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Thalamic and Cerebellar Gray Matter Volume Reduction in Synthetic Cannabinoids Users

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Key Words
Addiction · Neuroimaging · Synthetic drugs · Synthetic cannabinoids · Thalamus · Cerebellum · Gray matter · Volumetric magnetic resonance imaging

Abstract
Background: Synthetic cannabinoids are compounds that bind cannabinoid receptors with a high potency and have been used widely in Europe by young people. However, little is known about the pharmacology and morphological effects of this group of substances in the brain. This study is aimed at investigating the morphological differences among synthetic cannabinoids users and healthy controls. ***Methods:*** Voxel-based morphometry was used to investigate the differences in brain tissue composition in 20 patients with synthetic cannabinoids use and 20 healthy controls. All participants were male. ***Results:*** Compared to healthy controls, voxel of interest analyses showed that regional grey matter volume in both left and right thalamus and left cerebellum was significantly reduced in synthetic cannabinoids users ($p < 0.05$). No correlation has been found between the age of first cannabis use, duration of use, frequency of use and grey matter volume. ***Discussion:*** These preliminary results suggest an evidence of some structural differences in the brain of synthetic cannabinoids users, and point the need for further investigation of morphological effects of synthetic cannabinoids in the brain.

Introduction

Cannabinoids have been used widely, for recreational or medical reasons, for a very long time. The primary active compound of the hemp plant *Cannabis sativa* is delta9-tetrahydrocannabinol (delta9-THC). Psychoactive cannabinoids cause euphoria, enhancement of sensory perception, tachycardia, anti-nociception, difficulties in concentration, and acute impairment of memory in humans; cognitive disturbances may remain after withdrawal.

G protein-coupled cannabinoid receptors are responsible for the acute effects of, and development of tolerance to, cannabinoids. The hippocampus, cerebellum, and striatum are the main areas in the brain where cannabinoid 1 (CB1) receptors and its variant, CB1A, have been found [1].

In last five years, substances named 'Spice' in Europe, 'K2' in the United States, 'Bonzai', 'Jamaika', 'Jamaika

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gold' or 'Jamaika supreme' in Turkey are available and widely used, especially by young people [2]. These substances are synthetic cannabinoids (SCs) described as 'legal highs', 'designer drugs', and 'herbal highs' in the market, which make users think that they are legal or safer. Availability of these illicit drugs via Internet and coffee shops makes them more dangerous and, as a result, SCs use has become a contagious and mortal problem for youth. Although, many cannabis users who may otherwise meet the criteria for being at the moderate risk for problematic use are nonetheless able to successfully integrate cannabis use into everyday life with few associated problems [3], SCs users seem that they are not able to do this.

SCs bind to the cannabinoid receptors like cannabis, but have greater potency than cannabis. SCs have a chemical structure similar to cannabis; these compounds have varied and highly potent physiological effects [4]. SCs are more potent than delta9-THC because they are full agonists of the CB1 receptor, as opposed to the partial CB1/CB2 receptor agonism of delta9-THC. Full agonism on the CB1 receptors leads to maximum activation, even at significantly lower doses of SCs [5, 6].

Volumetric Studies

Magnetic resonance imaging (MRI) enables researchers to visualize brain structures and the contrast between gray and white matter and cerebrospinal fluid. This neuroimaging method led to the application of image-processing techniques to quantify various parameters of brain regions of interest. In the 2000s, many studies explored the effects of cannabis in the brain using MRI [7, 8]. Two studies described the differences between chronic cannabis users and controls [9, 10]. Matochik and colleagues described lower grey matter density in the right parahippocampus and greater grey matter density in the precentral gyrus and right thalamus in cannabis users, while Cousijn et al. found a larger anterior cerebellum in cannabis users, compared to controls and grey matter volume in the amygdala, and hippocampus correlated negatively with the amount of cannabis use or dependence within the group of heavy cannabis users. Matochik et al. also found differences in the density of white matter of cannabis users compared to controls, such as lower density in the left parietal lobe and higher in parahippocampus, fusiform gyrus, lentiform nucleus, and pons. Lorenzetti and colleagues demonstrated that heavy cannabis users have smaller hippocampal and amygdala volumes [11]. Additionally, Demirakca et al. also indicate that cannabidiol may exhibit a neuroprotective effect [12].

Inconsistent findings were reported in the neuroimaging studies conducted on cannabis users, but there is lack of literature regarding the effects of SCs on the brains of users.

