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ORIGINAL PAPER

Psychobiological responses to unpleasant emotions in cannabis users

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Abstract Aim of this paper is to investigate the psychobiological reactions to experimentally induced negative emotional states in active marijuana-dependent smokers and whether changes in emotional reactivity were reversed by prolonged abstinence. Twenty-eight patients were randomly included into group A (fourteen active marijuana-dependent smokers) or group B (fourteen abstinent marijuana-dependent subjects). Emotional response evaluation of group B subjects was assessed after 6 months of abstinence. Fourteen healthy volunteers, matched for age and sex, were used as controls. Psychometric and emotional response evaluations were performed by administering Symptoms Check List-90 and State-Trait Anxiety Inventory Y-1 (STAI). Neutral and unpleasant set of pictures selected from the international affective picture system and the Self-Assesment Manikin procedure (SAM) have been used to determine ratings of pleasure and arousal. Before and after the experimental session, blood samples were collected to determine ACTH and cortisol plasma levels. Active cannabis users displayed significantly higher levels of pleasantness SAM scores and lower levels of arousal SAM scores compared to abstinent cannabis users and controls in response to emotional task. In a close parallel with psychological data, hormonal findings indicate a persistent hyperactivity of hypothalamus–pituitary–adrenal (HPA) axis in cannabis users, particularly among active marijuana smokers, and an impaired hormonal reaction to negative emotions, in comparison with healthy subjects. The capacity of the HPA axis to respond to stressful stimuli/negative emotions seems to be only partially recovered after 6 months of abstinence. Ours findings, although obtained in a small number of subjects, suggest an association between active cannabis use, subjective reduced sensitivity to negative emotions and threat and HPA axis dysfunction.

Keywords Cannabis · Emotions · Psychobiological responses · HPA axis · SAM

The views expressed herein are those of the author(s) and do not necessarily reflect the views of the United Nations.

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Introduction

Previous studies in humans demonstrated abnormal emotional responses to liking, neutral and disliking stimuli among drug addicts [1, 13]. Accordingly, the influence of cannabis on the ability to perceive emotions was investigated many years ago, with the apparent evidence of a decline in affective sensitivity following active marijuana administration [4].

More recent findings indicate that the cannabinoid system is a key neurochemical mediator of anxiety and fear learning in both animals and humans [19, 35], the endogenous cannabinoids being involved in the modulation of amygdala emotional reactions [32, 35]. In particular, delta(9)-tetrahydrocannabinol (THC) has been reported to significantly reduce amygdala reactivity to social signals, indicating a possible mechanism underlying the anxiolytic role of cannabinoids [34].

The anxiogenic-like behavioral phenotype observed following both pharmacological and genetic blockade of cannabinoid CB1 receptors accounts for the participation of the endocannabinoid system in the control of emotional homeostasis [27]. Although euphoria has been identified as a primary factor in maintaining cannabis use, relaxation was the effect of marijuana reported most commonly [15], indicating cannabinoid capacity to modulate anxiety and attenuate the emotional response to stress.

Several pre-clinical and clinical data evidenced a gender-dependent differences in the biological and behavioral effects of different substance of abuse, including cannabis [9]. In fact, cannabinoids have been shown to exert sex-dependent effects in different physiological and behavioral aspects such as food intake and energy balance or anxiety and depression [9, 38].

The findings concerning the effects of long-term exposure to cannabis on emotional reactivity, arousal and anxiety remain controversial, particularly in humans. On the one hand, early cannabis use during adolescence appears to affect emotional development and mood, while, on the other, rebelliousness, antisocial behavior and mood disorders seem to be risk factors for early and regular cannabis use, probably pre-existing to cannabis use [16].

To make the interpretation of cannabis effects on emotional pattern more difficult, contrasting data suggest that cannabinoid receptor activation may produce an increased reactivity to stressful stimuli and significant anxiogenic effects. In experimental animals, THC has been demonstrated to induce anxiogenic and aversive effects [40]. Furthermore, more recently, anxiolytic response was found only at the high dose and anxiogenic response at low dose [11].

Considering human studies, intravenous use of delta-9-tetrahydrocannabinol in healthy individuals without a cannabis abuse disorder was reported to produce altered perception, increased anxiety and schizophrenia-like symptoms [7].

Although cannabis has been demonstrated to induce neurobiological changes that are significantly involved in reaction to stress and emotions, the effects of chronic exposure in humans on emotional arousal and on underlying neuroendocrine mechanisms are still unexplored. Findings suggest the involvement of noradrenergic pathways in some consequences of cannabis intake as an explanation of cannabinoid receptors in basic brain activities sustaining arousal and emotional states [31]. In fact, the endocannabinoid system has been proposed as an inhibitory modulator of neuronal and behavioral responses to stressful stimuli, thus suggesting its crucial involvement on the control of adrenocortical system [45].

