Reward and Affective Dysregulation in Cannabis Users

Inaugural-Dissertation

zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultät
der Heinrich-Heine-Universität Düsseldorf

vorgelegt von

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Köln, Januar 2018

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Gedruckt mit der Genehmigung der
Mathematisch-Naturwissenschaftlichen Fakultät der

Heinrich-Heine-Universität Düsseldorf

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Tag der mündlichen Prüfung: 23.05.2018

SUMMARY

Cannabis is the most commonly used illicit drug worldwide and current legalization debates are bringing about policy changes despite increasingly recognized adverse effects of chronic use. Dysregulations in reward and affect processing are at the core of substance use disorders. However, whether similar dysregulations can be observed in cannabis dependence remains a subject of debate.

Against this background, the presented three functional magnetic resonance imaging (fMRI) studies within the framework of this dissertation focused on neural and behavioral substrates associated with reward and emotional functioning in cannabis users.

The first study addressed processing of social reward in dependent cannabis users after 28 days of abstinence. Dependent cannabis users, as compared to controls, exhibited a reduction in differential reward perception and a blunted activation of the dorsal striatum that varied depending on social context. The extent of striatal alterations increased with a greater cumulative lifetime amount of cannabis.

The second study investigated emotion processing in dependent cannabis users after 28 days of abstinence. Relative to controls, dependent cannabis users showed no difference in emotional experience, but an increased orbitofrontal cortex (OFC) activation upon viewing negative scenes. Beyond that, striatal and amygdala connectivity each to the OFC was enhanced during the processing of negative scenes and at rest.

The third study assessed emotion regulation of negative affect in regular recreational cannabis users. Compared to controls, regular cannabis users showed lower emotion regulation success accompanied by increased prefrontal activation and decreased amygdala-prefrontal functional coupling. Regulation success decreased with stronger craving.

Together, these findings provide evidence that cannabis use and dependence are linked to motivational and affective dysregulation that compare to other substances dependences.

ZUSAMMENFASSUNG

Cannabis ist die am häufigsten konsumierte illegale Droge. Aktuelle Legalisierungsdebatten führen zu Gesetzesänderungen trotz der wissenschaftlich anerkannten negativen Auswirkungen des chronischen Konsums. Jedoch gibt es wenig Evidenz für Veränderungen der Belohnungs- und Emotionsverarbeitung bei Cannabiskonsumenten, wie sie bei anderen Substanzstörungen beobachtet werden.

Die vorgelegten Studien im Rahmen dieser Dissertation untersuchen mittels funktioneller Magnetresonanztomographie, ob funktionelle Veränderungen in Hirnarealen und neuralen Netzwerken der Belohnungsverarbeitung und emotionaler Funktionen bei Cannabiskonsumenten vorliegen.

In der ersten Studie wurde die Verarbeitung von sozialen Belohnungsreizen bei abhängigen Cannabiskonsumenten nach 28tägiger Abstinenz untersucht. Cannabiskonsumenten zeigten im Vergleich zu gesunden Kontrollen eine verminderte differentielle Wahrnehmung der Belohnung und eine geringere Aktivierung im dorsalen Striatum welche sich in Abhängigkeit vom sozialen Kontext zeigte. Das Ausmaß der striatalen Veränderungen nahm mit höherem kumulativen Cannabiskonsum zu.

Die zweite Studie untersuchte inwiefern abhängige Cannabiskonsumenten eine veränderte Verarbeitung emotionaler Reize nach 28tägiger Abstinenz aufweisen. Im Vergleich zu gesunden Kontrollen zeigten abhängige Cannabiskonsumenten eine vergleichbare Emotionswahrnehmung, allerdings eine erhöhte Aktivierung des orbitofrontalen Cortex (OFC) welche spezifisch für negative Stimuli beobachtet wurde. Zudem war die Konnektivität des Striatums und der Amygdala zum OFC bei Cannabiskonsumenten während der Verarbeitung negativer Stimuli, wie auch im Ruhezustand, erhöht.

In der dritten Studie wurde die Regulation negativer Emotionen bei Freizeit-Cannabiskonsumenten untersucht. Cannabiskonsumenten zeigten dabei im Vergleich zu Kontrollen einen verminderten Emotionsregulationserfolg. Damit einhergehend wurde eine

präfrontale Überaktivierung sowie eine verminderte Kopplung zwischen Amygdala und präfrontalem Kortex beobachtet. Innerhalb der Gruppe der Konsumenten nahm der Emotionsregulationserfolg mit zunehmendem Craving ab.

Zusammenfassend lässt sich beobachten, dass Cannabiskonsum und Abhängigkeit mit motivationaler und affektiver Dysregulation einhergehen. Die beobachteten Auffälligkeiten stehen im Einklang mit Veränderungen, welche bei anderen Abhängigkeitsstörungen beobachtet werden.

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1. Introduction

1.1. Prevalence of cannabis use

Many young adults experiment with cannabis. For most individuals exposure is pertained to an experimental and controlled phase (Orth, 2016), yet a significant number of people will develop problematic patterns of use that interfere with academic performances, daily activities and in case of a cannabis use disorder even require professional intervention. Accumulating evidence from different lines of research suggests adverse health effects associated with regular cannabis use, while social and legal acceptance for medicinal and recreational use of cannabis are steadily increasing. Particularly in the US where current policy changes are taking place, public opinion of cannabis as a relatively harmless drug contributes to widespread use and to half of the adult population supporting the legalization of cannabis (Cressey, 2015). Even though scientific literature provides evidence for adverse effects, many questions remain to be answered.