Voxel-based morphometry (VBM) is an automated and non-biased technique to explore the differences in the composition of brain tissue on a voxel-by-voxel basis, using a measure of tissue density or concentration [13]. VBM also detects differences in tissue composition at the voxel level that may not be apparent with analysis of regional volumes or from visual examination of MR images.

In this study, we investigated the differences in brain regions in a group of synthetic cannabinoid users who abstained from it for at least 7 days, in terms of comparison with 'never cannabis-used' healthy controls. We hypothesized that SCs users would have volume reductions in the areas which have many cannabinoid receptors.

Methods

Participants

We analyzed the medical records of patients that were treated in an addiction clinic in Istanbul between January 2013 and December 2014. The medical records of 35 patients were evaluated, and 15 were excluded due to lack of sufficient data. Participants who had another Axis-I psychiatric disorder, a past or current substance use disorder other than nicotine, or neurological disorders were excluded. The use of cannabis and other illegal drugs in the previous 3–4 days was excluded by immunoassay urine test. All participants were diagnosed as having cannabis use disorder, based on DSM-V, by two separate psychiatrists. The data derived from patient records included socio-demographic data, including sex (male/female), age, marital status, duration of education, age at first cannabis and SC use, duration of use (months), duration of problematic use of SCs (months), weekly frequency of SCs use in the last year, weekly number of SCs uses in the last year, and the presence of criminal records. The study was approved by the Ethical Committee of Uskudar University. All participants in the study were male and right-handed and all had done complete biochemical examinations and urine toxicology tests. Twenty healthy males who fulfilled inclusion criteria and were matched in terms of age, level of education, and socio-demographic status with substance users enrolled and were grouped as controls in the study.

Depressive symptoms and anxiety symptoms of patients were assessed by the Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI), respectively. The psychological symptom patterns of patients were assessed by the Symptom Checklist-90 (SCL-90).

Structural Magnetic Resonance Image Acquisition

Imaging was performed on a 1.5T MR scanner (Achieva, Philips Healthcare, Best, The Netherlands) with a SENSE-Head-8 coil at NPISTANBUL Neuropsychiatry Hospital, Istanbul. T1-

weighted MPRAGE sequence was employed as high-resolution anatomical scan (voxel size 1.25/1.25/1.2 mm; 130 slices; field of view 240 mm).

VBM Analyses

We examined the between-group differences in grey matter volume by using VBM. Data were processed and examined using the SPM software (Welcome Department of Imaging Neuroscience Group, London, UK; http://www.fil.ion.ucl.ac.uk/spm) and the VBM8 Toolbox (http://dbm.neuro.uni-jena.de/vbm.html) with default preprocessing parameters. Adaptive Nonlocal Means (SANLM) and a classical Markov Random Field (MRF) model were applied to the images in order to remove inhomogeneities and to improve the signal-to-noise ratio. Registration to standard MNI-space consisted of a linear affine transformation and a nonlinear deformation using high-dimensional DARTEL normalization. Subsequently, analyses were performed on segmented GM images, which were multiplied by the nonlinear components derived from the normalization matrix to preserve actual GM values locally (modulated GM volumes). To check the quality of the normalization procedure, the normalized unsegmented images were visually inspected. Sample homogeneities were controlled using covariance to identify potential outliers. Lastly, the segmented and modulated images were spatially smoothed with an 8-mm full-width, half-maximum Gaussian kernel.

Urine Toxicology Test

The Immunalysis K2 assay is intended for the qualitative assay of JWH-018, JWH-073, AM-2201 and their metabolites in participants' urine (Catalog Number: 344–0025EX). The Immunalysis K2 enzyme immunoassay kit provides only a preliminary analytical test result. Nonetheless, this kit is used to conduct a sensitive in vitro test to detect the presence of JWH-018, JWH-073, AM-2201 and their metabolites in human urine samples.

Data Analyses

The two groups were compared using the independent sample t-test, as implemented in the SPM second-level model. To account for differences in brain sizes, total intracranial volumes were entered in the model as covariates. The clusters were deemed significant if they survived FEW correction at a p level of 0.05 (cluster forming threshold = 20 voxels). Finally, to identify the associations between structural abnormalities and clinical scales, we conducted voxel of interest (VOI) analyses on cerebral tissues where group differences were identified. These areas were extracted using the MarsBaR toolbox and transferred to SPSS statistical software (SPSS Inc., Chicago, Ill., USA) for further analysis. Pearson's correlation coefficients were computed between the extracted VOIs of the clusters and outcome variables.