In order to better understand the effects of cannabis exposure on emotional reactivity and its biological correlates in humans, in this study, we investigated the psychobiological reactions to experimentally induced negative emotional states. To this end, we examined individuals currently smoking marijuana and who interrupted drug use 6 months before the experiment, as compared with healthy abstinent control subject.

We hypothesized that long-term cannabis users could be affected by a dysregulation of the emotion-processing mechanism, probably due to a dysfunction of the endogenous cannabinoid system and related changes in HPA axis activity. On one side, these brain changes could have been provoked by direct cannabis pharmacological effects and, on the other, could be related to a possible individual condition of neurobiological vulnerability, pre-existing to cannabis exposure.

Aims of the present study were to investigate: (1) the possible dysregulation of emotional arousal/anxiety among chronic cannabis users and related changes in HPA axis function (2) whether the possible changes in emotional reactivity, in terms of anxiety, perception of disliking effects and HPA axis responses, were attributable to cannabis exposure per se and reversed by prolonged abstinence or related to persistent individual psychobiological as part of substance abuse susceptibility.

Methods

Subjects

Twenty-eight (28) cannabis-dependent subjects (21 men and 7 women), aged 19–28 years (24.1 ± 2.7 years), with a history of cannabis use alone of 3–14 years (8.8 ± 3.1), were included in the study, after informed written consent. They were not paid for their participation and accepted to enter the study as volunteers. They were smoking marijuana twice or three times a day for at least 3 years without

any abstinence period (range from 3 to 14 years). All the participants used the same street-cannabis available in the illegal market in the province of Biella (North of Italy), with an estimated average concentration of 10% (data obtained from the Department of Antidrug Policies—Presidency of the Council of Ministers).

Cannabis dependence was diagnosed following DSM IV criteria; all the dependent subjects have had also cannabis abuse episodes in the history. Previous continuous consumption of other drugs of abuse and psychotropic agents or excessive alcohol intake has been excluded.

The subjects contacted the Addiction Treatment Centre of Biella (Italy) in 2007–2008 because of the administrative provisions of the Italian law, including a coerced program in outpatient treatment centers, as measures alternative to sanctions for cannabis possession, or voluntarily, for a problematic clinical condition related to drug use.

The participants in the study were the first 28 who accepted to take part in the study and completed the procedure, in chronologic order, randomly assigned to group A (14 subjects), to be submitted to the experiment, under the effect of marijuana, or to group B (14 subjects), to be submitted to the experiment after 6 months of abstinence from marijuana without any signs of marijuana withdrawal.

Among 36 participants (subjects who initially accepted), 16 have been randomly selected and included into group A (subjects to be studied as active marijuana-dependent smokers). Two subjects (2) were excluded because they did not complete the experimental procedure.

Twenty subjects have been randomly selected to participate in group B (abstinent marijuana-dependent subjects): among 20 subjects, 14 remained abstinent from cannabis and have been studied 6 months after they have interrupted cannabis use, testing negative for cannabinoids and other drugs' metabolites for the entire period of the program.

Baseline and two-time-a-week analysis for urine metabolites of the main substances of abuse (morphine, methadone, cocaine, cannabis, amphetamine derivatives, benzodiazepines and barbiturate) excluded their consumption of other drugs and confirmed (for group A) or excluded (for group B) the use of cannabis. Group B cannabis users have been submitted to urinalyses for 6 months to demonstrate stable abstinence conditions. Exclusion criteria included the following: poly-drug use, severe chronic liver or renal diseases or other chronic physical disorders, recent weight loss or obesity, endocrinopathies, immunopathies and, in particular, HIV disease. In agreement with the rules of the program, the subjects were smoking not more than 3–5 cigarettes per day and drinking not more than two cups of coffee daily, during the week before the study. They abstained from smoking or drinking caffeinated beverages 12 h before our biochemical investigation.

Fourteen healthy volunteers, recruited from the hospital staff and among university students, and matched for age and sex (20–32 years; 25.4 $\pm$ 3.6), were used as controls. Exclusion criteria from the study were the same as those used for the patients. Volunteers were also controlled by urinary drug screening for 1 week before the study and immediately before (2 h) the experimental days and abstained from smoking or drinking caffeinated beverages 12 h before the biochemical investigations. After complete description of the study to the subjects, written informed consent was obtained also from healthy volunteers.

Psychiatric assessments

Cannabis-dependent subjects and controls were submitted to structured interviews and a diagnostic evaluation by a trained psychiatrist, utilizing the SCID (Structural Clinical Interview) for Axis I disorders [42], Italian version: Clinical Interview structured for the DSM-III-R [10] and the SIDP (Structured Interview for DSM IV Personality Disorders) for Axis II disorders [26, 33]. A second clinical interview in the presence of a family member was performed by the same psychiatrist to avoid denial of symptoms. Personality disorders and other comorbid psychopathologies were assessed by the Minnesota Multiphasic Personality Inventory II (MMPI II) [17].

