relationship between hippocampus and memory scores (larger volume linked to better scores), larger hippocampi were associated with poorer memory in MJ + Nic users. Taken together, the MJ + Nic users exhibited abnormal links between hippocampal volume and memory scores, and these relationships significantly deviated from the same patterns among control subjects.

3.6. Memory, hippocampal volumes and substance use

The multiple regression analyses showed a significant interaction between brain volume and nicotine use intensity predicting immediate memory [$F(1,47) = 5.61$, $R^2_{\Delta} = .11$, $B = -0.26$, $t = -2.37$, $p = 0.02$] as well as a main effect of cigarettes/day [$F(1,47) = 4.81$, $R^2_{\Delta} = 0.09$, $B = -.17$, $t = -2.19$, $p = 0.03$] depicting an inverse link between nicotine use and memory scores. Fig. 3 displays the decomposed interaction and highlights negligible links between immediate memory scores and hippocampal volume among non-smokers and light smokers (1–2 cigarettes/day), suggesting that the altered relationship between memory and hippocampal volume was driven by heavy smokers who used 3+ cigarettes/day. This interaction was not detected when modeling delayed memory scores ($n = 51$, $p > 0.05$), though nicotine use intensity remained a significant predictor of worse delayed recall [$F(1,46) = 6.62$, $R^2_{\Delta} = 0.13$, $B = -0.20$, $t = -2.57$, $p = 0.01$]. To determine whether MJ use characteristics mediated this relationship, we re-ran the regression three times, adding estimated lifetime MJ use occasions as: (1) covariate (2) hippocampal volume*MJ use interaction and (3) hippocampus*MJ interaction with cigarettes/day removed from the model. Including lifetime MJ use did not alter the significant interactions between tobacco smoking intensity and hippocampal volume in predicting memory scores and lifetime MJ use (alone or in interaction with hippocampal volume) did not significantly account for memory scores. Taken together, among the MJ users, lifetime MJ use did not mediate the unique relationships between intensity of nicotine exposure, hippocampal volume and memory.

4. Discussion

No known studies have characterized the differential impact of independent versus combined marijuana and nicotine use on brain structure and related function. Here, we found that marijuana use

