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Psychiatric Symptoms, Salivary Cortisol and Cytokine Levels in Young Marijuana Users

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Abstract

Psychological maturation continues into young adulthood when substance abuse and several psychiatric disorders often emerge. Marijuana is the most common illicit drug abused by youths, typically preceding other illicit substances. We aimed to evaluate the complex and poorly studied relationships between marijuana use, psychiatric symptoms, and cortisol levels in young marijuana users. Psychiatric symptoms and salivary cortisol were measured in 122 youths (13-23 years old) with and without marijuana use. Psychiatric symptoms were evaluated using the Symptom-Checklist-90-R and Brief Psychiatric Rating Scale. Mid-day salivary cortisol levels were measured. Additionally, salivary cytokine levels were measured in a subset of participants. Although the cortisol levels and salivary cytokine levels were similar, the young marijuana users had more self-reported and clinician rated psychiatric symptoms than controls, especially anxiety-associated symptoms. Moreover, marijuana users with earlier age of first use had more symptoms, while those with longer abstinence had fewer symptoms. Greater cumulative lifetime marijuana use was also associated with greater psychiatric symptoms. The discordant anxiety (feeling stressed or anxious despite normal cortisol) in the marijuana users, as well as symptom exacerbations with early and continued marijuana use in young marijuana users suggest that marijuana use may contribute to an aberrant relationship between stress response and psychiatric symptoms. The greater symptomatology, especially in those with earlier initiation and greater marijuana usage, emphasize the need to intervene for substance use and perceived anxiety in this population.

Keywords

Marijuana; Anxiety; Cortisol; Adolescent

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Background

The brain and all its consequent functions (cognitive, social, emotional and personality) continue to develop into the third decade of life (Giedd, 2004). Adolescents and young adults are the most common age-groups to develop substance abuse disorders (Kessler et al., 1997) and new onset Axis 1 psychiatric disorders (Upadhyaya et al., 2002). Marijuana use often precedes other illicit substance use, and is therefore considered a “gateway drug” (Fergusson and Horwood, 2000). Furthermore, substance abuse, including marijuana abuse, is a common comorbidity and possible aggravation for several Axis 1 disorders (Johnston et al., 2012; Lai and Sitharthan, 2012). Nationwide, more than 1 in 5 twelfth graders report using marijuana during the previous 30 days and nearly half of twelfth graders have tried marijuana (Adam, 2006; Johnston et al., 2012). In addition, 80% of individuals who use illicit drugs have also used marijuana (Koshibu et al., 2004; SAMHSA, 2009).

The primary psychoactive ingredient in marijuana, Δ^9 -tetrahydrocannabinol (THC), acts on the brain’s cannabinoid system. This system plays a role in early development of several brain regions that may be abnormal in many psychiatric disorders, including the nigrostriatal pathway, prefrontal cortex, and hippocampus (Fride, 2004). However, less is known about how cannabinoids might influence adolescent developmental periods and their association with psychiatric outcomes.

In addition to the more publicized effects of marijuana on the brain and corresponding behavior, marijuana interacts with the immune and endocrine systems. Furthermore, adolescents are learning to cope with physical and emotional stressors, while their responses to stress (Romeo, 2010) and their immune system are changing, during puberty and into early adulthood (Jaspan et al., 2006).

Stress and drugs of abuse often interact with each other. Stress can affect drug consumption and relapse (Marinelli and Piazza, 2002), as well as enhance drug craving (Sinha, 2008). Most addictive drugs activate the hypothalamic-pituitary-adrenal (HPA) axis directly or indirectly (Armario, 2010). However, the influence of cannabinoids on stress and the HPA axis is controversial as cannabinoids can have both anxiogenic and anxiolytic properties that vary with dose and stress levels (Fokos and Panagis, 2010). Marijuana also interacts with the immune system both directly through cannabinoid receptors on immune cells and indirectly via the brain’s endocannabinoid system (Klein et al., 2003); in adults, marijuana also directly and indirectly activates the HPA axis (Steiner and Wotjak, 2008). Adult marijuana users, most of whom started using during adolescence, have altered brain activation (Chang et al., 2006) that is mediated in part by cortisol levels (King et al., 2011). However, less is known about how the brain and the immune system are affected by marijuana use in adolescents. Cortisol measures correlate with cytokines, including IL-6, and TNF- α in healthy adults (DeSantis et al., 2012); these correlations reflect the complex feedback systems that are often perturbed by stress, and may be the cause or consequence of illness.