For several decades cannabis has been the most commonly used illicit substance worldwide (UNODC, 2017) with 183 million, approximately 3.8 percent of the global population, having used the drug at least once in 2015. This number is relatively high compared to, for instance, 37 million amphetamine and stimulant users or 22 million ecstasy users. Although global use of cannabis has remained stable in the past decade, rates of use have increased in the US and Western Europe, including Germany with a peaking prevalence of 6.1 percent in 2015 and a particularly concerning increase in the number of cannabis users entering treatment for cannabis use disorder (EMCDDA, 2017). It is estimated that 9 percent of cannabis users will develop a clinically relevant cannabis dependence (Anthony et al., 1994; UNODC, 2016) which currently amounts to 1-1.5 percent of the adult population in the US and Germany (UNODC, 2017; EMCDDA, 2017). The risk of developing a cannabis dependence is significantly higher for individuals who start using in adolescence (Winters and Lee, 2008;

Volkow et al., 2014) and increases up to 50 percent for regular cannabis users (Hall and Degenhardt, 2009). Even though the addictive potential of cannabis and related harms of the drug in measures of physical and social harm are considered relatively low compared to other substances of potential abuse (Nutt et al., 2007), such as heroin or cocaine, the prevalence of cannabis use disorders is high due to its widespread use. The rise in numbers of cannabis users in the US and Western Europe placing more individuals at risk of developing a cannabis use disorder, together with increases in cannabis potency that can negatively impact the development and severity of dependence (Freeman and Winstock, 2015) and the public perception of cannabis as relatively harmless are alarming. Although withdrawal from cannabis may be less severe than for other drugs, cannabis dependence is comparable to other substance use disorders regarding motives for treatment initiation and high relapse rates (McRae et al., 2003) which range between 50 and 70 percent (Budney et al., 2008; Chauchard et al., 2013). Consequently, the identification of subjects at risk for developing a dependence and the advancement of specific treatment strategies are becoming increasingly relevant.

Since the recognition of cannabis dependence as a psychiatric disorder and the inclusion of this disorder into the DSM catalogue third edition in 1993, still little is known about the underlying neurobiological mechanisms of cannabis dependence and whether these align with other substance use disorders or rather have unique pathological profiles. Comprehensibly, therapeutic interventions for cannabis dependence have largely been based on programs for 'hard' drugs and alcohol (Stephens et al., 2002) and evidence-based programs specifically for cannabis dependence are still under evaluation. In Germany, as of recent, the CANDIS (*short for* CANnabis DISorders) program could show some success for behavioral and psychosocial interventions including cognitive behavioral therapy (CBT) and motivational enhancement therapy (MET) (Hoch et al., 2014) which is consistent with a common use of these therapy forms in the US (Bonnet and Scherbaum, 2005; Danovitch and Gorelick, 2012). However,

these interventions strongly rely on intact cognitive (Stevens et al., 2014), social and emotional functioning (Charlet et al., 2014) posing a direct challenge to longterm success rates when impairments in these domains are part of the psychopathology. Despite recent progress of behavioral (Hoch et al., 2014) and pharmacological (Copeland and Pokorski, 2016) treatment approaches, the overall treatment efficacy regarding longer term cannabis abstinence remain moderate to low.

Together, this suggests a pressing relevance of understanding adverse health effects of cannabis use, including the underlying neurobiological substrates of cannabis dependence, to develop more effective prevention and treatment programs. Moreover, within debates on matters of legalization it is pivotal to have sufficient empirical evidence relating to potential harms of the drug. Within the context of a rising interest in individualized therapies, uncovering behavioral and neural biomarkers associated with relapse risk could beyond that be beneficial for improved clinical outcome.

1.2. Acute and chronic effects of cannabis

Cannabis use has been associated with a range of acute and chronic adverse effects on the brain – effects predominantly mediated by the stimulation of CB1 receptors of the endocannabinoid system (ECBs). Central components of the ECBs, abundant throughout the body, are the cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2) and the endogenous activators thereof (anandamide and 2-arachidonoylglycerol) (Howlett et al., 2004). CB1 receptors are expressed on presynaptic neurons that are mainly responsible for endocannabinoid signaling in the central nervous system (CNS) and CB2 receptors are found in the periphery on immunocompetent cells and on microglia (Atwood and Mackie, 2010). In the CNS, the ECBs functions as a prominent neuromodulator of synaptic transmission affecting neurotransmitter systems including glutamate (Colizzi et al., 2016) and GABA (Lee et al., 2015) that can in turn

fine-tune dopaminergic neurotransmission (Covey et al., 2017). Consequently, it is implicated in the regulation of processes ranging from neuronal maturation (Meyer et al., 2018) to learning and memory (Riedel and Davies, 2005), to higher order behavioral functions such as emotional behavior, the regulation of stress (McLaughlin et al., 2014) and reward (Panagis et al., 2014).

The most studied principal components of cannabis are the psychoactive delta-9-tetrahydrocannabinol (THC) and the non-psychoactive cannabidiol (CBD) (Atakan, 2012). Whereas THC exposure has largely been linked to the adverse effects of cannabis exposure, CBD is increasingly recognized to have neuroprotective effects (Bhattacharyya et al., 2012a; 2012b) and has been shown to ameliorate negative effects of THC (Iseger and Bossong, 2015). The following section will focus on the acute and chronic effects of THC as this dissertation aims at uncovering adverse effects of cannabis use on motivational and emotional functioning.

Effects of acute and chronic THC exposure on brain structure and function in humans have been investigated through indirect measures such as neuropsychological assessments and functional and structural neuroimaging techniques including magnetic resonance imaging (MRI) and positron emission tomography (PET). Functional neuroimaging studies suggest that acute THC effects on cognitive and emotional processes are mediated by cortico-limbic structures with high CB1 receptor densities. THC acts as a partial agonist at CB1 receptors richly concentrated in the striatum, amygdala, hippocampus and prefrontal cortex (PFC) (Burns et al., 2007; Mackie et al., 2008) and acutely induces desired psychoactive effects such as mild euphoria and relaxation, as well as undesired effects on cognitive functions including impairments in short-term memory, verbal fluency and attention (Curran et al., 2002; Atakan, 2012; Broyd et al., 2016). Depending on the dose, social context and previous experience with the drug, THC can produce anxiety, paranoia or panic reactions (Martín-Santos et al., 2012).

