Impaired cognitive performance under psycho-social stress in cannabis dependence is mediated by attenuated precuneus activity

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Abstract

Deficient regulation of stress plays an important role in the escalation of substance use, addiction and relapse. Accumulating evidence suggests dysregulations in cognitive and reward-related processes and the underlying neural circuitry in cannabis dependence. However, alterations in stress regulation have not been systematically examined. Against this background the present fMRI study examined psycho-social stress processing in cannabis-dependent males and matched controls using an adaptive mental arithmetic task accompanied by negative social evaluation. During psycho-social stress exposure, but not the non-stress condition, cannabis users demonstrated decreased performance. In contrast, levels of experienced stress and cardiovascular stress responsivity did not differ from controls. Neuroimaging data revealed that stress-induced performance deteriorations in cannabis users were accompanied by decreased precuneus activity and increased connectivity of this region with the dorsal medial prefrontal cortex. Together, the present findings provide the first evidence for exaggerated stress-induced cognitive performance deteriorations in cannabis users. The neural data suggests that deficient stress-related dynamics of the precuneus may mediate the deterioration of performance on the behavioral level.

Keywords: Cannabis dependence / stress / cognitive performance / fMRI / precuneus

1. Introduction

Drug addiction is a chronically relapsing disorder of the brain characterized by escalating drug use, craving, drug bingeing and withdrawal accompanied by a loss of control over behavior (Koob & Volkow, 2010, 2016). Neurobiological models of addiction postulate that the transition from occasional or volitional to compulsive and addictive use involves a series of sequential neuroadaptations in neural circuits underlying reward processing, associative learning, executive control, and stress reactivity (Koob & Volkow, 2010).

Worldwide, cannabis is the most widely used illicit drug, with about 192.2 million people, or 3.8% of the world's population aged 15-64 consuming cannabis on a regular basis (UNODC, 2018). During the last two decades cannabis-use associated alterations in the domains of reward processing and cognition have been extensively studied (overview e.g. in Crean, Crane, & Mason, 2011; Curran et al., 2016; Solowij & Battisti, 2008; Volkow et al., 2016) and evidence from functional imaging studies suggesting neuroplastic adaptations in striato-limbic-frontal circuits subserving these functions is growing (Bossong & Kahn, 2016; Martin-Santos et al., 2010; Wrege et al., 2014). With respect to cognitive functions, problematic patterns of cannabis use (i.e. long-term or early-onset use) have primarily been associated with impairments in the domains of attention, working memory and associative memory beyond the acute intoxicated state (Becker, Wagner, Gouzoulis-Mayfrank, Spuentrup, & Daumann, 2010a, 2010b; Curran et al., 2016; Indlekofer et al., 2009; Messinis, Kyprianidou, Malefaki, & Papathanasopoulos, 2006; Volkow et al., 2016; Wagner, Becker, Gouzoulis-Mayfrank, & Daumann, 2010).

In addition to dysregulations in reward and cognitive processes, maladaptive stress reactivity and deficient regulation of stress represent important contributing factors for the initiation of substance use and are considered a hallmark of addiction (Sinha, 2007, 2008). Stress represents an adaptive response to environmental demands appraised as potentially exceeding the individual's coping capacity and consequentially can negatively impact the individual's well-being (Lazarus & Folkman, 1984). The acute stress response is an adaptive mechanism that improves threat detection and habitual behavior but comes at the cost of an impaired capacity to maintain attention and make complex decisions (van Leeuwen et al., 2018).

Impairments in this adaptive mechanism and maladaptation in the underlying neural circuitries thus may contribute to reduced access to adaptive coping, such that response inhibition and decision making during increased levels of emotional stress become compromised (Li & Sinha, 2008; Sinha, Garcia, Paliwal, Kreek, & Rounsaville, 2006). Deficient regulation of stress represents a risk-factor for the initiation of substance use and escalation of use (Cheetham, Allen, Yücel, & Lubman, 2010; Quinn & Fromme, 2010), including cannabis (Hyman & Sinha, 2009). Moreover, stress and impaired stress regulation considerably contribute to increased relapse risk across substance use disorders (Li & Sinha, 2008; Sinha, 2007, 2008; Sinha et al., 2006; Sinha & Li, 2007).

Functional MRI studies in cannabis users reported altered neural reactivity during subliminal processing of social-emotional signals (Gruber, Rogowska, & Yurgelun-Todd, 2009), negative emotional stimuli (Wesley, Lile, Hanlon, & Porrino, 2016; Zimmermann et al.,

2018) and the volitional regulation of the initial emotional response towards these stimuli (Zimmermann et al., 2018), as well as associated neural alterations in fronto-striato-limbic circuits critically engaged in emotional reactivity and regulation (Etkin, Büchel, & Gross, 2015; Ochsner & Gross, 2005). Psychosocial stress has been associated with a broad neurocircuitry including the cingulate, insula, precuneus, hypothalamus and frontotemporal regions (Dedovic et al., 2009; Eckstein et al., 2014) and increased cerebral blood flow in these regions with increasing levels of stress (Dedovic et al., 2009; Wang et al., 2005).