Marijuana and several other drugs of abuse interact with the immune system (Friedman and Eisenstein, 2004). Cannabinoids modulate inflammation through neuronal and non-neuronal (e.g., cytokine-producing cells like microglia and macrophages) cells. This pathway

influences multiple cytokines, including, IL-1 β , IL-6, and TNF- α . Of these, higher IL-6 levels have been associated with multiple psychosocial support and risk factors in adults, including lower self-esteem, more hopelessness, or more hostility (Sjogren et al., 2006).

Despite the high prevalence of marijuana use by youths, the relationships between marijuana use, stress and immunological responses, and psychiatric symptoms are not well studied in adolescents. Therefore, the current study aims to compare cortisol levels and psychiatric symptoms, as assessed by the Symptom Checklist-90-R (SCL-90R) and the Brief Psychiatric Rating Scale (BPRS), in adolescent marijuana users and non-users. We hypothesized that young marijuana users would have more psychiatric symptoms and elevated mid-day salivary cortisol levels. For exploratory purposes, we also evaluated three immune markers to gain further insight into how marijuana, cortisol, and psychiatric symptoms interact with the adolescent immune system. In addition, we assessed cortisol responses to stress after the subjects completed a battery of computerized assessments (described as “frustrating” or “annoying” by the participants) or the Trier Social Stress Test (TSST). Since THC is immunosuppressive (Cabral, 2006), we hypothesized that the marijuana users would have reduced IL-1 β , TNF- α and IL-6 levels, and a blunted stress response compared to the controls, and that mid-day cortisol would positively correlate with IL-6 levels and depressive symptoms.

Methods

All subjects provided informed written consent (or subject assent and parental consent if <18 years old) to participate in studies of adolescents with or without substance use, approved by the University of Hawaii Committee on Human Studies. 122 participants (80 marijuana users and 42 controls) were recruited from the local community. Each was evaluated by a physician using a structured neuropsychiatric examination, and provided a detailed drug use history and urine for toxicology. A structured interview regarding substance use history included self-reported age of first use, frequency (days per week), duration (months) and amount of use (converted to standard units i.e. joints, drinks, cigarettes), from which a cumulative estimate of lifetime use was determined. In addition to face-to-face interviews, structured surveys (SCL-90R and BPRS) were used to quantify subclinical psychiatric symptoms. A follow-up evaluation by a board-certified Adolescent Psychiatrist (DA) was conducted in anyone with any regular substance use or notable psychiatric responses during the interviews or on surveys, to determine if the subject met dependence for exclusionary substances and evaluate reported symptoms (by Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision criteria) and to assess for possible need for referral to psychiatric or substance use disorder treatment.

Participants were included in the study if they fulfilled the following criteria: 1) male or female ages 13-23 years; 2) not dependent on substances other than marijuana or tobacco; 3) no confounding medical conditions; 4) never treated with psychiatric medications. In addition, control subjects were enrolled only if they had tried marijuana <5 times in their lifetime, and marijuana users were included only if they had used marijuana at least weekly for at least six months, and had a cumulative use of >20 joints. None of the subjects were acutely intoxicated (admitted using within the last 4 hours or had overt signs of intoxication

upon interview and medical evaluation). Subjects were excluded if they had a positive urine drug screen for amphetamines, cocaine, opiates, or benzodiazepines, or reported recent (last 30 days) illicit (other than marijuana) drug use.

Psychiatric Symptom Assessments

The SCL-90R is a self-administered psychometric questionnaire. Each subject answered 90 questions using a 5-point scale to assess their distress over the past week. Nine domain scores and 3 global scores were generated from their responses. Only raw scores were used for the current study due to the artificial differences introduced by t-scores for those under versus over 18 years old.