Under acute THC administration, impaired response inhibition during cognitive interference and lower memory performance have been associated with attenuated task-related activation

in frontal, temporal and parietal cortices (Bossong et al., 2012; 2013; Bhattacharyya et al., 2015). Furthermore, in healthy individuals THC attenuates caudate nucleus activation while increasing PFC activation during attentional salience processing (Bhattacharyya et al., 2012a). Endocannabinoid signaling can also modulate emotional processes. Impaired emotion recognition has been observed (Ballard et al., 2012; Hindocha et al., 2015) while THC acutely attenuates subgenual anterior cingulate cortex (ACC) reactivity during processing of negative affective stimuli (Rabinak et al., 2012) and mediates the induction of anxiety via CB1 receptors in the amygdala (Bhattacharyya et al., 2017). Moreover, THC acutely modulates prefrontal-limbic brain circuits during fear extinction (Rabinak and Phan, 2014) while preventing the recovery of fear (Rabinak et al., 2013) and has been shown to increase amygdala activation and attenuate amygdala-dorsolateral prefrontal cortex (dlPFC) functional connectivity during cognitive reappraisal of negatively valenced images (Gorka et al., 2016).

The highly debated question whether THC acutely modulates dopamine function such as observed for other addictive drugs (Nutt et al., 2015) has been answered in animal studies showing acute elevations in dopamine release in the PFC, striatum and nucleus accumbens (Bloomfield et al., 2016) and in human PET studies demonstrating a moderate dopamine increase in the striatum (Bossong et al., 2009; Bossong et al., 2015).

Together, these studies show that acute administration of THC modulates cognitive and emotional processes, and dopaminergic neurotransmission. It is conceivable that chronic exogenous stimulation of the ECBs may lead to homeostatic adaptations that can impact neuronal efficiency, neuronal integration and communication.

In rodents, repeated exposure to THC can affect receptor expression in the dopamine (Ginovart et al., 2012; Tournier et al., 2016), glutamate (Fan et al., 2010) and endocannabinoid system (Romero et al., 1998). A downregulation in dopamine function is thought to be central to addictive behavior and an adaptation in reward processes, while a downregulation and

desensitization of CB1 receptors may be related to tolerance (González et al., 2005). However, the mechanisms of these cellular adaptations may be more complex than changes to receptor expressions alone, as it has been shown that chronic stimulation by THC may impact intracellular signaling cascades and transcription factors (Fratta and Fattore, 2013).

Currently, prior neuropsychological and brain imaging studies regarding neuroadaptations related to chronic cannabis exposure in humans remain conflicting, possibly related to a large heterogeneity of the study samples, i.e. in the extent of cannabis exposure or the dependence and abstinence status. Therefore, the precise make-up, extent and duration of observed cannabis-associated effects remains to be further elucidated. Most commonly reported are cognitive dysfunctions particularly in the domains of verbal learning, memory and attention that partially persist with prolonged abstinence (Broyd et al., 2016; Nader and Sanchez, 2017) and structural changes to gray matter of the hippocampus (e.g. Matochik et al. 2005; Yücel et al. 2008; Ashtari et al. 2011;) and cortical regions (e.g. Medina et al., 2009; Churchwell et al., 2010; Kumra et al., 2012).

Previous fMRI research in chronic cannabis users has predominately focused on cognitive processes. Converging lines of evidence suggests functional changes in cortical and hippocampal regions associated with memory function (e.g. Block et al., 2002; Kanayama et al., 2004; Jager et al., 2006; Becker et al., 2010a), altered ACC and lateral PFC activations during inhibitory processing (e.g. Eldreth et al., 2004; Gruber et al., 2005; Hester et al., 2009) and altered activation in the OFC, dlPFC and ventromedial PFC (vmPFC) during decision-making (e.g. Bolla et al., 2005; Vaidya et al., 2012; Cousijn et al., 2013). While some studies could link altered brain activation to impairments in cognitive performance (Block et al., 2002; Gruber et al., 2005), others showed increased activation (e.g. Jager et al., 2006) or recruitment of additional task-untypical regions (e.g. Eldreth et al., 2004; Kanayama et al., 2004) in the

absence of behavioral differences suggesting additional compensatory effort with higher cognitive demand.

However, whether chronic cannabis use is linked to neurobiological changes in emotional and motivational functioning related to natural reinforcers has gained less attention in scientific literature, although dysfunctions in both domains are highly implicated in substance addictions (see section 2.3.).

Naturally, cues that have become associated with cannabis induce craving for the drug (Charboneau et al., 2013) which is thought to be mediated by a network including the ventral tegmental area (VTA), striatum, thalamus, insula, amygdala, ACC and OFC (Filbey et al., 2009; Cousijn et al., 2013; Wetherill et al., 2014), as comparable to other substances of abuse (Jasinska et al., 2014). Activation thereby in the OFC, ventral striatum (Filbey et al., 2009; Cousijn et al., 2013) and the dorsal striatum (Vingerhoets et al., 2016) associate with cannabis use problem severity. Furthermore, greater reward circuitry connectivity between the ventral striatum and OFC has been observed in dependent cannabis users (Filbey and Dunlop, 2014). Together, these studies show reward-related drug memory that can promote continued drug use and beyond that indicate that the extent of activation may more intensely challenge the maintenance of abstinence.

Additionally, emerging evidence has demonstrated that long-term cannabis use is associated with both attenuated (van Hell et al., 2010) and increased ventral striatal activation (Nestor et al., 2010) during monetary reward fMRI tasks in non-dependent subjects. Changes to striatal processing of drug and non-drug rewards in the progression and maintenance of substance addictions has been linked to underlying altered dopaminergic functioning (see section 2.3.). Whether this can be observed in cannabis abusers has been addressed. A PET study indirectly measuring dopamine synthesis capacity with a radio ligand tracers could show that apathy in cannabis users associated with lower dopamine synthesis capacity in the striatum possibly

linked to lower reward sensitivity (Bloomfield et al., 2014a) and higher levels of cannabis use (Bloomfield et al., 2014b). Furthermore, dopamine transporter availability in the dorsal striatum is lower in dependent cannabis users (Leroy et al., 2012) collectively suggesting that striatal reward processing in regular non-dependent cannabis users and underlying dopaminergic functioning in dependent cannabis users appear to be compromised. Exaggerated striatal reactivity to drug-reward cues in conjunction with reduced sensitivity for natural (non-drug) rewards (Volkow et al., 2012) may contribute to the progression of addiction during which drug seeking becomes the central motivational drive. However, whether altered processing of natural rewards can be observed in cannabis dependent individuals remains to be investigated.