The assessment of symptoms of comorbid psychopathologies also included a psychometric evaluation with Symptoms Checklist 90 (SCL-90-R) [6].

Emotional response evaluation

Thirty-six pictures (18 neutral and 18 unpleasant) were selected from the international affective picture system (IAPS) [21, 41]. The pictures were arranged in two sets: a neutral set of pictures (household objects) and an unpleasant set of pictures (mutilated bodies). The average ratings expressed as an ordered pair were 3.2 and 2.5 for the neutral slide set, 2.1 and 6.7 for the unpleasant slide set. Within each set, the 18 pictures were presented three times in three different blocks. Pictures were presented in different orders in the three blocks. Each picture was presented for 30 s followed by 3-s inter-picture interval, so each set lasted for 30 min.

All the subjects (active cannabis-dependent subjects, abstinent cannabis-dependent subjects and normal controls) were submitted to two experimental sessions, each on one of the 2 experimental days, a week apart in a counterbalanced order: the first session included unpleasant set and the second neutral set. Experimental session time was always 3.00 p.m., to exclude diurnal variability of hormonal plasma levels and cortisol morning peak.

Group A subjects reported cannabis use the night before the emotional experimental task and showed positive urinalyses during the week before the experiment and the week of the experiment.

In each experimental session, participants sat in a recliner in a small room (room A). State-Trait Anxiety Inventory Y-1 (STAI) [20] has been administered to the participants. Then, a catheter was inserted into an antecubital vein kept patent by saline infusion. After 30 min, a first basal blood sample was drawn (baseline). Previous studies [14, 20] have shown that the baseline hormonal values, sampled 30 min after the i.v. insertion, were not influenced by the emotional reaction to venipuncture. Then, the subjects were transferred into room B. Participants sat in a recliner in front of a monitor. The size of the screen was 17 inches, and the subjects were at 50 cm from the screen during the 30-min session. The participants were then instructed that a series of pictures will be displayed and that they should attend to each picture the entire time it was exposed on the screen. Following this, each participant was familiarized with the Self-Assesment Manikin (SAM) procedure [22], which involves ratings of pleasure and arousal. After one of the three slide sets was presented, subject was told to evaluate the slide show, using the SAM. Pleasantness and unpleasantness were rated for emotional "valence", and the word "valence" was used in the previous studies utilizing imagery exposure [43].

After slide viewing, a second blood sample for hormonal assays was collected. Hormonal levels were measured at a single time point, coinciding with the end of the visual stimulus presentation, not to influence emotional reactions during the 30-min session. In fact, more blood samples, and the frequent contacts with the nurse to obtain serial endocrine measures, could have induced stress-related hormonal changes.

A video camera in room B permitted to the research assistant to observe the subjects during the experimental session.

Neuroendocrine measures

For the determination of cortisol and ACTH plasma levels, the blood samples were centrifuged at $1{,}400 \times g$ for 15 min at 4°C; the supernatant (plasma) was then analyzed by a full automatic chemiluminescence system (Immulyte Medical System USA). The plasma concentrations of cortisol and ACTH were expressed as μg/dl and pg/ml, respectively.

Statistical analysis

Differences between the three groups of active cannabis users (A), abstinent subjects (B) and controls (C) were tested by means of one-way ANOVA (Analysis Of Variance). This test was used for differences between means of SCL-90 scores, of SAM scores of pleasantness and arousal and of STAI scores of anxiety after vision of neutral and unpleasant slides. Bonferroni Post hoc test was used after significant ANOVA to check which group(s) contributed to and determined the overall statistical significance. Mean differences in SAM and STAI scores after vision of neutral and unpleasant were tested by means of paired t-tests.

To check for differences in neuroendocrine and anxiety responses to neutral and disliking slides among the three groups of subjects, a two-way ANOVA with repeated measures was adopted. The effect of cannabis use on cortisol and ACTH levels as well as on STAI scores is therefore tested in two situations, after viewing of neutral slides and after viewing of disliking ones. Analysis of statistical significance was coupled with effect size measures. These indicators have been often used in psychological studies as a way to measure and interpret direction and magnitude of an effect of a treatment. In our study, standardized mean differences index has been used in tests of mean comparison. More specifically, we have used Coehn's d_z for matched pairs t test and Cohen's f for ANOVA. In the former case, it represents the ratio between the population mean of the difference z and standard deviation of the same difference z. In the latter case, the effect size is measured as the ratio between the between-group variance and the within-group variance. Effect size figures below 0.2 are regarded as small, medium around 0.5 and large over 0.8 [5].

Results

Psychiatric and psychometric evaluation

No significant differences at the clinical evaluation have been evidenced in the rate of psychiatric symptoms between group A (cannabis-dependent subjects actively smoking marijuana) and group B (abstinent cannabis-dependent subjects) subjects (data not shown). Cannabis users (A–B) showed symptoms of anxiety disorders, depression, antisocial personality disorders and borderline personality disorders, paranoid ideation, but no one fulfilled the entire list of symptoms for DSM diagnoses.