For the BPRS, subjects were interviewed and observed for 24 items using a 1-7 scale, indicating the presence and intensity of current symptoms. For this study, three global BPRS scores and five domain scores were calculated for each participant. Global BPRS scores parallel the SCL-90R scores to include Total BPRS (sum of all scores), Presence (number of scores >1 indicating presence of symptoms), and Intensity (average score for all symptoms present). Domain scores are a modification of a patient study (Ventura et al., 2000) that further separates the Depression and Anxiety components resulting in the following domains: Manic Excitement (motor hyperactivity, elated mood, excitement, distractibility, hostility, grandiosity), Negative Symptoms (blunted affect, motor retardation, emotional withdrawal, self-neglect), Positive Symptoms (bizarre behavior, unusual thought content, disorientation, hallucinations, suspiciousness), Depression (depression, somatic concerns, suicidality, self-neglect) and Anxiety (anxiety, guilt, suspiciousness, tension).

Salivary Cortisol & Cytokine Measurement

Saliva was collected using a Salivette (Sarstedt, Inc., Newton, NC) in the late morning or afternoon, more than an hour into their supervised study visit. Forty-nine participants also completed the TSST: participants were read a six-sentence story and asked to complete the story in any way, while two investigators observed and recorded their responses for 5 minutes. The subjects were then instructed to count backwards by 13 starting at 1,023. If they made a mistake, they had to start over. After 5 minutes of this mental arithmetic, a saliva sample was taken to measure the acute response to the stressor. The subjects were then allowed to read, listen to music, or watch a movie of their choice to relax for 60 min, after which another saliva sample was collected to determine delayed stress response or recovery. Furthermore, 69 participants completed a one-hour battery of computerized tests (mental flexibility, attention, working-memory, and risk-taking tasks). Saliva samples were collected before and immediately following these tests. All samples were centrifuged and frozen at -70°C until the day of the assay. Cortisol and cytokine levels were assayed in duplicates, following kit instructions with a 96-well plate enzyme-linked immunosorbent assay (High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit: Salimetrics LLC, State College, PA; High Sensitivity Human IL-1 beta/IL-1F2, Human IL-6, and Human TNF-alpha Quantikine ELISA Kits R&D Systems Inc, Minneapolis, MN), using a BioTek Powerwave XS 96-well plate reader (Winooski, VT) and Sigma Plot (San Jose, CA) software.

Statistical analyses

Data were analyzed using StatView (SAS Institute Inc., Cary, NC). Due to the wide range of marijuana usage, marijuana users were split into light and heavy user groups, with a median split near 500 estimated lifetime joints. SCL-90R scoring norms indicate that females typically have more psychiatric symptoms and greater intensity of symptoms compared to males on several measures. Therefore, we first evaluated our data using two-way ANOVA, with group and gender as independent variables. However, since no interactions between group and gender were found, all further analyses combined the measurements from both sexes. MANOVAs were used to assess group (Control-CON, Light Marijuana-LMJ, Heavy Marijuana-HMJ) effects and interactions for the multiple measures within the SCL-90R (3 global, 9 domains) and BPRS (3 global, 5 domains), while repeated-measures-ANOVAs were used to assess multiple time points for cortisol measures. Those measures with significant effects or interactions were further evaluated with post-hoc t-tests. Associations between cortisol or cytokine levels and symptoms were tested with Pearson correlations. Possible associations between variables that showed group differences and drug usage (cumulative lifetime joints, tobacco cigarettes, and standard drinks, age of first marijuana use and abstinence from marijuana use) were also explored with Pearson correlations. The three cumulative measures and the duration of abstinence were log transformed to normalize the data for analyses. Results were considered significant if they survived Bonferroni correction for multiple comparisons (for 3 ANOVAs, 3 SCL-90R global scores, and 3 global BPRS scores $p < 0.017$; for 5 BPRS domains and 5 drug use measures $p < 0.010$; for 9 SCL-90R domains $p < 0.006$). Statistical trends were defined as p-values ≤ 0.10 without correction for multiple comparisons. Due to the smaller sample size and the exploratory nature of the cytokine evaluations, these measurements for all marijuana users were combined into one group (MJ) and compared with the controls using unpaired t-tests (two-tailed).