Finally, chronic cannabis use has been shown to impact neural processing of emotions impairing fear extinction (Papini et al., 2017), attenuating medial PFC activity during emotional evaluation (Wesley et al., 2016) and amygdala and cingulate cortex activation during implicit emotion processing (Gruber et al., 2009) in early phases of abstinence. Despite a report of deficient identification and discrimination of facial emotions in dependent cannabis users after a minimum of 28 days of abstinence (Bayrakçı et al., 2015), the neural substrates of impaired emotional functioning in dependent cannabis users has yet to be uncovered.

Interestingly, the extent of neuroadaptations revealed by structural and functional studies has been shown to increase with an earlier age of onset (e.g. Becker et al., 2010b; Harding et al., 2012), a larger cumulative lifetime exposure (e.g. Yücel et al., 2008; Nestor et al., 2010) and a greater severity of problems associated with cannabis use (e.g. Cousijn et al., 2012; Vingerhoets et al., 2016) indicating that (a) adolescence may be a period of particular vulnerability to pharmacological insult that can induce persisting neural alterations, (b) THC may in fact, in line with animal studies, induce dose-dependent neuroadaptations in humans,

and (c) the severity of functional impairments may directly be related to the severity of the disorder.

However, a majority of studies have focused on non-dependent users allowing a delineation of effects induced by drug exposure, predispositional factors that may have contributed to the development of regular use patterns or adaptations involved in initial stages of dependence. Yet, neurobiological markers of cannabis dependence disorder have not been systematically characterized.

Alterations in neural functioning related to cue-reactivity (Filbey and Dunlop, 2014; Wetherill et al., 2014) and reductions in hippocampal volume (Yücel et al., 2008; Ashtari et al., 2011) have been observed in dependent cannabis users. Furthermore, neural alterations have been connected to dependence severity and treatment failure. For instance, in cannabis dependent individuals, increased striatal activity during receipt of losing outcomes as well as smaller putamen volumes (Yip et al., 2014) and lower inhibitory control related ACC, dlPFC and striatal activity (Kober et al., 2014) have been associated with less abstinence success during (Yip et al., 2014) and after (Kober et al., 2014) treatment. Together, these findings indicate that neuroadaptations in both cognitive control and reward-based learning regions may differentiate dependent from non-dependent cannabis users and that these alterations may be a neurobiological substrate of lasting vulnerability to drug relapse over time.

However, at the same time, a number of studies have highlighted the relevance of assessing effects after longer abstinence periods by showing that brain activation (Sneider et al., 2008), regionally selective CB1 receptor downregulation (Hirvonen et al., 2012) and cognitive functions (Pope et al., 2001) may normalize with prolonged abstinence of > 4 weeks, although other studies could observe lasting impairments (Eldreth et al., 2004; Bolla et al., 2005). A dominant aspect of addictions is the vulnerability to relapse even with prolonged abstinence

after withdrawal symptoms have ceased indicating that lasting cognitive and affective changes may be central to this disorder and therefore need greater attention in scientific literature.

1.3. Neurobiological substrates of substance addiction

Although each drug has distinct molecular targets and therefore unique pharmacological profiles, substance addictions share common neurobiological substrates such as changes to the dopaminergic system and clinical similarities in form of a chronic relapsing neurobehavioral disorder. Addictive behavior encompasses recurring phases of drug binging/intoxication, withdrawal/negative affect and preoccupation/anticipation (Koob and Le Moal, 1997) and with the progression of this disorder a transition from initial volitional drug use to continued compulsive habitual drug administration despite the awareness of negative consequences can be observed. Addiction theories have formulated underlying processes in terms of increased motivational drive to use drugs as a result of changes to the brain's reward system (incentive sensitization) (Robinson and Berridge, 1993; 2008), continued drug use to alleviate negative emotion states (negative reinforcement) (Baker et al., 2004), and counteradaptive mechanisms in line with opponent process theory (Solomon, 1977; Koob et al., 1993). At the core of these models is the idea of a shift in brain signaling that tips motivational and appetitive responses in favor of continued drug use. In healthy individuals, a balance between cognitive control, motivational and stress circuits allows an efficient selection of choices that guides behavior. It is conceivable that disruptions at either node of this dynamic communication can result in maladaptive behaviors such as addiction. Essentially, motivational and appetitive urges in addictive behavior reflected in strong emotional drives may conflict with higher order goals. Addiction thereby has been conceptualized as a failure to solve this conflict (Heatherton and Wagner, 2011).

Three neurobiological systems are referred to across influential addiction models and are thought to promote the progression and maintenance of addiction: (1) the reward neurocircuitry with a focus on the striatum related to the incentive salience of the drug (Robinson and Berridge, 1993; 2008), (2) the amygdala stress system underlying withdrawal and increased emotional distress (Koob, 2015) and (3) prefrontal cortical circuits associated with diminished behavioral control (Goldstein and Volkow, 2011). Together, an imbalance between prefrontal top-down and bottom-up subcortical signaling related to reward and emotion thereby is considered as a core characteristic of addiction (Heatherton and Wagner, 2011).