The scores of psychometric evaluation with Symptoms Checklist 90 (SCL-90-R) evidenced in group A, group B and healthy control subjects (group C) have been reported in Table 1. Anova F test resulted statistically significant for each of the symptoms analyzed, proving the existence of significant differences in scores between groups. Post hoc Bonferroni test allowed then to highlight the differences between SCL-90-R scores means. SCL-90-R scores were

Table 1 One-way ANOVA of Symptoms Checklist 90 (SCL 90) scores between active cannabis users (group A), abstinent cannabis users (group B) and controls (group C)

SCL-90 scores	Means ± SE			*F*	*P* value	Bonferroni post hoc test
	A	B	C			
Somatisation	1.30 ± 0.20	1.15 ± 0.17	0.17 ± 0.12	191.6	<0.001	All means different
O.C.D.	1.35 ± 0.18	1.16 ± 0.27	0.14 ± 0.16	133.2	<0.001	Only C different
Interpersonal sensitivity	1.06 ± 0.26	1.34 ± 0.18	0.29 ± 0.24	79.1	<0.001	All means different
Depression	1.39 ± 0.33	1.20 ± 0.23	0.13 ± 0.14	107.5	<0.001	Only C different
Anxiety	1.42 ± 0.22	1.20 ± 0.21	0.01 ± 0.04	261.6	<0.001	All means different
Anger hostility	0.45 ± 0.16	0.45 ± 0.24	0.04 ± 0.1	26.7	<0.001	Only C different
Phobic anxiety	0.54 ± 0.18	0.46 ± 0.17	0.00 ± 0.0	56.8	<0.001	Only C different
Paranoid ideation	0.96 ± 0.22	0.76 ± 0.19	0.00 ± 0.0	126.2	<0.001	All means different
Psychoticism	1.27 ± 0.20	0.47 ± 0.19	0.01 ± 0.03	219.6	<0.001	All means different

significantly higher in active cannabis users and abstinent cannabis users in respect of control subjects who never smoked cannabis, for somatisation, obsessive–compulsive, interpersonal sensitivity, depression, anxiety, anger hostility, phobic anxiety, paranoid ideation and psychoticism. Active cannabis users showed significantly higher scores than abstinent cannabis users on the scales of psychoticism, paranoid ideation, somatisation and anxiety. In contrast, abstinent cannabis users showed higher scores on the scale of interpersonal sensitivity respect to active cannabis users.

Emotional response and anxiety evaluation

Self-Assesment Manikin scores of pleasantness have been reported in Table 2. Pleasantness after disliking slides showed significantly lower scores than after neutral images in normal ($t = 11.706$, P value < 0.001, $df = 13$, $d_z = 3.128$) and in abstinent subjects ($t = 5.037$, P value < 0.001, $df = 13$, $d_z = 1.346$), with very high effect size figures. On the contrary, pleasantness scores were not significantly different after neutral and disliking slides in active cannabis users ($t = 1.989$, P value $= 0.068$, $df = 13$, $d_z = 0.53$), showing also not such a large effect size.

After unpleasant slides, pleasantness scores were significantly higher in both active and abstinent cannabis users with respect to control subject scores possibly evidencing a reduced sensitivity to disliking images. Pleasantness scores were in turn higher in active cannabis users than in abstinent users. After neutral images, pleasantness was vice versa higher in control subjects than in active and non-active cannabis users, which presented very close figures at SAM. Accordingly, in both ANOVA models, effect size figures resulted quite large, 1.230 and 0.918, respectively. Arousal scores have been reported in Table 3. The control group ($t = -6.176$, P value < 0.001, $df = 13$) and abstinent subjects ($t = -6.600$, P value < 0.001, $df = 13$) showed significantly higher scores of arousal (and very

Table 2 One-way ANOVA of pleasantness scores measured with the Self-Assesment Manikin (SAM) procedure between active cannabis users (group A), abstinent cannabis users (group B) and controls (group C)

	Means ± SD			ANOVA		Bonferroni test
	A	B	C	*F*	*P* value	
Neutral	4.7 ± 0.8	4.6 ± 1.0	6.1 ± 0.9	12.13	<0.001	Only C different
Unpleasant	4.2 ± 0.7	3.2 ± 0.9	1.8 ± 0.7	35.26	<0.001	All means different

Table 3 One-way ANOVA of arousal scores measured with the Self-Assesment Manikin (SAM) procedure between active cannabis users (group A), abstinent cannabis users (group B) and controls (group C)

	Means ± SD			ANOVA		Bonferroni test
	A	B	C	*F*	*P* value	
Neutral	4.7 ± 0.7	4.4 ± 0.9	4.4 ± 0.9	0.54	0.589	–
Unpleasant	4.9 ± 0.9	6.8 ± 0.8	7.4 ± 1.0	27.09	<0.001	Only A different

large effect size figures, respectively, 1.651 and 1.764) after vision of unpleasant slides than after vision of neutral ones. On the contrary, no differences in arousal levels at SAM were evidenced between emotional and neutral sessions among active cannabis users, with unpleasant and neutral slide sets obtaining similar responses in rated arousal ($t = -0.999$, P value $= 0.336$, $df = 13$, $d_z = 0.266$). The comparison of mean arousal scores between groups showed that no significant differential ratings were detected after viewing of neutral slides (Cohen's $f = 0.157$). On the contrary, active cannabis users displayed significantly lower levels of arousing compared to abstinent cannabis users and controls during emotional session (Cohen's $f = 1.184$).