Results

The participant groups were similar in age and gender proportion (**Table 1**). Although the heavy marijuana users were significantly older than the controls, by only one year, the three groups were not different on ANOVA. All participants had physiological measures (blood pressure, heart rates and body mass index) that were within normal limits and a normal physical examination.

Most marijuana users were poly-substance (e.g., alcohol, tobacco) users, but marijuana was their drug of choice. When light and heavy marijuana users were combined, the age of first marijuana use was 14 years, and the participants used marijuana almost daily for approximately 4 years. Conversely, only 8 of the 42 control subjects experimented with marijuana, starting at an older age of 18 years (**Table 1**). Controls who had tried marijuana typically reported “trying a hit once” or “sharing a joint with friends” once or twice; therefore, their averaged estimated lifetime use was less than one joint. Marijuana users smoked more tobacco and drank more alcohol than the controls, although none met dependence criteria for alcohol. Most (74%) HMJ subjects were current users, while most (72%) LMJ subjects were not currently using marijuana as indicated by self-report and urine toxicology.

Psychiatric Symptoms (Figure 1)

A MANOVA of SCL-90R (group effect $p=0.002$, group by measure interaction $p<0.0001$) and BPRS (group effect $p<0.0001$, group by measure interaction $p<0.0001$) measures indicates group differences. Marijuana users had more symptoms than controls, especially the HMJ. Overall psychological distress was higher in marijuana users on the SCL-90R Global Severity Index (GSI, group- $p=0.020$; HMJ > CON, $p=0.006$) and Total BPRS (group- $p=0.012$; LMJ > CON, $p=0.05$; HMJ > CON, $p=0.004$). The number of symptoms present also was elevated in marijuana users, as indicated by the SCL-90R Positive Symptom Total (PST, group $p=0.017$; HMJ > CON, $p=0.004$) and BPRS Presence (group $p=0.010$; LMJ > CON, $p=0.03$; HMJ > CON, $p=0.004$). Trends for group differences were found on the symptom intensity (the SCL-90R Positive Symptom Distress Index (PSDI, $p=0.07$) and BPRS Intensity ($p=0.09$), with the highest intensity in the HMJ group.

Post-hoc analyses showed that anxiety-associated symptoms were most commonly reported. The SCL-90R primary symptom dimension raw scores indicate worse symptoms for marijuana users, especially the HMJ, in 2/9 dimensions: Paranoid Ideation (group- $p=0.004$; HMJ > LMJ, $p=0.001$; HMJ > CON, $p=0.03$), and Phobic Anxiety (group- $p=0.002$; HMJ > LMJ, $p=0.016$; HMJ > CON, $p=0.0007$); with similar trends for Obsessive-Compulsive (group- $p=0.021$; HMJ > CON, $p=0.006$) and Somatization (group- $p=0.007$; HMJ > LMJ, $p=0.030$; HMJ > CON, $p=0.003$). Similarly, marijuana users had worse BPRS symptoms: Anxious (group- $p=0.002$; HMJ > LMJ, $p=0.05$; HMJ > CON, $p=0.0005$) and Manic (group- $p=0.018$; LMJ > CON, $p=0.012$; HMJ > CON, $p=0.017$).

Associations between marijuana use and psychiatric symptoms (Figures 2 & 3)

Several psychiatric measures that showed group differences also showed associations with marijuana usage. Younger age-of-first marijuana use was associated with higher SCL-90R PST ($r=-0.24$, $p=0.032$), PSDI ($r=-0.30$, $p=0.006$), and Paranoid ($r=-0.24$, $p=0.031$) scores (**Figure 2 a, b, & d**). However, longer duration of abstinence (log days) was associated with lesser Obsessive Compulsive symptoms ($r=-0.33$, $p=0.004$) (**Figure 2 f**). Additionally, more lifetime usage (log lifetime joints) was associated with higher PSDI ($r=0.25$, $p=0.025$), Obsessive Compulsive ($r=0.26$, $p=0.016$), and Phobic Anxiety ($r=0.22$, $p=0.042$) scores (**Figure 2 c, e & g**).