REWARD SYSTEM

The mesocorticolimbic reward system has been attributed a pivotal role in the development and maintenance of substance addictions. With the ventral striatum at its center receiving cortical input from the OFC and ACC and dopaminergic projections from the VTA, this circuit extends to regions such as the amygdala and hippocampus (Haber and Knutson, 2010). Across species, all drugs of abuse lead to elevated dopamine release in the striatum (e.g. Koob, 1992; Volkow et al., 1999; Bossong et al., 2009) which is associated with the positive reinforcing effects (reward) of the drug experienced as subjective 'high' in humans (Volkow et al., 2009). Chronic stimulation of the dopaminergic reward system may lead to lasting adaptive processes that lend to the exaggerated salience of the drug and consequently strong motivational drive to continue the use despite accumulating adverse consequences. Addicted individuals show significant reductions in dopamine receptors levels (Volkow et al., 1996), as well as a decrease in dopamine release capacity in the striatum associated with a blunted pleasurable response to the drug (Volkow et al., 2007). Moreover, drug cues potently activate the brain's reward system including the striatum and OFC (Jasinska et al., 2014) while striatal activations associated with natural rewards are attenuated (Volkow et al., 2010). Together, these observations may underlie

the incentive salience theory that posits an increased reward expectancy toward the drug at the expense of other essential rewards.

As mentioned above, PET studies could show lower dopamine transporter availability in dependent cannabis users (Leroy et al., 2012). However, whether this translates into reduced reward responses to natural rewards has yet to be investigated.

EMOTION / STRESS SYSTEM

Counteradaptive mechanisms in the reward system causing a blunted response to natural rewards and in the stress system causing increased sensitivity to negative events and stressors together can result in emotional distress promoting continued drug use. In healthy individuals, the amygdala, central to the brain's stress system, is implicated in manifold emotional functions including emotional influences on attention, learning and memory and the induction of negative affect (Phan et al., 2002; Phelps and LeDoux, 2005), while frontal-amygdala connectivity is essential for emotion regulation (Banks et al., 2007). Extended neurobiological substrates involved in the perception and appraisal of negative and stressful events are, amongst others, the hippocampus, OFC, medial PFC and ACC (García-García et al., 2016). Recruitment of the brain stress system is mediated by corticotropin-releasing factor (CRF) and norepinephrine in the extended amygdala which play a critical role in modulating brain motivational pathways (Heinrichs, 2005; Koob, 2015).

All abusive drugs have been shown to produce chronic alterations to the brain stress system (Koob, 1999), including heightened CRF activity (Sinha, 2008), which may contribute substantially to states of heightened emotional distress and hypersensitivity to stressful stimuli (Koob and Le Moal, 1997). Adaptations may reflect compensatory mechanisms to reinstate homeostatic balance (Feltenstein and See, 2008). Both a heightened (de Arcos et al., 2008) and an attenuated (Verdejo-García et al., 2006) response to negative affective stimuli have been observed in substance dependent individuals, as well as an altered brain response of prefrontal

regions involved in processing of negative events (Gilman and Hommer, 2008; Wesley et al., 2016). Increases can potentially be explained in the context of a heightened sensitivity to stressful events and decreases as a blunted response that reflects the neglect of adverse consequences of the drug. Moreover, activation in the amygdala upon cue-exposure (Kühn and Gallinat, 2011) may be linked to heightened emotional distress related to strong craving (Goudriaan et al., 2010).

The interaction between heightened negative affect related to withdrawal arising from physiological counteradaptive mechanisms and the absence of the drug and a hypersensitive stress system may have detrimental effects on behavioral choices. Dependent individuals, including cannabis users (Simons et al., 2000), commonly report alleviating negative affect as the primary motivation for continued drug intake (Wetter et al., 1994). Emotional distress (Sinha, 2001; Li and Sinha, 2008), persistent anhedonia (Lubman et al., 2009) and exposure to drug cues (Beck et al., 2012) have been shown to contribute to relapse even after longer abstinence periods suggesting that reinstating drug use is strongly related to imbalanced affective states. Moreover, the ability to efficiently regulate emotions and sustain goal-directed behavior in the face of emotional distress leads to higher treatment success in substance abuse treatment programs (Hopwood et al., 2015) indicating that the compromised interplay between cognition and affect is central to the maintenance of substance use disorders. Difficulties in keeping elevated negative emotional states at bay may reflect a failure of cognitive control systems (Li and Sinha, 2008).

COGNITIVE CONTROL SYSTEM

In addition to disrupted reward-related and affective functioning, addiction encompasses deficits in self-regulatory brain circuits that lead to impaired attentional and inhibitory control and decision-making, as well as imbalanced emotion and stress regulation (Volkow et al., 2010). A broad field of literature has focused on elucidating cognitive impairments related to

substance dependence (Baler and Volkow, 2006) and could link cognitive disruptions to lower treatment success (Aharonovich et al., 2006). Compromised PFC function is thought to be at the core of loss of control over drug intake (Goldstein and Volkow, 2011). Altered function of both the dlPFC, implicated in conflict resolution, and the ACC, implicated in conflict detection, have been associated with impaired behavioral control (Goldstein and Volkow, 2011) while compromised ACC and vmPFC function in the context of emotion regulation deficits have been observed across substance addictions (Wilcox et al., 2016). It has been argued that a change in dopaminergic (Volkow et al., 2009) and glutamatergic (Kalivas et al., 2005) neurotransmission may be at the basis of these functional impairments mirrored in the loss of behavioral control. Prefrontal control mechanisms have been shown to be domain general (Cohen and Liebermann, 2013) and prefrontal resources have been postulated to be subject to depletion (Heatherton and Wagner, 2011). Therefore, if a low efficiency of prefrontal control is present due to addiction-associated impairments, resources may not be sufficient to meet the challenge of strong negative affect and increased urges for the drug.

Together, these neurobiological changes related to chronic drug intake and addiction progression render a dependent individual vulnerable to relapse even after years of abstinence indicating that substance addictions are related to persisting neuroadaptations that need to be considered in treatment programs.