Further support for a potential association between escalating cannabis use and impaired stress processing comes from studies examining cannabis use motivation and stress reactivity in cannabis users. Large scale surveys have demonstrated that the regulation of negative affective states represents a primary motivational drive for cannabis use (Simons, Correia, & Carey, 2000) and that these coping-oriented use motivations associate with an increase risk to develop addictive patterns of use (Schlossarek, Kempkensteffen, Reimer, & Verthein, 2016). Moreover, evidence from experimental studies suggests altered emotional reactivity and regulation in response to negative emotional stimuli in cannabis users, including decoding deficits for threatening social-emotional signals (Platt, Kamboj, Morgan, & Curran, 2010) and an exaggerated hormonal stress response towards these stimuli (Somaini et al., 2012). Despite the important role of the endocannabinoid system in stress reactivity and regulation (Clapper, Mangieri, & Piomelli, 2009; Hirvonen et al., 2012) and initial studies suggesting cannabis-induced adaptations in endocannabionoid signaling following chronic exposure (Clapper et al., 2009; Hirvonen et al., 2012), associations between cannabis use and altered stress processing on the neural level have not been systematically examined.

Against this background, the present study employed an evaluated psychosocial stress induction fMRI paradigm (Montreal Imaging Stress Task, MIST (Dedovic et al., 2005; Lederbogen et al., 2011)) during which subjects are required to perform an adaptive arithmetic tasks combined with negative performance feedback and critical social evaluation to determine altered stress processing in cannabis dependent males in comparison to carefully matched non-using controls. Based on previous studies, we expected that cannabis users would exhibit deficient stress regulation in the context of altered neural reactivity in circuits engaged in psycho-social stress processing.

2. Materials and Methods

2.1 Participants

N = 34 cannabis dependent males and n = 28 non-using healthy controls were recruited. All cannabis users fulfilled the DSM-IV criteria for cannabis dependence (assessed using the Mini-International Neuropsychiatric Interview, MINI, Sheehan et al., 1998). To reduce non-cannabis-use associated variance in the data, the present study enrolled only male participants. The decision was based on previous studies reporting sex-differences in the subjective experience and associated neural activity in response to experimental psycho-social stress induction (Kogler, Gur, & Derntl, 2015) and an influence of menstrual cycle

phase (Chung, Peisen, et al., 2016) and hormonal contraceptives on emotion regulation (Chung, Springer, et al., 2016) as well as emerging evidence for sex-differences in stress induced drug-carving (Potenza et al., 2012) and sex-differences in the effects of chronic cannabis use on stress-processing (Fattore, 2013). A similar approach has been employed in previous studies on stress reactivity (Eckstein et al., 2014) and emotional alterations in cannabis dependence (Jager, Block, Luijten, & Ramsey, 2013; Zhou et al., 2018; Zimmermann et al., 2017, 2018).

Inclusion criteria for all subjects were (1) age between 18-40 years, and, (2) right-handedness. Exclusion criteria for all participants included (1) history or current DSM-IV axis I disorders, such as psychotic or bipolar symptoms (assessed using the MINI), (2) Beck Depression Inventory (BDI-II) score > 20 indicating a moderate depression (Beck, Steer, & Brown, 1996) , (3) current or history of a medical disorder including neurological and endocrinological abnormalities (4) current or regular use of medication, (5) usage of other illicit substances > 75 lifetime occasions or during the 28 days prior to the experiment, (6) positive urine screen for cocaine (300 ng/ml), methamphetamine (500 ng/ml), amphetamine (500 ng/m), methadone (300 ng/ml) or opiate (300 ng/ml) (Drug-Screen-Multi 7TF, nal von minden GmbH, Moers, Germany), (8) breath alcohol level >0.00‰ (assessed by breath sample using TM-7500, Trendmedic, Penzberg, Germany). For the control group the following additional exclusion criteria were applied: (1) cumulative lifetime use of cannabis > 15g (M = 1.29, SD = 1.02), (2) use of any other illicit substance >10 lifetime occasions. To control for confounding sub-acute effects of cannabis all users were required to remain abstinent from cannabis 24 h prior to the experiments. To increase adherence with the abstinence period participants were informed that a urinary drug test for cannabis use would be performed on the day of the experiment (for the implementation of a similar bogus pipeline in cannabis users see (Filbey & Dunlop, 2014; Roese & Jamieson, 1993)).

Dependent cannabis users were recruited in cooperation with regional addiction treatment centers, advertisements and flyers. Healthy control subjects were recruited through advertisements and underwent identical study procedures. All subjects provided written informed consent and the study had full ethical approval by the local ethics committee at the University of Bonn, Germany. All study procedures were in accordance with the latest revision of the Declaration of Helsinki.