Similarly, BPRS measures showed associations with marijuana usage. Longer duration of abstinence was associated with fewer symptoms (Present $r=-0.31$, $p=0.017$), lesser Anxiety ($r=-0.31$, $p=0.017$), and lesser Manic ($r=-0.29$, $p=0.030$) and Total symptoms ($r=-0.32$, $p=0.015$) (**Figure 3 a, b, c, & d**). Additionally, more lifetime marijuana usage was associated with more Anxiety symptoms ($r=0.29$, $p=0.021$) (**Figure 3 e**).

The marijuana user groups, especially the HMJ group, used more tobacco and alcohol than controls, and both lifetime amounts of tobacco and alcohol used correlated with lifetime joints of marijuana smoked ($r>0.4$; $p<0.0001$). Therefore, the group comparisons for psychiatric symptoms were re-analyzed to include lifetime number of tobacco cigarettes smoked as a covariate. Three (paranoid ideations, phobic anxiety and obsessive-compulsive) of the six SCL90 measures and three (presence, mania and anxiety) of the four BPRS

measures that previously showed group differences remained significant. Similarly, when lifetime alcohol use was included as a covariate in the group comparisons, five of the six SCL90 measures (except somatization) and all four BPRS measures that previously showed group differences remained and became more significant.

Salivary Cortisol and Cytokine levels and associations (Figure 4)

All baseline saliva collection was performed in the late morning or afternoon. The time of day for subjects completing the computer testing did not differ between groups ($p=0.92$). Testing was performed on average 6.6 ± 0.5 hours after waking in controls and 5.6 ± 0.5 hours after waking in marijuana users. Both groups averaged 7 hours of sleep the night before testing. The TSST was administered approximately 1.5 hours later in the day than the computer tests; consequently cortisol levels approximated mid-day levels. Baseline cortisol levels did not differ between groups ($p=0.46$; **Figure 4 a**). Salivary cortisol levels decreased ($p<0.0001$) after the computerized testing for all groups (CON -31%, LMJ -43%, HMJ -35%). Within the subset of participants who completed the TSST, the cortisol levels did not differ between groups but both groups showed a modest decrease in cortisol levels across the three time points of cortisol measurements (repeated measure ANOVA $p=0.05$). Pre-stress cortisol levels were not significantly different from post-stress or from recovery cortisol levels, but the cortisol level was 16% lower at one-hour after recovery compared to the post TSST measures (paired- t - $p=0.01$, **Figure 4 b**). Baseline cortisol levels correlated with the intensity of BPRS symptoms ($r=0.26$, $p=0.017$, **Figure 4 c**), but not with any drug usage parameters.

In our exploratory sample, levels of IL-1 β and TNF- α were similar between MJ users and controls; however, IL-6 levels tended to be higher in marijuana users relative to controls (5.5 ± 1.5 vs. 2.1 ± 0.6 , $p=0.10$). Similar to the correlations found with baseline cortisol levels, higher IL-6 levels correlated with BPRS Symptom Intensity ($r=0.62$ $p=0.004$ **Figure 4 d**), but not with drug usage or with cortisol levels. Furthermore, higher TNF- α correlated with greater SCL-90R Symptom Total ($r=0.45$ $p=0.017$ **Figure 4 e**).

Discussion

Several abnormalities were found in our adolescent marijuana users. First, compared to control subjects, young marijuana users had more psychiatric symptoms, even after we accounted for the concurrent tobacco smoking and mild alcohol use in many of the heavy marijuana users. The higher scores on these symptom scales also indicate multiple forms of elevated but sub-clinical anxieties, despite normal mid-day salivary cortisol levels. Second, the participants did not have a significant stress response to the TSST, but their cortisol levels decreased significantly after a recovery period. Third, the elevated psychiatric symptoms were associated with earlier age of first marijuana use, more recent marijuana use, and greater lifetime marijuana exposure. Lastly, contrary to our hypothesis, exploratory analyses of three proinflammatory cytokines found non-significant and mild elevation of IL-6 and TNF- α levels, which were related to greater psychiatric symptoms.