2. Methodology

2.1. fMRI: Biological markers of substance addiction

Neuroimaging techniques have allowed to map behavioral processes to neural circuits and understand how these are affected in psychopathology (Fowler et al., 2007; Suckling and Nestor, 2017). fMRI has consistently revealed differences in brain function between substance dependent individuals and healthy controls and thereby allows to determine the neural basis of

addiction. Particularly, human brain imaging studies have been able to detect cannabis-associated alterations and reveal altered brain functions as observed in a broad range of substance use disorders.

At the basis of fMRI methodology are actively signaling neurons that increase the demand of oxygen transported by hemoglobin in the arterial blood stream (Norris, 2006) which in turn shifts the ratio of oxygenated and deoxygenated hemoglobin. This shift results in local magnetic field changes that can be detected by MRI and are measured by the blood oxygen level dependent (BOLD) contrast (Ogawa et al., 1992). Therefore, the BOLD signal can be used as an indirect measure of neural activity at rest or of reactivity to a task-challenge shedding light on neurobiological processes related to complex behavioral functions. Furthermore, by comparing study samples of healthy individuals with psychiatric populations, neural markers of psychopathology can be determined. The noninvasive methodology of fMRI therefore appears to be highly suited for uncovering neuroadaptations in dependent cannabis users that map maladaptive addictive behavior and moreover, can together with structural, PET and neuropsychological data allow a broad description of the disorder. Studying adaptations of the neurocircuitry could thus allow to create more effective treatment options by using these biomarkers (Moeller and Paulus, 2018) for neurofeedback, pharmacological or behavioral therapies that directly target the underlying circuits. In this context imaging techniques can further strengthen our understanding of the neurobiology of addiction and help improve, extend and generate treatment resources.

2.2. Cannabis dependence: Diagnostic criteria

Cannabis dependence and mental health were determined based on Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 2000) classifications that are briefly outlined in the following section. Addiction is defined in terms

of pathological substance use that gives rise to clinically relevant impairment or distress. Importantly, substance use is continued despite the adverse consequences.

DSM-IV criteria classify substance dependence based on four dimensions including:

(1) physiological dependence symptoms such as tolerance and withdrawal (2 criteria),

(2) loss of control directly related to the amount and duration of drug intake (3 criteria),

(3) social problems related to maladaptive behavior (1 criterion) and

(4) engaging in risky behavior associated with the use of the drug (1 criterion).

Cannabis users meet a diagnosis of cannabis dependence if they fulfil at least 3 of the 7 criteria in the past 12 months. DSM-IV furthermore distinguishes drug dependence and drug abuse. However, the presented studies 1 and 2 of this dissertation included participants based on dependence criteria irrespective of abuse status.

As the prevalence of psychiatric disorders such as major depression, anxiety and bipolar disorders is high among individuals with cannabis dependence (Agosti et al., 2002; Fergusson et al., 2003) and these disorders can be characterized by neural changes in both the domains of reward and emotional functioning (e.g. Etkin et al., 2007; Smoski et al., 2009; Stuhrmann et al., 2011), this must be considered in empirical studies addressing neural substrates of cannabis dependence. To exclude individuals with such disorders, mental health (other than cannabis dependence) was assessed according to DSM-IV.

In addition, the co-occurrence of cannabis and tobacco use is widespread (Agrawal et al., 2012) which is mirrored in a high number of cigarette smokers in the groups of dependent and non-dependent cannabis users of the presented studies. This is of particular relevance as changes to reward and emotional function have been observed in regular and dependent cigarette smokers (Bühler et al., 2011; Onur et al., 2011). Therefore, groups were matched for the number of cigarette smokers and cigarette use patterns. As a trade-off between confounding effects of

acute nicotine and nicotine craving on reward and emotional functions, all smokers abstained from nicotine for 1.5-2 hours prior to the assessments.

3. Rationale of the present dissertation

As can be observed across substance classes, chronic exposure to addictive drugs is linked to neuroadaptations affecting cognition, motivation and emotions. Previous literature regarding the impact of cannabis on neurobiological processes has provided converging evidence for cognitive impairments and deficient underlying neural networks. Yet, to date it has not been determined whether cannabis dependence is linked to natural reward deficits and affective dysregulations, as well as compromised associated neural circuits as observed for other substance use disorders.

Given the pivotal role of the brain's reward system and stress system in addiction along with the involvement of the ECBs in these functions, the objective of the presented dissertation was to investigate lasting motivational and emotional functioning and the neural substrates thereof in dependent cannabis users. Furthermore, neuroadaptations may render dependent individuals vulnerable to relapse even after withdrawal symptoms have ceased. As cannabinoid metabolites that may have an effect upon outcome measures remain in the body for up to 4 weeks, it is essential to assess substrates of motivational and emotional functioning after prolonged abstinence phases when aiming at uncovering lasting adaptations related to the disorder.

The presented dissertation comprises three empirical studies addressing this issue.

Firstly, we assessed whether cannabis dependence is associated with lasting adaptions in behavioral and neural substrates of (a) social reward processing and (b) emotion processing after 28 days of abstinence.

Secondly, we addressed whether alterations in emotion regulation and underlying functional brain networks can be observed in chronic, recreational cannabis users with the future outlook of examining emotion regulation capacity in dependent cannabis users.

4. Original studies

4.1. Cannabis dependence after 28 days of abstinence

4.1.1. Cannabis Dependence is Associated with Lasting Context-dependent Reductions in Reactivity to Social Reward

The exact nature and extent of long-term consequences of cannabis dependence, and whether they are comparable to other substance dependences, remain under debate (Volkow et al., 2014; Curran et al., 2016). A key feature of addictive drugs is the ability to acutely induce dopamine release in the striatum while chronic use can lead to lasting disruption in reward function that increases the response to drug-cues and blunts the response to natural rewards (Volkow et al., 2010). This core imbalance of the brain's reward circuit is thought to shift the hedonic set-point and drive the maladapted behavior in substance dependent individuals. Altered striatal function in response to monetary incentives that may have attained drug cue properties has been observed in chronic cannabis users (Nestor et al., 2010; van Hell et al., 2010). Therefore, whether striatal processes related to natural rewards are persistently disrupted in dependent cannabis users remains to be determined.