To control for confounding effects of between-group differences in emotional state and baseline cognitive performance, anxiety (assessed using the State Trait Anxiety Inventory, STAI, (Spielberger, 1983)), mood (assessed using the Positive and Negative Affect Schedule, PANAS, (Watson, Clark, & Tellegen, 1988)) and attention (d2 test of attention, (Brickenkamp, 2002)) were assessed on the day of the examination. Given the high prevalence of tobacco co-use in cannabis dependence (Agrawal, Budney, & Lynskey, 2012) the experimental groups were matched in terms of tobacco use characteristics. However, both, acute nicotine administration as well as abstinence-induced nicotine craving may impact stress processing and underlying neural mechanisms (Ashare et al., 2016; Becker & Hurlemann, 2016). Nicotine craving is reported to peak around 3-6 hours following the last cigarette (Jarvik et al., 2000; Schuh & Stitzer, 1995) and a recent study reported craving-associated neural activity changes after 4h of abstinence (Franklin et al., 2018). As a trade-off, subjects were

allowed to smoke as usual on the day of the fMRI experiment, however underwent a 1.5h supervised abstinence period before the start of the experimental paradigm (similar approach in Zhou et al., 2018; Zimmermann et al., 2017).

Two cannabis users were excluded due to excessive use of other illicit substances (see exclusion criteria). After initial data quality assessments, data from four cannabis users and three controls were excluded due to excessive head movement (> 3.5 mm) during the fMRI acquisition. Two control subjects were excluded because they consistently rated subjective stress during the control as well as stress condition very low (stress experience was assessed using a 1-8 scale, one subject consistently rated the stress experience as 1 throughout all runs, the other consistently as 2, indicating that the paradigm did not successfully induce stress in these subjects). The final dataset for the subsequent analyses thus included n = 28 dependent cannabis users and n = 23 healthy controls (age M = 25.10, SD = 4.46).

2.2 Experimental design

Psychosocial stress during fMRI acquisition was induced using the previously evaluated Montreal Imaging Stress Task (MIST) procedure (Dedovic et al., 2005; Lederbogen et al., 2011). During the paradigm subjects were asked to perform mental arithmetics and were confronted with negative feedback about their performance indicating that they performed worse than the other study participants (Dedovic et al., 2005) (detailed procedures also given in (Eckstein et al., 2014)). Briefly, before the experiment subjects were instructed to perform the task with high accuracy and speed. The instructions emphasized that it would be very important that subjects match the average performance of the other participants and that the experimenters would monitor and evaluate the performance online via monitor. Together, this experimental setting has shown to reliably induce stress. To further increase the psychosocial stress the experimenters criticized the participants' "bad" performance via intercom and reminded them of the importance to perform similar to the other participants between the runs of the task.

The blocked design fMRI paradigm consisted of six runs of approximate 6 min duration: three runs that included negative feedback (stress condition) and three runs without feedback (no-stress control condition). The order of runs was fixed (a no-stress run always followed by a stress run). Each run incorporated four 60 s blocks that were preceded by a visual attention cue (5 s) and followed by a 20 s inter-block interval that served as low-level baseline (fixation cross). During the blocks subjects were required to perform an arithmetic task and to select the correct answer using a rotary dial. The subjects received feedback ("correct" or "incorrect") on whether their response was correct or incorrect. During the stress blocks additional performance indicators were shown to the subjects, including his own performance and average performance of the other subjects. To further increase stress a time limit was implemented that was indicated by a progressing bar moving from the left to right and "timeout" was displayed, in case no response occurred during the given time. Unbeknownst to the subject, an algorithm was employed that adopted the response times to the performance of the subject to increase the failure rate. First, the average response time of the participant was determined in a pre-scan training session of 2 minutes without a time limit per arithmetic task and the time limit for the task during fMRI was set to 90% of

the subject's individual baseline response time. Furthermore, the time limit was decreased by 10% after three correct responses and likewise increased by 10% after three incorrect responses (details see also Dedovic et al., 2005).

After each run subjects rated their stress level on a scale from 1 (very low) to 8 (very high). To determine baseline and stress-induced cannabis craving, all subjects rated their level of cannabis craving (visual analog scale, VAS, 0-100) before and after the paradigm. To control for between-group differences in task engagement and self-perceived performance, subjects rated task enjoyment (1-9 points, 1 - very unpleasant, 9 - very pleasant) and their own performance (1-9 points, 1 - very negative, 9 - very positive) at the end of the experiment. As a measure of a physiological indicator of stress blood pressure (systolic and diastolic) was repeatedly measured at four different time point (at rest 30 minutes after arrival - t1, immediately before the task - t2, immediately and 60 minutes after the task - t3 and t4). Blood pressure data for five subjects was lost due to technical failure, leading to a final sample size of n = 23 controls and n = 23 users for the corresponding analysis.