Psychiatric Symptoms

Adolescent marijuana users, especially the heavy users, had more symptoms than controls. In particular, the anxiety-related measures (general anxiety, obsessive-compulsive, paranoid ideation, and phobic anxiety) were significantly elevated in the marijuana users. Associations between anxiety and marijuana use or THC were reported previously in both humans and animal studies, but the relationships were complex. A naturalistic study of college students showed no anxiolytic or anxiogenic effects of marijuana in their daily life, but found that those with an anxiety disorder were more likely to use marijuana (Tournier et al., 2003). Animal models suggest both stress and THC dose contributed to the anxiolytic or anxiogenic effects of THC. In unstressed rats, low doses of THC induce anxiolytic-like effects; however, in stressed rats, lower doses induced anxiogenic-like effects, while higher doses induced anxiolytic-like effects (Fokos and Panagis, 2010). Our marijuana users did not appear physiologically stressed (based on cortisol levels), despite their self-report of feeling anxious.

Anxious paranoia is of particular interest considering a large survey reported that paranoia was associated with marijuana use and since other characteristics common to adolescents (youth, social stress, perceived isolation) were also associated with paranoia in the general population (Freeman et al., 2011). Therefore, young marijuana users may be a particularly vulnerable population. These findings are consistent with those in a large study of normal subjects (ages 16-74 years), which found that those with psychotic symptoms were more likely to be marijuana-dependent and those with paranoid symptoms were more likely to be under 35 years old (Johns et al., 2004), suggesting earlier marijuana use may be a risk factor for paranoia. Additionally, a prospective longitudinal study reported that early marijuana users (before age 18 years) had more schizophrenia symptoms than non-users at age 26 years, even when preexisting symptoms were taken into account (Arseneault et al., 2002), again suggesting that early marijuana use increased the likelihood of developing psychosis. Furthermore, cannabinoid receptor dysregulation during adolescence may interfere with maturation of the prefrontal cortex, leading to vulnerabilities to psychiatric disorders (Caballero and Tseng, 2012). While several of these studies focused on schizophrenia, our subjects had elevations in some symptoms commonly found in schizophrenia (paranoia and suspiciousness) but not others (psychoticism and hallucinations). Consistent with these prior studies, we found an association between earlier marijuana use and more severe paranoid ideation symptoms.

Cortisol and Stress

Our young marijuana users showed non-significant elevations in basal measures of mid-day salivary cortisol, which is consistent with prior studies of marijuana users in different age groups. While one study of adult (18-42 years old) marijuana users also did not show significantly different morning and afternoon cortisol levels than controls (Block et al., 1991), a longitudinal study of adolescent marijuana users (10-14 years old) found higher evening cortisol levels (blunted evening decrease), particularly in those who started using marijuana at an earlier age (Huizink et al., 2006). The current study did not evaluate nocturnal cortisol levels. Therefore, follow-up studies with a more detailed assessment of

cortisol circadian patterns, and a larger sample size for the TSST, might better define the basal stress and stress response in this population.

We did not observe a significant response to the TSST, in contrast to our previous study of adolescent methamphetamine users, which used the same TSST procedures to show an enhanced cortisol response (+30%) in adolescent methamphetamine users, especially in the younger users (< 17 years) (King et al., 2010a). Therefore, a larger sample size that includes younger marijuana users may allow us to further delineate the age-dependent TSST response in the marijuana users. Furthermore, the salivary cortisol levels decreased significantly after an hour of recovery in all of our participants. These findings suggest that pre-test anxiety in some of our participants might have contributed to a higher “basal” cortisol level, as suggested by a prior report (van Leeuwen et al., 2011). Our findings of modest elevation in IL-6, which others have observed in anxious adults (O'Donovan et al., 2010), further support this interpretation.