The results of STAI scores are shown in Table 4. The basal levels of anxiety were not significantly different at the beginning of the two sessions (neutral and unpleasant slides) for any of the three groups here considered (cannabis user, $t = 0.916$, P value $= 0.376$, $df = 13$; abstinent, $t = 0.094$, P value $= 0.927$, $df = 13$; control, $t = -0.118$, P value $= 0.908$, $df = 13$). Existence of very small differences is also supported by the very low values of effect size, respectively, 0.032, 0.025 and 0.245. However, basal STAI scores were significantly higher in active cannabis users (group A) compared to abstinent cannabis users (group B), which in turn resulted significantly higher with respect to control subjects (group C) in both sessions (Cohen's f 1.890 and 2.035). Variation of anxiety scores in each group after viewing of neutral and unpleasant slide sets with respect to basal level is shown in Table 5 and Fig. 1. There is evidence of a non-significant variation in STAI scores for all the three groups in case of neutral slides, while significant changes in anxiety emerged after viewing of unpleasant slides. In particular, control subjects and non-active cannabis users show important and significant increases in STAI scores, while active cannabis users do not.

Neuroendocrine measures

Tables 6 and 7 and Fig. 2 show the results of two-way ANOVA with repeated measures performed on the effects of cannabis use and slide sets viewing on neurohormone levels. Serum cortisol and ACTH basal levels were significantly different in the three groups. Cortisol and ACTH basal levels were higher in active cannabis users in comparison with abstinent cannabis users and controls in both neutral and unpleasant sections. Similarly, abstinent cannabis users showed higher basal levels of those two neurohormones with respect to control subjects who never smoked cannabis.

Cortisol and ACTH levels did not increase significantly after vision of a neutral slide set, but they did after viewing of an unpleasant one in comparison with baseline levels. This increase in neurohormone levels occurred especially among healthy controls and abstinent cannabis users, while among cannabis users the increase was much more limited. In particular, the response after vision of unpleasant images resulted statistically significant in each of the three groups for cortisol, while it was non-significant for ACTH among

Table 4 One-way ANOVA of basal anxiety scores measured with State-Trait Anxiety Inventory Y-1 (STAI) between active cannabis users (group A), abstinent cannabis users (group B) and controls (group C)

	Means ± SD			ANOVA		Bonferroni test
	A	B	C	F	P value	
Pre-neutral	48.3 ± 6.1	42.1 ± 3.8	28.4 ± 3.3	69.83	<0.001	A, B, C significantly different
Pre-unpleasant	47.4 ± 5.2	41.9 ± 2.8	28.5 ± 3.6	83.57	<0.001	A, B, C significantly different

Table 5 Two-way ANOVA with repeated measures

Slides	Cannabis users		Abstinents		Controls		P values		
	Pre	Post	Pre	Post	Pre	Post	G[1]	T	$G \times T$[2]
Neutral	48.3 ± 6.1	48.3 ± 5.7	42.1 ± 3.8	39.9 ± 4.9	28.4 ± 3.3	28.9 ± 3.1	<0.001	0.429	0.227
Unpleasant	47.4 ± 5.2	48.1 ± 5.8	41.9 ± 2.8	54.8 ± 4.4	28.5 ± 3.6	49.9 ± 3.4	<0.001	<0.001	<0.001

Effect of cannabis use and vision of neutral and unpleasant slides viewing on anxiety scores

Values are means ± SD. G is for main effect of groups (cannabis users vs. abstinents vs. controls); T is for main effect of time (pre-viewing vs. post-viewing); $G \times T$ is for interaction effect between group and time

[1] For neutral slides, the three means are all statistically different one another, while for Unpleasant slides only Controls are significantly different (Bonferroni post hoc test)

[2] For Unpleasant slides, only active Cannabis users show an anxiety response that was not significantly different from basal levels (Bonferroni post hoc test)