In total, the experimental task lasted approximately 40 min. Stimuli were presented using Presentation 14 (Neurobehavioral Systems, Albany, CA) via liquid crystal display video goggles (Nordic NeuroLab, Bergen, Norway).

2.3 fMRI acquisition

Data were acquired on a Siemens Trio 3T MRI system (Siemens, Erlangen, Germany). Functional data was acquired using a T2* echo-planar imaging (EPI) BOLD sequence [repetition time (TR) = 2500 ms, echo time (TE) = 30 ms, 37 slices, slice thickness = 3.0mm, no gap, voxel size = 2 × 2 × 3 mm, flip angle = 90°, field of view = 192 mm]. To exclude subjects with apparent brain pathologies and to facilitate normalization of the functional data, a high-resolution T1-weighted structural image was additionally acquired (TR = 1660 ms, TE = 2540 ms, 208 slices, field of view = 256 mm, voxel size = 0.8 × 0.8 × 0.8 mm).

2.4 fMRI data processing

fMRI data were analyzed using SPM12 (Wellcome Department of Cognitive Neurology, London, UK, http://www.fil.ion.ucl.ac. uk/spm/software/spm12; Friston et al., 1994). The first five volumes were discarded to achieve magnet-steady images. Next, slice time correction was employed to correct for slice acquisition time and images were realigned using a six-parameter rigid body algorithm to correct for head movement. Subsequently, the images were normalized using a two-step procedure that included co-registration with the T1 image, segmentation of the T1 image and application of the resulting transformation matrix to the functional time-series. Images were written out at 3 × 3 × 3mm resolution and subsequently smoothed with a Gaussian kernel (full width at half maximum, FWHM, 8mm).

The first level design matrix employed a boxcar function to model the 'stress' and 'no-stress' condition. The six motion parameters were additionally included as nuisance regressors, and the experimental regressors were convolved with the canonical hemodynamic response function (HRF).

2.5 fMRI BOLD level analyses

On the second level, we first investigated the stress-network in the present study using a one sample t-test on the pooled data from cannabis users and controls (contrast [stress > no-stress]). Secondly, to determine altered neural stress processing in cannabis users, a two-sample t-test was conducted comparing stress-related activity between the groups (contrast [stress > no-stress]. To increase the sensitivity of the analysis, the task-specific stress neural networks were initially defined with an independent data set from a previous study using the identical paradigm to determine the effects of oxytocin on psycho-social stress (data from Eckstein et al., 2014). Based on the results from this independent dataset (contrast [stress > no-stress], one-sample *t*-test, family-wise error, FWE, corrected at $p < 0.05$; $n = 31$; only non-oxytocin treatment subjects included) the middle temporal gyrus, precuneus, (para-)hippocampal gyrus and inferior parietal lobule were identified as stress sensitive regions. Based on these results, bilateral masks for the four regions were defined using the Wake Forest University (WFU) PickAtlas (Maldjian, Laurienti, Kraft, & Burdette, 2003). The bilateral masks for the middle temporal gyrus, precuneus, hippocampal formation and inferior parietal lobule were determined using the Automatic Anatomical Labeling (AAL) atlas as implemented in the WFU PickAtlas and subsequently used for small volume correction (SVC) employing an FWE-corrected $p < 0.05$**.** For further post hoc analyses signal changes from 6mm radius spheres centered at maximum coordinates of between-group differences were extracted using MarsBar [(Brett, Anton, Valabregue, Poline, & others, 2002), http://marsbar.sourceforge.net/].

2.5 fMRI functional connectivity analyses

To further explore whether neural activity alterations in cannabis users were associated with altered network-level communication, a generalized form of context-dependent psychophysiological interaction (gPPI) analysis was performed (McLaren, Ries, Xu, & Johnson, 2012). To this end, the functional connectivity of the regions that exhibited significant between-group BOLD activity differences were examined. The first level gPPI models were modelled after deconvolution and included a psychological factor, physiological factor and the interaction between the two factors (PPI term). The two experimental conditions as well as the motion parameters were included. In line with the analysis of stress-reactivity differences on the level of BOLD-activity, cannabis-associated alterations on the network level were explored by means of two-sample t-tests using the contrast [stress > no-stress]. Between-group differences on the network level were explored using an uncorrected $p < .001$ and a minimum voxel size of 10.

3. Results

3.1 Potential confounders and drug use patterns

The groups were comparable with respect to several important confounders, including mood and anxiety, use of nicotine and alcohol as well as task enjoyment and perceived performance (*ps* > 0.10 in all cases). Use of other prevalent illicit drugs was low in both groups, however, cannabis users reported more occasions of ecstasy use (use on 9.72 ±2.19, controls use on 2.33 ±2.31 occasions). Details on demographics, confounders and drug use, including cannabis use parameters are given in **Table 1**.