Descriptive analyses were presented using means and standard deviations for normally distributed variables.

Results

The SCs group consisted of twenty males who claimed SCs as their drug of choice, used SCs for a minimum period of one year, or currently used SCs for five or more times per week. Fourteen of the 20 participants (70%) in SCs group had positive results in urine toxicology. The MR scans were acquired on day 7 after the last SCs usage.

Table 1. Socio-demographic characteristics of participants

	Control group (n = 20)	SCs group (n = 20)
Age, years, mean ± SD	25.85±3.62	23.95±4.74
Duration of education, years, mean ± SD	11.1±2.94	9.75±2.67
Marital status, n (%)		
Single/divorced	14 (70)	14 (70)
Married	6 (30)	6 (30)
Presence of criminal records, n (%)		
Yes	1 (5)	9 (45)
No	19 (95)	11 (55)
Employment status, n (%)		
Unemployed	11 (55)	6 (30)
Employed (full-time)	5 (25)	3 (15)
Employed (part-time)	4 (20)	11 (55)
Residential state, n (%)		
Alone	2 (10)	0 (0)
With friends	5 (25)	1 (5)
With family	13 (65)	19 (95)

The comparison group consisted of 20 healthy male who had no history of psychopathology and use of any psychoactive drug. The sociodemographic characteristics of participants of the two study groups are presented in table 1, and the clinical characteristics of SCs users are presented in table 2. Participants in the SCs group reported SCs as their drug of choice and did not report the current use of other drugs, including alcohol. Comparing the control group with SCs users, VOI analysis showed that regional grey matter volume in both the left and right thalamus and left cerebellum was significantly decreased in SCs users (table 3).

There was no relationship of age at first cannabis and SCs use, duration of use, weekly frequency of SCs use in the last year, or the weekly number of SCs uses in the last year with gray matter tissue volume (fig. 1).

Discussion

In the 2000s, many studies explored the effects of cannabis in the brain using MRI [7, 8]. Lorenzetti and colleagues demonstrated that heavy cannabis users have smaller hippocampal and amigdala volumes [11]. Mato-

Table 2. Clinical characteristics of SCs users

	SCs group (n = 20)
Age of onset of cannabis use, years	18.8±3.94
No. of cannabis smoked before SCs use, months	9.78±7.36
Age onset of SCs use	21.45±4.76
Regular SCs use, months	17.50±8.85
Problematic SCs use, months	11.80±7.14
Frequency of SCs use weekly in past year	8.75±4.11
No. of SCs use in past year	317.75±143.99
BDI	17.2±8.02
BAI	14.5±11.33
SCL-90 symptom dimensions	
Hostility	2.11±0.94
Paranoid ideation	1.59±0.70
Psychoticism	0.95±0.42

Values are mean ± SD.
BDI = Beck depression inventory; BAI = Beck anxiety inventory; SCL-90 = symptom checklist-90.

Table 3. Gray matter differences between control group and SCs users

Brain regions control > users	Peak coordinate			Cluster size (voxel)	t
	x	y	z		
Thalamus	4.5	−6	−4.5	1,532	7.50*
	−4.5	−7.5	−1.5		7.43*
	4.5	−18	−1.5		7.02*
Cerebellum L	−	−63	−58.5	848	7.46*
	34.5	−66	−48		6.76*
	−				
	43.5				

L = Left hemisphere.
* Significant at voxel of interest level, $p < 0.001$, FWE cluster-corrected $p < 0.05$.

chik and colleagues described lower grey matter density in the right parahippocampus and greater grey matter density in the precentral gyrus and right thalamus in cannabis users, while Cousijn et al. found a larger anterior cerebellum in cannabis users, compared to controls and grey matter volume in the amygdala and hippocampus correlated negatively with the amount of cannabis use or dependence within the group of heavy cannabis users [9, 10].

As described below, inconsistent findings were reported in the neuroimaging studies conducted on cannabis users, but there is lack of literature regarding the effects of SCs on the brains of users.

There is very limited literature about SCs and, according to Papanti et al., most of the available reports on SCs were limited to retrospective toxicology surveys, case reports/case series, human laboratory studies assessing potential acute toxicological effects of SCs, and interviews/surveys focusing on self-reported harms/side effects identified among SCs users [14].

While VBM cannot provide a clear evidence for a particular mechanism underlying the reductions in brain tissue volume, the method is valuable for identifying focal brain regions for further investigation and for detecting subtle structural reductions that may not be apparent on visual examination of MR images or with analysis of regional volumes.