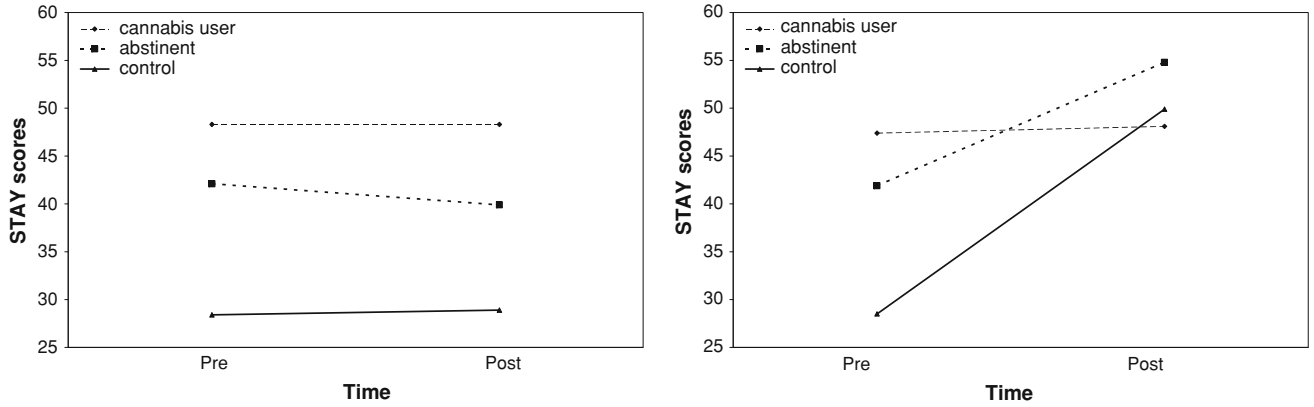

Fig. 1 STAI anxiety scores before and after slide set viewing (neutral, on the *left*; unpleasant, on the *right*) in cannabis users, abstinent subjects and controls

Table 6 Two-way ANOVA with repeated measures

Neurohormones	Cannabis users		Abstinents		Controls		*P* values		
	Pre	Post	Pre	Post	Pre	Post	G^1	T	$G \times T$
Cortisol	36.1 ± 5.4	35.9 ± 3.2	30.4 ± 5.1	31.3 ± 8.3	18.3 ± 2.0	18.6 ± 2.4	<0.001	0.636	0.800
ACTH	75.6 ± 7.6	74.8 ± 6.4	54.0 ± 9.8	53.2 ± 8.6	37.9 ± 2.8	39.2 ± 3.1	<0.001	0.912	0.297

Effect of cannabis use and neutral slide set viewing on neurohormones levels

Values are means ± SD. *G* is for main effect of groups (cannabis users vs. abstinents vs. controls); *T* is for main effect of time (pre-viewing vs. post-viewing); $G \times T$ is for interaction effect between group and time

[1] For both hormones, the three means are all statistically different one another (Bonferroni post hoc test)

Table 7 Two-way ANOVA with repeated measures

Neurohormones	Cannabis users		Abstinents		Controls		*P* values		
	Pre	Post	Pre	Post	Pre	Post	G^1	T	$G \times T^2$
Cortisol	37.1 ± 4.7	39.3 ± 4.5	30.6 ± 5.1	35.3 ± 4.9	17.8 ± 2.5	39.0 ± 2.9	<0.001	<0.001	<0.001
ACTH	75.9 ± 4.9	76.9 ± 6.2	51.9 ± 6.5	55.2 ± 6.4	39.7 ± 2.3	62.9 ± 5.3	<0.001	<0.001	<0.001

Effect of cannabis use and unpleasant slide set viewing on neurohormones levels

Values are means ± SD. *G* is for main effect of groups (cannabis users vs. abstinents vs. controls); *T* is for main effect of time (pre-viewing vs. post-viewing); $G \times T$ is for interaction effect between group and time

[1] For cortisol, the three means are all statistically different one another, while for ACTH only cannabis users show significantly different concentrations (Bonferroni post hoc test)

[2] For cortisol, the three groups show responses statistically different from one another after viewing of an unpleasant slide set. For ACTH, only cannabis users show a non-statistically significant response after unpleasant slide set viewing (Bonferroni post hoc test)

active cannabis users, likely due to the already high basal levels of ACTH in this group. This is the logical consequence of the effects associated with the interaction between group (cannabis user, abstinent, control) and time (before and after viewing of slides), and the following Bonferroni post hoc test performed on the six means of such an interaction.

Considering that the proportion between female and male in the present sample of cannabis users was 1:3, the number of female subjects included in our study was very small, not permitting a reliable statistical analysis.