3.2 Craving, stress experience, task performance and blood pressure

Examining experienced cannabis craving using a mixed ANOVA with group (control vs. user) as a between subject factor and time (pre- vs. post-stress task) as within-subject factor revealed significant main effects of group [$F_{(1, 49)} = 51.20$, $p < 0.001$, partial $\eta^2 = 0.51$] and time [$F_{(1, 49)} = 6.23$, $p = 0.02$, partial $\eta^2 = 0.11$], and a significant interaction effect [$F_{(1, 49)} = 4.23$, $p = 0.05$, partial $\eta^2 = 0.08$]. Post-hoc analyses showed generally higher craving ratings in the cannabis group. Importantly, within the group of cannabis users craving strongly increased after stress exposure (cannabis group, $F_{(1, 49)} = 11.49$, $p = 0.001$, Cohen's $d = 0.38$, control group $F_{(1, 49)} = 0.09$, $p = 0.77$, see **Figure 1A**).

The subjective experience of stress during the paradigm was analyzed by means of a mixed ANOVA with the between subject factor group (control vs. user) and the within subject factor condition (stress vs. no-stress). The main effect of condition was significant [$F_{(1, 49)} = 131.91$, $p < 0.001$, partial $\eta^2 = 0.73$] indicating successful stress-induction. However, there was no significant interaction effect indicating that both groups experienced comparable levels of subjective stress.

Examining accuracy in terms of percent correct responses using a concordant mixed ANOVA revealed a main effects of condition [$F_{(1, 49)} = 60.00$, $p < 0.001$, partial $\eta^2 = 0.55$] reflecting that both groups performed better during the no-stress condition. Furthermore, there were a main effect of group [$F_{(1, 49)} = 4.54$, $p = 0.04$, partial $\eta^2 = 0.09$] and a significant group x condition interaction effect [$F_{(1, 49)} = 5.15$, $p = 0.03$, partial $\eta^2 = 0.10$]. Bonferroni-corrected post-hoc tests revealed that the groups exhibited comparable performance during the no- stress condition ($F_{(1, 49)} = 1.05$, $p = 0.31$). However, under stress cannabis users performed significantly worse than controls ($F_{(1, 49)} = 5.66$, $p = 0.02$, Cohen's $d = 0.65$, see **Figure 1B**).

Examination of blood pressure data revealed a significant main effect of time (t1/ t2/ t3/ t4) for both systolic blood pressure [$F_{(3, 132)} = 6.19$, $p = 0.001$, partial $\eta^2 = 0.12$] and diastolic blood pressure [$F_{(3, 132)} = 4.63$, $p = 0.005$, partial $\eta^2 = 0.10$]. Bonferroni- corrected pairwise comparisons illustrated that systolic blood pressure after the task was higher compared to 30 minutes after arrival ($p = 0.03$) and immediately before the task ($p = 0.001$) reflecting successful stress induction. Diastolic blood pressure was higher at t3 compared to t2 ($p = 0.006$). In line with the lack of between-group differences in self-reported stress experience, both groups displayed comparable cardio-vascular stress reactivity.

3.2 fMRI – BOLD level

In line with previous studies (e.g. Eckstein et al., 2014), the paradigm induced widespread activity in the psycho-social stress networks encompassing middle frontal regions, precuneus and posterior cingulate cortex (see **Table 2**).

Examining neural differences between the cannabis users and controls revealed significantly decreased stress-reactivity in the right precuneus (3, -70, 50, $k = 32$, $t = 3.50$, FWE $p < 0.05$) of the cannabis users as compared to controls. Post-hoc analyses on extracted precuneus parameter estimates revealed that cannabis users showed a lower increase during stress relative to the non-stress condition ($t_{(27)} = 2.47$, $p = 0.023$, Cohen's $d = 0.32$,

corresponding to a small effect size) as compared to controls ($t_{(23)} = 5.71$, $p < 0.001$, Cohen's $d = 0.83$, corresponding to a large effect size). This was also reflected by marginally significant lower precuneus activity during stress itself in the cannabis users as compared to controls ($t_{(49)} = 1.74$, $p = 0.088$) (**Figure 2**). No between-group differences were observed for the other regions of interest. Moreover, an exploratory whole brain analysis did not reveal between group differences in other regions.

3.2.1 Associations between neural activity and cannabis use parameters

In the cannabis dependent group no significant associations between precuneus activity and cannabis use parameters (age of onset, cumulative lifetime use, frequency of use) were observed (all $p > 0.22$).

3.3 Functional connectivity

An exploratory analysis focusing on altered stress-related connectivity of the precuneus employing identical first and second level models as for the BOLD level analysis revealed increased connectivity of the precuneus with the dorsal medial prefrontal cortex (dmPFC; 12, 26, 56, $k = 34$, $t = 4.16$) in cannabis users relative to controls during stress exposure (**Figure 3**).