This is the first volumetric MRI study conducted in SCs users that aimed at investigating the structure of the brain. Using VBM, we detected volume reductions in both left and right thalamus and left cerebellum in a sample of SCs users, compared with the healthy control group.

The thalamus functions as an information-processing and relay station; it is like a bridge for bidirectional signal flow between cortical and subcortical regions, links different cortical regions via trans-thalamic pathways, and is a point of convergence for frontostriatal and cerebello-thalamo-cortical circuits [15].

According to the findings of this study, SCs users demonstrated reduced thalamic volumes.

Although the lack of preexisting findings may limit the generalizability of our findings for the association of SCs use with the thalamic volume reduction, this study support that SCs use may have an impact on the reduction of the thalamic volume.

On the other hand, cannabinoid receptors are highly expressed in the cerebellum, and deficits in cerebellar-dependent functions follow acute or chronic cannabis use in human. These cerebellar-mediated processes are aberrant in schizophrenia and long-term heavy cannabis use, and lead to cognitive deficits that are similar to those in schizophrenia [16, 17]. The accumulating evidence suggests that cannabis use may lead to cognitive disturbances, psychotic symptoms, and specific regional brain alterations. Nonetheless, the causality of SCs use with cerebellar structural integrity in SCs users, with or without psychosis, has not been examined yet. Solowij et al. determined that cannabis use may have a relatively greater

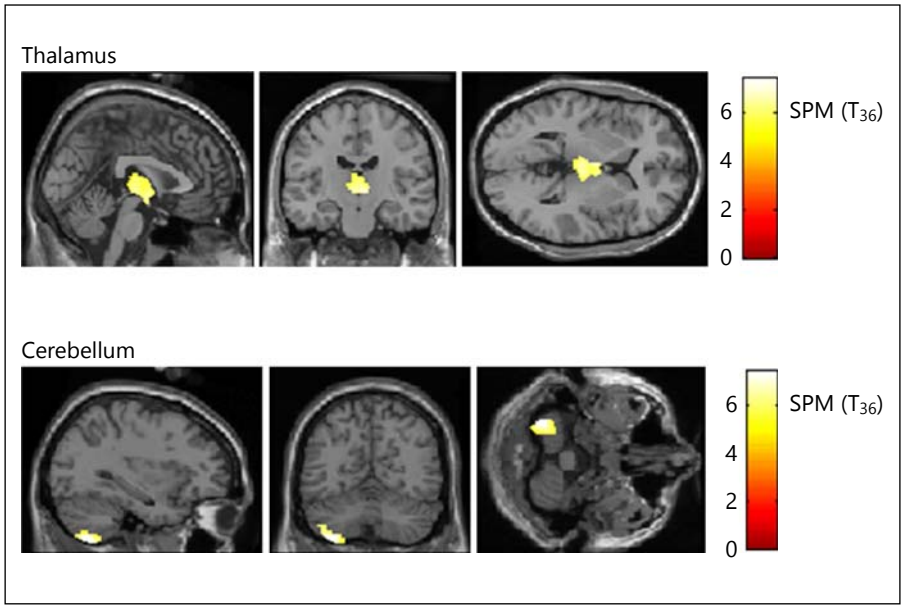

Fig. 1. VBM results on gray matter density. More dense regions in healthy control group compared to SCs users.

adverse effect on cerebellar white matter than schizophrenia [18]; the volume reduction in the left cerebellum in a sample of SCs users, compared to a healthy control group might be the result or the cause. Participants had higher scores in hostility and paranoid ideation subscales and scores that reached the upper limit in the psychoticism subscale of the SCL-90, which was compatible with the volumetric findings of this study. In this study, because VBM cannot provide information about the microstructure of a brain region, the mechanisms that underlie that volume reduction in the thalamus and cerebellum of SCs users, compared with the healthy control group, are unclear.

It is also unclear why no differences were found in other brain regions that are known for CB1 receptor expression.

The results of this study did not clarify if the differences between groups existed prior to the initiation of SCs use, or if other variables, either not controlled for or unrecognized, contributed to the volume reduction in thalamus and cerebellum.

In conclusion, we observed a gray matter density reduction in the right and left thalamus and lower gray matter density in the left cerebellum among SCs users, compared to healthy controls. Findings of this study need to be replicated with neuropsychiatric examination among both patients and controls in larger samples.

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