Discussion

The results of the present study seem to suggest an association between cannabis use and subjective reduced sensitivity to negative emotions and threat, particularly in those who actively smoked marijuana at the time of the experiment. Abstinent cannabis users in our sample presented a lower perception of unpleasantness, compared to control subjects, in front of negative emotion slides, but arousal responses similar to healthy controls, possibly indicating that their reactivity to stressful stimuli was in

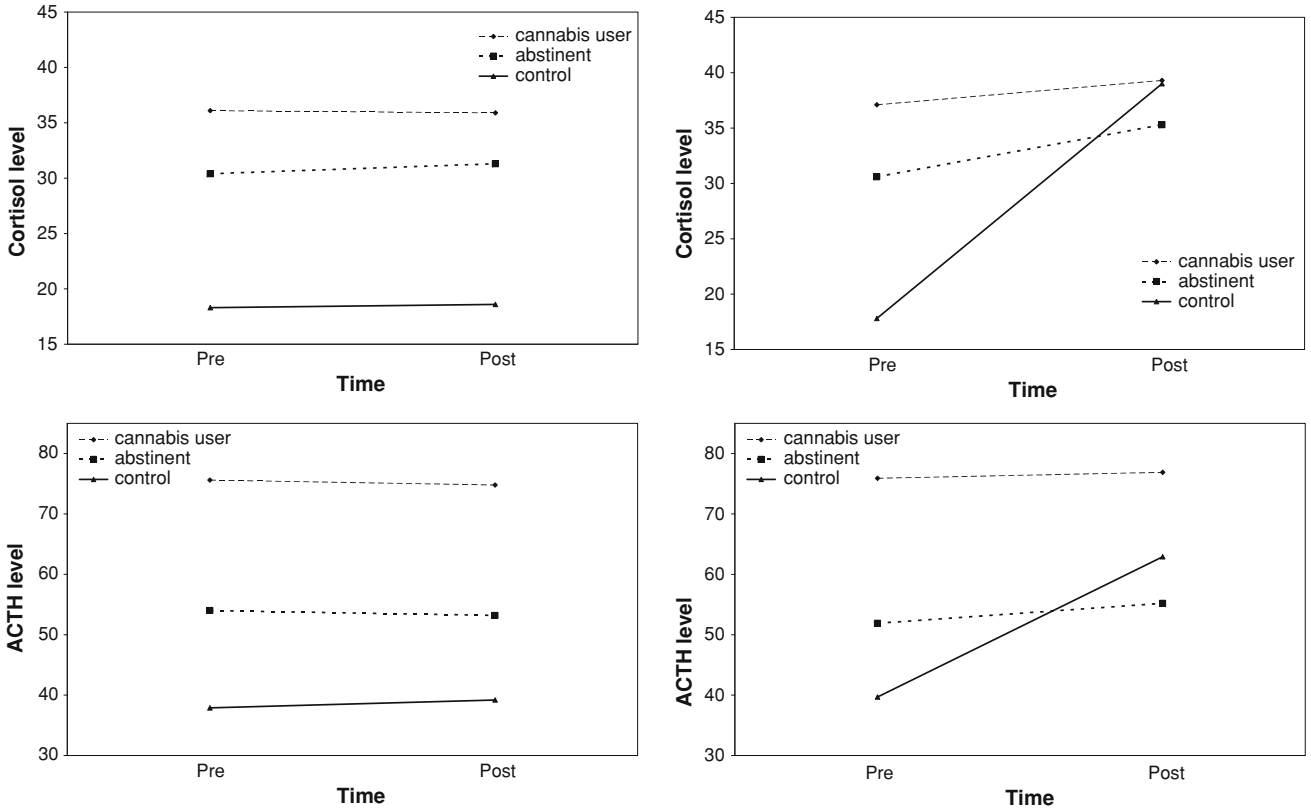

Fig. 2 Cortisol (*upper* figures) and ACTH levels (*lower* figures) before and after slide set viewing (neutral, on the *left*; unpleasant, on the *right*) in cannabis users, abstinent subjects and controls

part recovered. In line with this evidence, a possible derangement of safety system sensitivity, particularly of the reactions to threat, dangerous situations, negative and unpleasant emotions, has been previously reported in cannabis users [2].

The ability of cannabis to affect emotional arousal and anxiety levels in response to adverse experiences could be related to the influence of the endocannabinoid system on different neurotransmitter systems that have been demonstrated to be differently modulated in each brain area by the activation or deactivation of cannabinoid CB1 brain receptors [24]. Moreover, cortico-releasing hormone (CRH), the hypothalamic peptide activating HPA axis, has been found implicated with the endocannabinoid system, which seems to be an inhibitory modulator of adrenocortical responses to stressful stimuli [44].

A reduced response to negative emotions in terms of anxiety scores at STAI was evidenced in our experiment among active smokers of marijuana, suggesting a lack of reactivity to stressful stimuli in these subjects. In addition, active cannabis smokers showed high basal levels of anxiety, also before the exposure to the emotional task with negative emotions, indicating a state of potential persistent arousal and impaired reactivity to stress.

Anxiety responses and activation of behavioral reactivity to stress have been demonstrated to be affected by cannabis in a complex way. Δ-9-Tetrahydrocannabinol (Δ9-THC) and cannabindiol (CBD) have distinct effects on the neural, electrodermal and symptomatic responses to negative emotional stimuli. In particular, the effects of CBD on activation in limbic and para-limbic regions may contribute to its ability to reduce autonomic arousal and subjective anxiety, whereas the anxiogenic effects of Δ9-THC may be related to effects on frontal and parietal areas [12]. The prolonged stimulation of cannabinoid receptors could have been responsible for a derangement in the anxiety modulation mechanism, with both a stable reduced inhibition on arousal activation mechanisms, when the system is quiescent, and a reduced capacity to react to stress in front of acute threat situation and negative emotions.