4 Discussion

The present study examined psycho-social stress processing in cannabis-dependent males using an adaptive mental arithmetic task accompanied by negative social evaluation. During psycho-social stress exposure, but not the no-stress condition, cannabis users demonstrated decreased performance relative to controls, despite normal stress experience and cardiovascular stress reactivity. Neuroimaging data revealed that performance deteriorations in cannabis users were accompanied by decreased precuneus activity and increased connectivity of this regions with the dmPFC.

In line with previous studies, the experimental task successfully induced stress in both groups as indicated by increased subjective stress experience and cardiovascular activity (Dedovic et al., 2009). Whereas no differences in cardiovascular and subjective stress reactivity were observed between groups, cannabis-dependent users demonstrated a significantly lower performance in the arithmetic task during stress-induction. Previous studies reported altered stress reactivity related to alcohol (Breese, Sinha, & Heilig, 2011) and nicotine use (Wardle, Munafò, & De Wit, 2011) as well as elevated levels of anxiety and depression (Dedovic et al., 2013; Ming et al., 2017). However, the lack of between-group differences in these potential confounders in our samples suggests rather cannabis dependence-specific effects. Both groups exhibited a high and comparable performance in the absence of stress indicating comparable baseline cognitive performance. In line with previous studies (e.g. McRae-Clark et al., 2011) psycho-social stress increased cannabis craving in dependent subjects, confirming the important role of stress in the driving factors of cannabis dependence and relapse (Hyman & Sinha, 2009). Against our expectations, however, the groups did not differ with respect to subjective stress experience (however,

see Gilman et al., 2016 reporting a normal distress experience in cannabis users during social exclusion), cardiovascular indices as well as self-perceived performance or task enjoyment. Together this suggests that while stress induction and the perception thereof may be intact in dependent cannabis users, psycho-social stress however increases cannabis craving and leads to marked deteriorations in cognitive performance.

On the neural level, lower performance in the cannabis group was accompanied by an attenuated stress-related increase in precuneus activity. The precuneus, located in the posteromedial parietal lobe, is considered to play central role in a range of highly integrated tasks, including basic cognitive (i.e. sustained attention, volitional attention shifting as well as mental arithmetical performance) as well as social cognitive, particularly self-referential and mentalizing, processes (Cavanna & Trimble, 2006; Menon, Rivera, White, Glover, & Reiss, 2000; Schurz, Radua, Aichhorn, Richlan, & Perner, 2014). The precuneus has received little attention in neurobiological models of addiction. However, an increasing number of studies reported altered precuneus activation in chronic cannabis users during cue-induced craving (Filbey, Schacht, Myers, Chavez, & Hutchison, 2009) as well as cognitive processing in emotional and social contexts such as risky decision making (De Bellis et al., 2013), suppression of emotional distractors (Aloi et al., 2018), evaluation of episodic memory episodes (Riba et al., 2015), or mentalizing (Roser et al., 2012).

Given the stronger engagement of parietal regions, including the precuneus, with increasing difficulty of mental arithmetic operations (Menon et al., 2000), additional recruitment of the precuneus may have attenuated stress-related task deteriorations in the controls. In contrast, decreased task performance during stress in cannabis users may be linked to failure of compensatory precuneus recruitment. Moreover, decreased stress-related dynamic precuneus recruitment was accompanied by increased functional connectivity of this regions with the dmPFC. The dmPFC is involved in both, mental arithmetic performance as well as regulation of negative affect (Etkin et al., 2015; Menon et al., 2000). Increased interactions with the dmPFC may thus reflect a deficient compensatory attempt to maintain task performance or a successful compensation of negative emotional experience during performance deterioration, with the lack of between-group differences in emotional distress arguing for the latter.

The observed psycho-social stress specific performance impairments in cannabis-dependent subjects align with previous studies reporting impaired cognitive performance in cannabis users in the context of negative emotional (Zimmermann et al., 2017) and social information (Gilman, Lee, et al., 2016; Gilman, Schuster, et al., 2016; Gilman, Curran, Calderon, Schuster, & Evins, 2016). Together these findings suggest an association between chronic cannabis use and deficient integration at the intersection between emotional and cognitive processes.

The impact of stress on addiction is multifactorial, and deficient stress regulation has been determined as risk-factor for the escalation of cannabis use and dependence (Cheetham et al., 2010; Quinn & Fromme, 2010; Hyman & Sinha, 2009). The retrospective nature of the present study does not allow to disentangle predisposing factors from consequences of chronic cannabis exposure or addiction-related maladaptation in stress

regulation. Therefore, impaired performance under stress as well as associated neural alterations may alternatively reflect a predisposing deficit for the development of cannabis dependence or changes in stress-related cannabinoid signaling due to cannabis exposure-related adaptations.