Lastly, cortisol measures have been shown to correlate with cytokines, including IL-6, and TNF- α in adult populations (DeSantis et al., 2012). However, we did not observe such correlations in our younger population, which may be a consequence of the small sample size or an indication that these younger individuals have an immature immune-endocrine interaction. Salivary and blood levels of cytokines do not show consistent correlations. For instance, serum and salivary IL-6 levels (in adults ages 30-65 years) correlated with psychosocial scales, but their IL-6 levels in serum, saliva, cell-free supernatants or in vitro mononuclear cells did not correlate with each other (Sjogren et al., 2006). Other studies in adult subjects found significant correlations between saliva and plasma levels of IL-6, but not for TNF- α or IL-1 β (Williamson et al., 2012) and significant correlations between saliva and serum levels of IL-6 and TNF- α (Monea et al., 2014). In addition to these tenuous associations between cytokines in various body fluids, possible interactions between cytokines and cannabinoids are also complicated. Animal models and in vitro studies indicate various effects on cytokine levels, depending on the cannabinoid (THC, synthetic, endogenous), the study preparation (cell, tissue, blood), and the cytokine measured (TNF- α , IL-6, IL-1 β and others)(Nagarkatti et al., 2009). Some of the variability may result from the different dosages or ages of the subjects; marijuana may have anti-inflammatory properties, but high doses of THC may promote inflammation (Berdyshev et al., 1997), also THC-induced inflammatory responses, including production of TNF- α and IL-1 β , in adolescent mice may reverse in adulthood(Moretti et al., 2014). Even less is known about how the peripheral immune responses might relate to neuroinflammation and the levels of cytokines in the brain of marijuana users. These uncertainties further underscore the importance of studying the actual amounts of marijuana used by youth as they transition into adulthood.

Marijuana and Psychiatric Symptoms Interactions

Previous studies reported mixed results regarding the associations between cortisol levels and psychiatric symptoms. A study of adolescents in a naturalistic setting showed associations between cortisol levels and negative mood (Degenhardt et al., 2003; Adam, 2006). However, in another study, although psychiatric symptoms and cortisol levels were both greater in adolescent methamphetamine users, these measures did not appear to be

related (King et al., 2010b). In the current study, only the overall intensity of BPRS measured symptoms was associated with cortisol levels, but several psychiatric symptoms were associated with marijuana usage measures. Taken together, these associations suggest that some symptomatology, such as BPRS intensity, may not be directly linked to marijuana use, but to the same biological or environmental influences that also affect the cortisol levels. For other symptoms, early and cumulative marijuana use in youths may contribute to their greater manifestation; in particular, PSDI was markedly higher in HMJ than LMJ, rather than between users and non-users. However, it is also possible that these symptoms were premorbid and contributed to early drug seeking or the use of marijuana to “self-medicate”. Other predisposing variables, such as genetic and environmental factors, also may contribute to both greater psychiatric symptoms and earlier or heavy marijuana use.

The current study has some limitations. First, our young marijuana users smoked more tobacco and used more alcohol than the controls, although marijuana was their drug-of-choice and none were dependent on alcohol. Therefore, the psychiatric symptoms, cortisol and cytokine levels may represent the interaction of these “gateway drugs” or an “at-risk” profile of adolescent marijuana users. Second, only mid-day cortisol measurements were obtained; therefore, our findings cannot be directly compared with prior studies that measured cortisol levels at other times of the day. Third, the modest sample size of the subgroups that completed the stress test and the cytokine measurements did not allow us to evaluate the possible relationships with the psychiatric symptoms fully. A larger sample size is needed to better assess age and marijuana dose effects on cortisol levels, stress response, and salivary cytokine levels.

The adolescents in our study were still undergoing social, hormonal, and neurological maturation. Brain regions that are often implicated in addiction and other psychiatric disorders such as the amygdala, hippocampus and prefrontal cortex, continue to mature into early adulthood (Romeo and Sisk, 2001; Koshibu et al., 2004; Paus et al., 2008; Cloak et al., 2011). Therefore, longitudinal follow-up of these participants are needed to determine if their symptoms would worsen or improve with age, with continuation or discontinuation of marijuana use. Future studies need to include neuroimaging measures that assess brain structural, neurochemical and functional alterations in relation to marijuana use, peripheral immune markers and other phenotypic and environmental measures. The greater symptomatology and the correlations between symptoms and marijuana usage patterns emphasize the need for early interventions for substance use and psychopathology in these youths. With the legalization of marijuana for both recreational and “medicinal” purposes in many regions of the United States, more studies are urgently needed, and a coordinated effort is underway to determine the influence of substance use on the developing adolescent brain (ABCD, 2014). The interaction of the brain and other systems, like the endocrine and immune systems, also needs to be addressed in this age group.