The alteration of hormonal plasma levels observed in our study indicates a possible persistent hyperactivity of HPA axis in cannabis users, particularly among active marijuana smokers, and an impaired hormonal reaction to negative emotions, in comparison with healthy subjects. This alteration seems to be only partially recovered after 6 months of abstinence.

Previous research found that cannabis-induced psychotomimetic effects and perceptual alterations were associated with increased levels of in plasma cortisol, suggesting the possible involvement of HPA axis dysregulation in the significant changes evidenced in the reaction to negative emotions [8, 36]. In agreement with our results, at socially relevant doses, Δ9-THC has been reported to raise plasma cortisol levels in a dose-dependent manner, but frequent users showed blunted increases relative to healthy controls, possibly suggesting the development of tolerance to the neuroendocrine effects of cannabinoids [36].

Basic research indicates that mutant mice lacking cannabinoid receptors (CB1) show anxiogenic-like and depressive-like phenotypes, as well as profound alterations in their adrenocortical activity, confirming the strict relationship between HPA axis dysfunction and altered response to stress [44]. In addition, withdrawal from abused drugs, such as cannabis, nicotine and alcohol, by increasing corticotrophin release factor could induce a persistent overstimulation of HPA axis [3].

The psychobiological changes evidenced in our study, including both reduced reactivity to unpleasant stimuli and reduced anxiety, with HPA axis persistent hyperactivity and blunted responses to stress in cortisol and ACTH, were more evident in active cannabis smokers and statistically correlated with cannabis exposure extent. This suggests a direct pharmacological effect of cannabis in modulating stress reactivity and emotion sensitivity.

Otherwise, a condition of dysfunction of both HPA axis and reaction to negative emotions pre-existing to cannabis exposure and related to individual personality traits, and vulnerability to substance use disorders, cannot be excluded by the results of our study.

Depressed adolescents who have anxiety traits and whose HPA axis is active when the system is normally quiescent, with less cortisol responsiveness to exciting stimuli, similarly to what was evidenced in our cannabis users, have been reported to be at risk for developing substance use disorders [37]. Accordingly, depressed adolescents who are at risk to make suicide attempts have been found to display significant elevations of cortisol prior to sleep onset, when the HPA axis is normally most quiescent [29]. Considering depressive symptoms revealed by SCL 90 in our cannabis users, the hypothesis that HPA dysregulation may not only be entirely related to cannabis pharmacological effects but also to individual neurobiological characteristics remains valid. In contrast to our results, a meta-analysis on depressed youth not exposed to drugs evidenced an overactive response to psychological stressors and not a blunted one [23], underlying the possible role of cannabis itself in the impairment of emotional stress response.

Other evidence on HPA axis dysregulation pre-existing to cannabis exposure demonstrated HPA axis hypo-activity at awakening in adolescents with early-onset of cannabis use compared to late-onset users, which might indicate an increased risk for early-onset users of seeking stimulation to restore arousal levels using substances [18]. The high level of cortisol and ACTH evidenced in our cannabis users would be entirely attributable, following this self-medication interpretation, to the long-term cannabinoid receptor stimulation in chronic marijuana smokers.

The main weakness of our research design, which should be addressed in further studies, was that the same cannabis users have not been investigated prospectively, before and after 6 months of abstinence. However, the results of a prospective experimental design could have been influenced by habituation, with reduced sensitivity to the reiterated emotional stimuli administered in the same group of drug users.

In conclusion, although obtained in a small sample of subjects and influenced by the wide variability of cultural factors in front of emotional stimuli, our findings strengthen the evidence of a possible dysfunction of HPA axis in relation to a maladaptive coping style with unpleasant emotions in cannabis users, particularly in active marijuana smokers.

This psychobiological derangement in response to stressful emotions may probably contribute, in heavy cannabis smokers, to the cannabinoid effects perception that seems to be not always pleasant, with anxiety and panic symptoms [30]. In the long term, HPA axis activity and threat response dysregulation may play a role in bad mood perception and an internal distress condition, in turn involved in the proneness to paranoia and paranoid ideation repeatedly evidenced in cannabis users by other authors [25, 28, 39] and demonstrated at SCL 90 also in the present study.

The psychobiological dysfunction in response to emotional stimuli emerging in our study in cannabis users could be responsible for impaired interpersonal relationships and social exclusion, reduced engagement and motivation, poor capacity to cope with stress. Ultimately, this clinical condition may explain the need of self-medication with opioids, alcohol and benzodiazepines, increasing the vulnerability of cannabis users for a complex substance abuse syndrome.

Conflict of interest None.

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