Findings from the present study need to be interpreted in the context of the following limitations: (1) To reduce sex- and menstrual cycle-dependent variations in stress processing with the experimental groups, only male subjects were included. The generalizability of the findings in female cannabis-dependent users thus needs to be determined in future studies. (2) The cannabis users and healthy controls were carefully matched with respect to the use of other illicit substances. However, the groups differed in the occasions of ecstasy use. Although a previous study indicates that emotional dysregulations in low-dose ecstasy users are predicted by cannabis rather that ecstasy use (Daumann et al., 2004) we cannot completely rule out that long term effects of low dose ecstasy use may have contributed to the observed effects. Finally, both groups were matched for nicotine use. However, we cannot exclude complex interactive effects between nicotine and cannabis use.

Summarizing, the present study provides the first evidence for stress-induced cognitive performance deficits in cannabis users. Importantly, deficits were observed specifically for acute stress in contrast to normal performance under non-stress and normal perceived stress intensity. The neural data suggest that deficient stress-related dynamics of the precuneus may mediate these effects.

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Table 1 Group characteristics and substance use (mean ± SD; *χ^2 test employed).

Measurements	Control	User	*t* (χ^2)-value	*p*-value
Age (years)	24.57±3.55	25.54±5.11	0.77	0.45
Education (years)	16.20±2.28	15.95±3.68	0.30	0.77
Number of smokers	19	26	1.09*	0.30
Years of nicotine use	7.21±2.94	9.65±5.75	1.68	0.10
Cigarettes per day	8.45±5.34	9.51±7.11	0.57	0.57
Packyears	3.04±2.44	5.56±6.33	1.63	0.11
Onset of alcohol use	16.57±1.85	15.98±3.80	0.66	0.51
Days per week with alcohol use	1.24±1.05	1.56±1.35	0.90	0.37
Quantity (units) of alcohol per week	6.31±5.67	10.14±9.01	1.70	0.10
Fagerström test, Nicotine dependence	1.00±1.31	1.86±2.22	1.63	0.11
Age of first nicotine use	15.71±1.74	14.96±2.49	1.22	0.23
PANAS P	21.17±5.51	20.00±6.43	0.69	0.49
PANAS N	1.96±3.14	1.29±2.75	0.81	0.42
BDI II	6.09±6.26	6.50±4.42	0.28	0.78
STAI -State	32.83±5.69	31.71±5.77	0.69	0.49
STAI -Trait	35.48±8.17	35.86±7.71	0.17	0.87
Age at first use of cannabis	16.92±2.57	15.68±2.82	1.51	0.14
Average frequency	-	23.61±7.27	-	-
(last 12 months, days per month)				
Lifetime quantity (g)	1.29±1.02	2309±1655	7.38	0.001
Participants with past ecstasy use	3	18	-	-
Occasions of ecstasy use	2.33±2.31	9.72±2.19	2.88	0.01
Participants with past cocaine use	2	14	-	-
Occasions of cocaine use	5.50±4.50	5.93±2.73	0.08	0.94
Participants with past amphetamine use	4	18	-	-
Occasions of amphetamine use	3.25±4.50	12.17±15.39	1.13	0.27
Participants with past hallucinogen use	-	17	-	-
Occasions of hallucinogen use	-		-	-
Participants with past opiate use	-	3	-	-
Occasions of opiate use	-		-	-
Participants with past solvents use	-	7	-	-
Occasions of solvents use	-		-	-
Post experiment				
Task pleasantness	6.09±1.93	6.11±1.99	0.04	0.97
Evaluation of performance	5.87±1.33	5.18±1.66	1.62	0.11

Table 2 Stress-related activity increases in the entire sample (stress > no stress)

				MNI coordinates		
Hemisphere	Region	Cluster size	Peak t	x	y	z
Stress vs. no- stress						
R	Precuneus/ Middle Occipital Gyrus	6864	16.30	9	-76	-10
			16.04	45	-64	8
			15.69	18	-94	14
R	Insula/ Middle Frontal Gyrus	400	10.48	33	20	5
			7.73	33	44	20
			7.61	33	41	32
R	Middle Frontal Gyrus/ Medial	603	9.72	39	-1	53
	Frontal Gyrus/ Cingulate Gyrus		9.14	45	8	32
			8.58	21	-1	59
L	Middle Frontal Gyrus	69	7.50	-39	-10	50

Figures and Legends

Figure 1 (A) Cannabis craving assessed before and after stress-induction, **(B)** performance accuracy during the no-stress and stress condition.