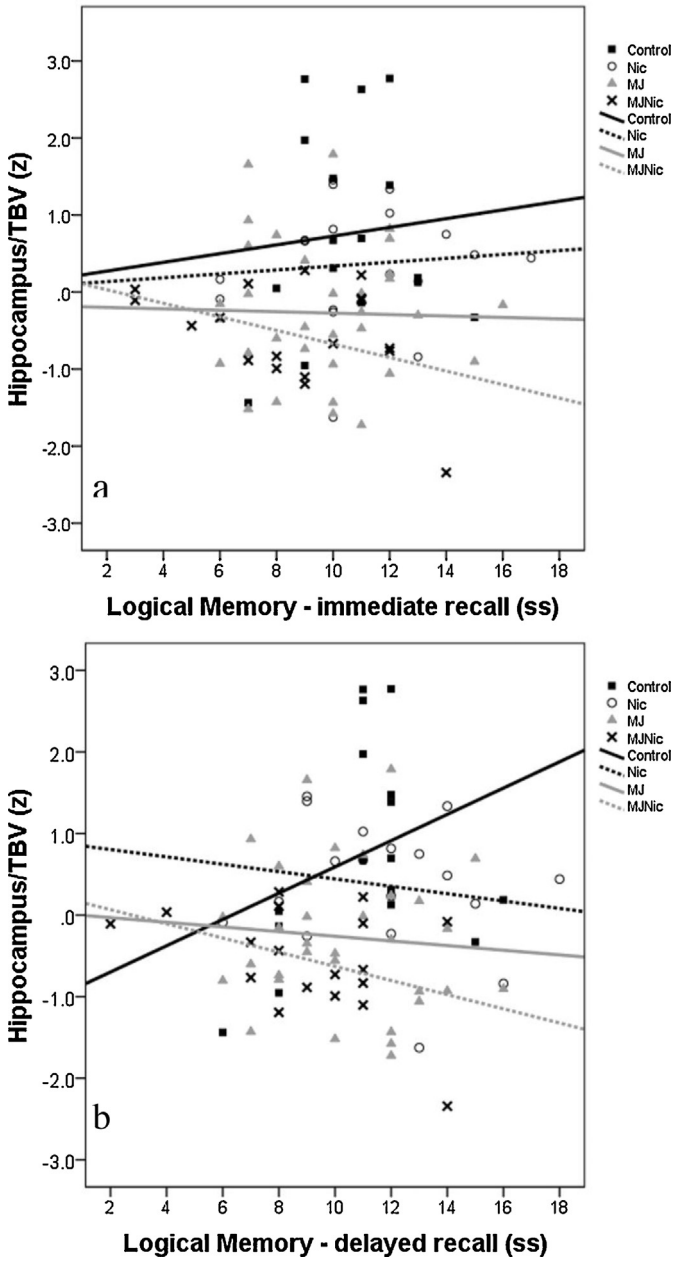

Fig. 2. Bivariate scatterplots with fit lines by group for hippocampal volumes (as TBV ratio z-score) with WMS-III logical memory (a) immediate recall scaled score (b) and delayed recall scaled score.

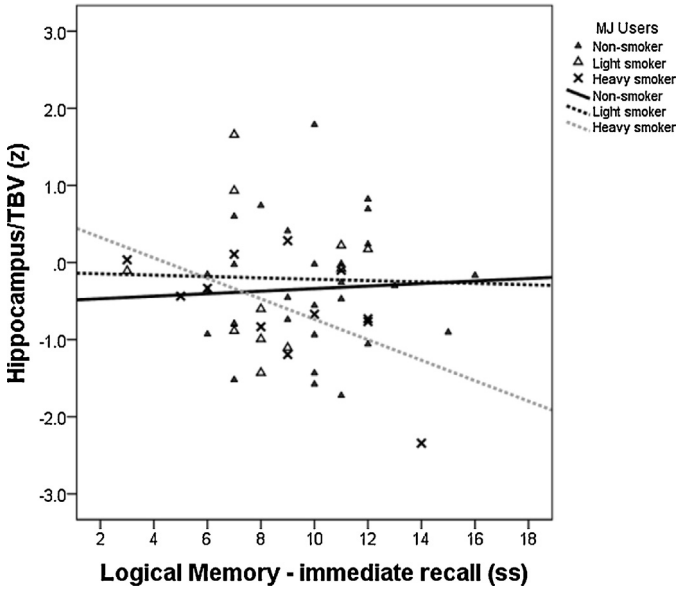

Fig. 3. Decomposing the interaction between hippocampal volumes (as TBV ratio z-score) and nicotine use intensity (cigarettes/day) predicting logical memory immediate recall scaled scores.

individually and combined with tobacco had smaller hippocampal volumes compared to tobacco users and non-using controls. We also found differential associations between brain and behavior such that smaller hippocampal volumes were associated with poorer memory performance for controls, while in MJ + Nic users, smaller hippocampal volumes were linked to relatively higher memory scores. Our findings of marijuana-related abnormalities in hippocampal morphology and relationship to impaired memory function is concordant with recent findings by Smith et al. [12].