Against this background, the present study addressed whether cannabis dependence is associated with lasting reductions of reward function after 28 days of abstinence. Cannabis dependence was determined according to DSM-IV criteria and abstinence was confirmed by negative qualitative urine toxicology for THC and self-reports. As pleasant interpersonal touch reliably conveys social reward and elicits activation in the cortico-limbic reward circuit (Ellingsen et al., 2016), 23 cannabis-dependent men and 24 non-using healthy controls were

led to believe they were in physical closeness of or were touched (CLOSE, TOUCH) by either a male or female experimenter (MALE, FEMALE). This previously established fMRI paradigm (Scheele et al., 2014) allowed the assessment of touch- and social context-dependent (i.e. female compared to male social interaction) reward dynamics. Behavioral measures of reward perception were assessed by pleasantness ratings.

Relative to controls, male dependent cannabis users displayed a blunted reward perception reflected in a significantly lower increase in pleasantness for female compared to male touch. Controls responded to female relative to male interaction with increased striatal activation whereas cannabis users displayed the opposite activation pattern. Stronger striatal alterations associated with a greater lifetime exposure to cannabis. Functional changes to the striatum pertained specifically to the contextual modulation, i.e. female compared to male interaction, while processing of touch in cannabis users was intact.

These findings demonstrate that, similar to neuroadaptations observed across substance addictions and in cannabis users after short abstinence, cannabis dependence is linked neuroadaptations in striatal responsivity to non-drug rewards. However, extending previous literature, we found that reward processing deficits persist with prolonged abstinence and appear to depend on the social context. This more intricate pattern adds to observed striatal changes in cannabis users that vary with social context (Gilman et al., 2016a; 2016b).

Reduced striatal dopamine release capacity has been linked to anhedonia and dependence severity (Van den Giessen et al., 2017) and a loss of endocannabinoid receptors in reward-related regions including the striatum (Ceccarini et al., 2015) has been observed in chronic cannabis users. Interestingly, endocannabinoid signaling can modulate dopaminergic neurotransmission in the striatum (Silveira et al., 2016). In this light, although our methodology does not allow a direct conclusion regarding the molecular mechanisms, the present findings may reflect lasting neuroplastic changes related to the interaction of these transmitter systems.

In view of the importance of intact hedonic experience (Lubman et al., 2009), striatal reward processing of non-drug rewards (cannabis dependence see e.g. Yip et al., 2014) and social factors (Nikmanesh et al., 2015) for the longterm success of addiction treatment interventions, lasting alterations in these domains in cannabis dependent individuals appear particularly concerning. Future developments in treatment programs may consider these neurobiological deficits in their approach.

4.1.2. Altered orbitofrontal activity and dorsal striatal connectivity during emotion processing in dependent marijuana users after 28 days of abstinence.

Treatment demand for problematic cannabis use is growing, yet current therapeutic options remain limited. Beyond that, efficient cognitive (Stevens et al., 2014) and emotional functioning (Charlet et al., 2014) promote longterm success of addiction treatment programs. In this light, converging lines of evidence suggesting an association between chronic cannabis use and lasting impairments in cognitive brain function (Broyd et al., 2016) are particularly concerning.

In contrast to extensive scientific literature addressing alterations in cognitive brain function, a limited number of fMRI studies have investigated the impact of chronic cannabis use on emotion processing. Given the role of affective dysregulation in substance dependence (Cheetham et al., 2010) and the involvement of the ECBs in emotional modulation (Batalla et al., 2014; McLaughlin et al., 2014), this is rather surprising. Studies with short abstinence periods observed decreased amygdala and cingulate activity during implicit emotional processing of masked faces (Gruber et al., 2009), attenuated activation during processing of negative stimuli (Heitzeg et al., 2015), and decreased prefrontal activity during explicit evaluation of affective scenes (Wesley et al., 2016) in chronic cannabis users. However, in this rather short cannabis abstinence phase of 12–24 h prior to fMRI assessment cannabis users

may experience withdrawal symptoms (Budney et al., 2003) and emotional distress (Jacobus et al., 2017), and may be subject to subacute effects of cannabinoids and cannabinoid metabolites, all of which may confound affective outcome measures. As of recent cannabis dependence has been linked to impairments in both the identification and discrimination of facial emotions after a prolonged abstinence of at least 28 days (Bayrakçı et al., 2015), yet neural alterations that underlie altered emotion processing in cannabis dependence after sustained abstinence remain to be determined.

With this in mind, the present fMRI study targeted emotion processing and associated fronto-limbic activation in 19 dependent cannabis users and 18 matched non-using controls after >28 days of abstinence. Cannabis dependence was determined according to DSM-IV criteria and abstinence was confirmed by negative qualitative urine toxicology for THC and self-reports. FMRI data was acquired while participants completed a passive emotion processing task displaying positive, negative or neutral scenes from the IAPS catalogue. Moreover, resting-state fMRI data was collected and IAPS images were rated post-MRI on measures of valence and arousal.

Relative to control subjects, negative emotional content induced greater medial orbitofrontal cortex (mOFC) activity and stronger mOFC-dorsal striatal and mOFC-amygdala functional connectivity in dependent cannabis users. Furthermore, increased mOFC-dorsal striatal functional coupling was also found in cannabis users at rest in the absence of task-challenge. However, neural processing of positive content as well as subjective ratings of valence and arousal for all emotional categories were comparable in both groups.

The present findings add to literature on cannabis use associated alterations of emotion processing (Gruber et al. 2009; Wetherill et al. 2014; Wesley et al. 2016) and additionally suggest that neural changes can also be observed in dependent cannabis users with prolonged abstinence.