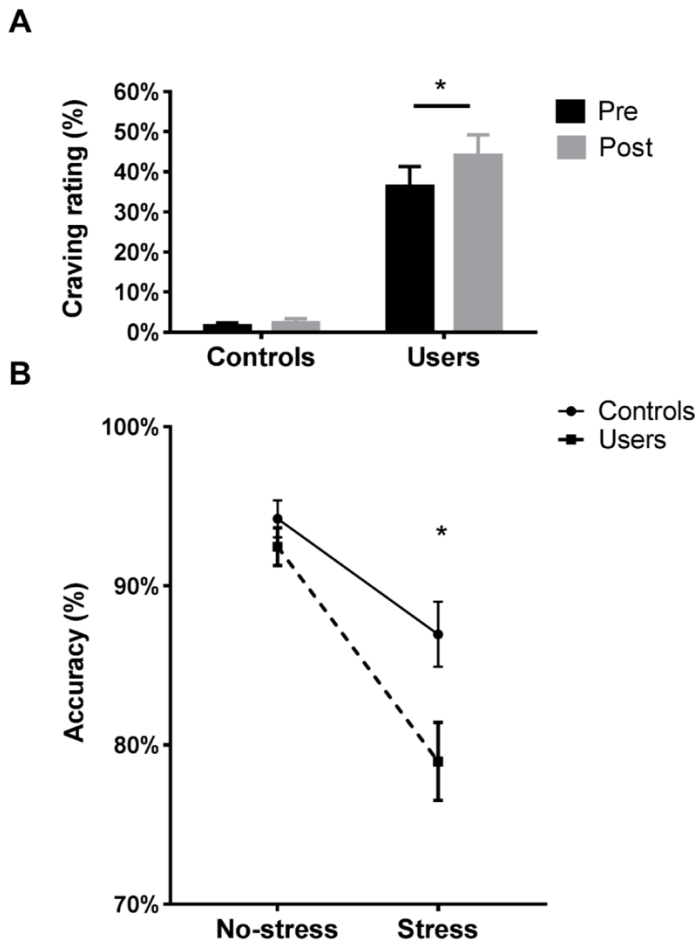

Figure 2 Cannabis users demonstrated decreased precuneus activity during psycho-social stress. Extracted parameter estimates from the precuneus for the contrasts (stress > no-stress) **(A)** and (no-stress vs baseline, stress vs baseline) **(B)** revealed that the effect was driven by lower activity during the stress condition.

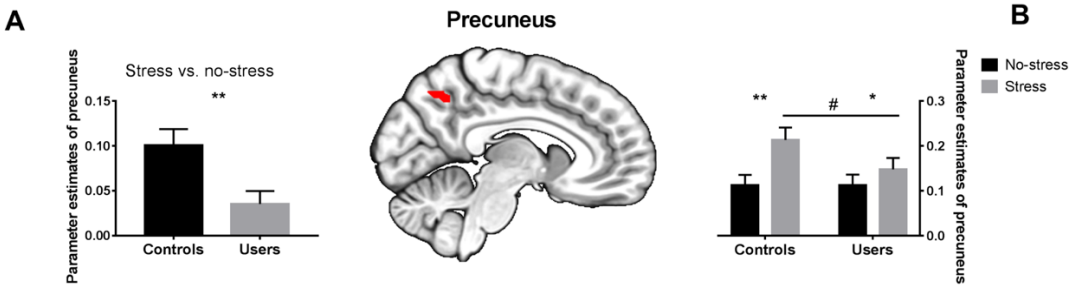
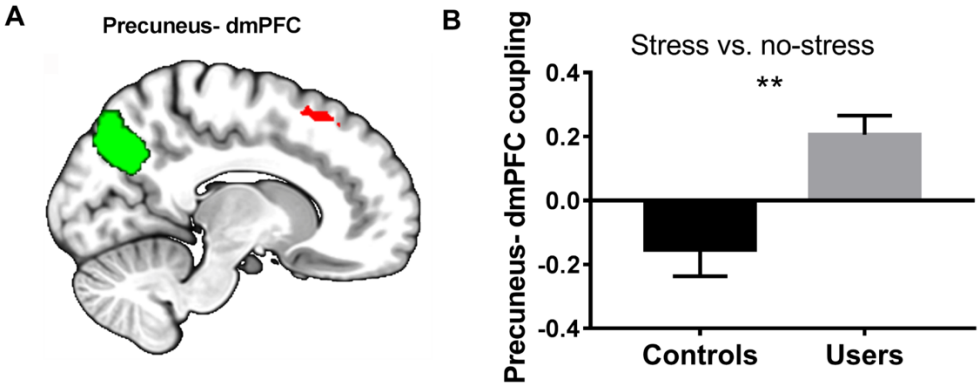
Figure 3 Cannabis users demonstrated increased precuneus – dorsal medial prefrontal (dmPFC) connectivity during stress. **(A)** Location of the precuneus-dmPFC pathway that exhibited group differences, **(B)** extracted connectivity estimates from the pathway for the contrast (stress > no-stress).