Several studies have previously reported reduced hippocampal volumes in chronic marijuana users (see reviews by [45,46]), which may reflect a potential neurotoxic effect (e.g., cell loss/breakdown). However, the best evidence for direct neurotoxic influence of THC has been primarily limited to well-controlled pharmacological manipulations of hippocampal neuron cultures [10]. Alternatively, studies of in vivo THC treatment in rodent models find reduced dendritic length and spine density [47,48], suggesting that gross volumetric deficits may reflect morphological changes to hippocampal neurons. Structural and functional abnormalities may also be obscured by THC-activation of glial cells [49], thereby yielding decreased neuronal densities by virtue of the presence of increased glial cells. It is of interest that the combined MJ + Nic users had the smallest hippocampal volumes. Given that MJ + Nic user hippocampal volumes were not statistically different from the MJ-only group, this finding does not support an additive detrimental effect of combined THC and nicotine exposure. However, MJ + Nic had the lowest memory performance out of all groups, thus functional interactions cannot be ruled out. Instead of a dual-toxic mechanism, it is possible that poorer memory performance in MJ + Nic users stems from a dual-withdrawal process, whereby combined withdrawal from MJ and nicotine may further weaken memory processes. There is some evidence that nicotine interferes with memory function in MJ users. For example, Jacobsen et al. [18] found abnormal fronto-parietal brain response during a verbal working memory task in conjunction with poorer word recall in MJ + Nic users following short periods of nicotine withdrawal (24 h). Interestingly, these effects normalized without abstaining from tobacco. In our study, however, the combined MJ + Nic using subjects were not required to abstain from tobacco for an extended period (>3 h); thus, nicotine withdrawal effects on short-term memory function would be minimal. It is possible that the functional relationship between hippocampal volume and memory in MJ + Nic users (better memory with smaller volumes) might invert during acute nicotine deprivation. Although nicotine selectively enhances memory function [54–57], we did not observe recovery of any memory function from smoking nicotine in the chronic MJ + Nic subjects. The lack of significant difference may result from heavier MJ use relative to tobacco use. Most of the MJ users in this study were heavy MJ users (average of 6.76 days per week). THC and other cannabinoids build up and are metabolized and excreted relatively slowly (~12 h for THC and up to 72 h for some psychoactive metabolites) [58], while the nicotine of a cigarette [59] can be metabolized in ~2–8 h. In the current study, participants were required to abstain from MJ for three days, but tobacco smokers were only abstinent for ~3 h. As such, poorer memory performance in MJ + Nic users is not likely driven by acute nicotine withdrawal.

Rather, memory functioning is more likely disrupted by chronic MJ and tobacco co-use, combined with MJ withdrawal. One of the positive acute effects of nicotine is normalization of withdrawal effects [60,61]. For example, File and colleagues (2001) observed no cognitive enhancing effects of nicotine in a sample of non-smoking students. Thus, the positive effects on short-term memory after nicotine administration appear to reflect the reversal of an acute deprivation state. Nevertheless, the nicotine users had the greatest WMS scores relative to the other groups including the non-using controls suggesting underlying sub-threshold effects that parallel the existing literature.

The results and conclusions from this study should be examined within the context of its limitations. First, the dataset examined in this analysis was derived from two larger parent projects, thus, certain variables of interest could not be manipulated or controlled to study more detailed MJ-related effects (e.g., episodic use, lifetime use fluctuations, intensity of use in MJ gram weight, etc.). In spite of these limitations, MJ users (MJ-only and MJ + Nic) were homogenous in their MJ use due to study inclusion criteria (near-daily without two or more consecutive days of MJ abstinence in the 90 days prior). In addition, we only had one memory measure common to examine across our combined sample. While the WMS-III story memory paradigm is a well-validated and researched instrument, it may not generalize to learning and memory processes in alternative nonverbal modalities. Further, using a memory measure with a somewhat higher executive loading such as an unstructured word list learning task may elucidate some of the differential effects of MJ versus nicotine on memory functioning. Lastly, the between-group differences observed in this study were characterized by small-to-medium effect sizes and future research would be needed to replicate these findings in larger samples.

5. Conclusions

This study offers an important example for the treatment of comorbid substance use in addictions research. Current heights and potential rising rates of marijuana and nicotine necessitates better understanding of their specific and interactive effects ("World Drug Report", 2013), especially given the probability that marijuana use tendencies may change with changing policies. In the present study, we observed hippocampal volume deficits in MJ users with further abnormal brain-behavior relationships specific to combined MJ + Nic users. As such, unique neurobehavioral consequences of co-morbid tobacco and MJ use convey significant social and clinical implications.

Conflicts of interest

The authors report no conflicts of interest.

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