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Fig. 1.

Sub-clinical psychiatric symptoms were assessed using the Symptom Check-List 90R (SCL-90R, **a & b**) and Brief Psychiatric Rating Scale (BPRS, **c & d**) in participants. Control, light marijuana use (LMJ), and heavy marijuana use (HMJ) groups were compared. Global scores shown for the three groups include: Global Severity Index (GSI; ANOVA- $p=0.02$), Positive Symptom Distress Index (PSDI; ANOVA- $p=0.07$), Positive Symptom Total (PST; ANOVA- $p=0.02$), BPRS Presence ($p=0.01$), BPRS Intensity (ANOVA- $p=0.09$), and BPRS Total (ANOVA- $p=0.01$). Significant or trend (ANOVA- $p<0.05$) domain scores also are illustrated. Significant post-hoc t-test results are indicated in the figures.

Fig. 2. Significant correlations between SCL-90R symptoms and marijuana use are illustrated. Symptoms were greatest in those who started using marijuana at a young age (**a & b & d**), more with more joints used (**c & e & g**), and less with longer abstinence (**f**).

Fig. 3.

Significant correlations between BPRS symptoms and marijuana use are illustrated. Lesser symptoms with longer abstinence (**a-d**) and greater anxiety with more cumulative lifetime use (**e**) was observed.

Fig. 4.

a) Cortisol and cytokine concentrations (mean±SEM) from mid-day saliva samples (sample size noted at base of bars): Cortisol levels (µg/L) did not differ between control (<1 lifetime joint), LMJ (1-500 lifetime joints), and HMJ (>500 lifetime joints) groups by ANOVA. IL-1 β and TNF- α levels (pg/mL) did not differ between control (<5 joints lifetime) and all MJ (>5 joints lifetime) groups, but there was a trend for IL-6 levels (pg/mL) to be higher in marijuana users by t-test. **b)** Salivary cortisol levels changed slightly over the course of the Trier Social Stress Test (TSST, $p=0.05$ on repeated measure ANOVA), with a significant decrease in cortisol during the one-hour recovery phase (post-hoc paired t-test: $p=0.01$ across all groups and $p=0.02$ for LMJ). **c & d)** Salivary cortisol and IL-6 was higher in individuals with the more intense BPRS symptoms. There was a trend for non-normal distribution for IL-6 levels as plotted. Following the removal of the outlier with an IL-6 value >15 pg/mL the data was normalized and the correlation was weaker but still significant ($r=0.48$, $p=0.04$). **e)** TNF- α levels were highest in those with the most SCL-90R symptoms.

Table 1

	CON n=42	LMJ n=37	HMJ n=43	ANOVA or χ^2	CON vs. LMJ	CON vs. HMJ	LMJ vs. HMJ
Age (years)	18.3±0.4	19.1±0.4	19.4±0.3	0.07	n.s.	0.02	n.s.
Gender (% male)	52%	51.4%	60.4%	χ^2	n.s.	n.s.	n.s.
Age First MJ Use (Years)	18.5±0.8	15.7±0.4	13.9±0.4	<0.0001	0.0030	<0.0001	0.0016
Abstinence (days)*	351±140	145±57	12±7	<0.0001	0.0087	<0.0001	0.0002
MJ Frequency (days/week)		4.1±0.4	6.9±0.1				<0.0001
Duration MJ (months)		34.4±4.0	56.9±3.6				0.0001
Daily MJ (grams)		0.8±0.1	2.5±0.3				0.0001
Est. Lifetime MJ (joints)*	0.08±0.03	153±25	3546±859	<0.0001	<0.0001	<0.0001	<0.0001
THC(+) Urine	0%	30%	74%	χ^2	<0.0001	<0.0001	<0.0001
Est. Lifetime tobacco use (#cigarettes)*	41±35	935±398	7647±2019	0.0019	n.s.	n.s.	0.0005
Est. Lifetime alcohol use (#drinks)*	50±18	678±191	1453±263	<0.0001	0.0008	<0.0001	0.0002