Pathological changes of the OFC and underlying circuitry have frequently been linked to substance addictions (Schoenenbaum and Shaham, 2008; Goldstein and Volkow, 2011). In healthy individuals, the OFC is involved in reward processing (Elliott et al., 2010) and decision-making (Cunningham et al., 2009) and together with the dorsal striatum has increasingly been linked to processing of negative emotions. Together with the amygdala, the dorsal striatum exhibits strong reactivity to negative visual stimuli (Carretie et al., 2009) while the OFC participates in the automatic downregulation of this limbic-striatal reactivity (Ochsner and Gross, 2005; Phillips et al., 2008). As such, the present findings of increased OFC activation in conjunction with stronger OFC-striatal and OFC-amygdala functional coupling may reflect elevated automatic top-down control in response to negative affective stimulation. Analogous increases in OFC-striatal connectivity at rest has been observed in disorders with comparable behavioral maladaptations such as cocaine dependence (Contreras-Rodriguez et al., 2016) as well as obsessive-compulsive disorder (Beucke et al., 2013). Moreover, neuroplastic changes to this fronto-subcortical circuitry have been proposed to lie at the core of the progression to substance addictions. In particular, the transition from voluntary to habitual drug use may be reflected in a shift from the ventral to dorsal striatum and deficient prefrontal control mechanisms (Everitt and Robbins, 2013). Therefore, the observed heightened OFC activation and connectivity may be characteristic to the pathology of addictive behaviors, including cannabis dependence.

Together, these findings reveal emotion processing alterations in cannabis-dependent individuals that persist with prolonged abstinence. Changes to neural function may be explained by neuroadaptations as a consequence of chronic cannabis use or may represent a predisposing vulnerability for the development of cannabis dependence.

4.2. A pilot study - Emotion Regulation Deficits in Regular Marijuana Users

Volitional regulation of emotions allows individuals to guide appropriate behavior in the context of negative and stressful events (Gross and Munoz, 1995). Successful regulation of negative emotions is reflected in downregulation of limbic activity, including the amygdala, in response to negative events by prefrontal regulatory networks (Ochsner and Gross, 2005; Etkin et al., 2015). A compromised regulatory capacity resulting in heightened emotional distress can adversely impact behavioral choices and may predispose an individual for drug use disorders (Cheetham et al., 2010; Quinn and Fromme, 2010). In accordance, substance dependence has been linked to lower emotion regulation success (Fox et al., 2007) in conjunction with attenuated prefrontal–amygdala communication during cognitive control of negative emotions (Albein-Urios et al., 2014).

Drug use itself can regulate negative emotions and cannabis users report the alleviation of emotional distress as the primary motivational drive for continued use (Simons et al., 2000), potentially related to dysfunctional emotion regulation capacities. Previous literature provides evidence for compromised function in key frontal nodes of inhibitory and behavioral control (Battisti et al., 2010). However, whether prefrontal control deficits in regular cannabis users translate into impaired volitional cognitive regulation of negative emotions remains unclear.

Against this background, this study assessed volitional regulation of negative emotions and the underlying neural networks in regular cannabis users (n = 23) relative to healthy non-using controls (n = 20). Participants underwent fMRI while completing a validated emotion regulation paradigm applying the regulation strategy of distancing. Neural measures and ratings of negative affect for the three conditions *neutral*, *spontaneous negative affect* and *regulating negative affect* were compared between groups. Emotion regulation success is thereby reflected in the decrease of negative affect ratings in the *regulation* compared to *spontaneous* condition.

Relative to controls, cannabis users displayed a significantly lower decrease in negative affect during emotion regulation. This lower efficiency in regulation success was accompanied by increased activity in a bilateral frontal network consistently implicated in emotion regulation including the precentral gyrus, middle cingulate cortex (MCC), and supplementary motor area (SMA). Furthermore, cannabis users showed a compromised downregulation of the amygdala along with weaker amygdala–dlPFC functional connectivity during volitional regulation of negative emotions. Lower emotion regulation success was associated with stronger craving for cannabis.

We are the first to provide evidence for reduced volitional control capacities at the interface of cognition and emotion within regulatory brain circuits in regular cannabis users. The present findings converge with the suggested role of deficient top–down control of emotions in the development and maintenance of substance use disorders across various substance classes (Wilcox et al., 2016). Increased activation in core nodes of cognition-emotion integration, such as the SMA and MCC (Shackman et al., 2011), may reflect a stronger effort to exert control. However, despite emotion regulation processes being initiated, a lower regulation success as well as a weaker reduction of amygdala activation in cannabis users indicates that the compensatory recruitment of neural resources is only partially successful. The attenuated amygdala-dlPFC coupling in the present sample of cannabis users may be central to the deficient regulation of negative affect. Endocannabinoid signaling can regulate stress (Volkow et al., 2017) and mediate anxiolysis (Phan et al., 2008), in line with a rich concentration of CB1 receptors in the fronto–limbic neural circuitry (Eggan and Lewis, 2007) and a modulation of dlPFC–amygdala coupling during emotion regulation by THC (Gorka et al., 2016). Therefore, cannabis may initially be used to downregulate negative emotions and repeated use may disrupt the efficiency of this regulatory circuit through interactions with neurotransmitter systems. However, the cross-sectional design of the present study does not allow to delineate whether

deficient emotion regulation predated the onset of cannabis use, or whether regulatory impairments are the result of regular cannabis exposure.

Together, the increased activation in regular cannabis users may reflect a compensatory recruitment of additional frontal regulatory resources that fail at top-down volitional regulation of negative emotions mediated by impaired amygdala–prefrontal interaction. Therefore, this paradigm is suitable for future investigations aiming to uncover neural adaptations associated with volitional control of negative affect in dependent cannabis users.

5. General discussion

In light of the increasing relevance of characterizing adverse effects of chronic cannabis use, and particularly cannabis dependence, the present dissertation converges with evidence regarding detrimental effects in the domains of reward and emotional processes strongly associated with the transition to dependent use and extends previous literature by the following aspects:

(a) Dependent cannabis users display attenuated differential reward coding and adaptations in the striatal response to social rewards that persist with prolonged abstinence lending partial support to the incentive salience theory of addiction. Attenuation of reward dynamics in the striatum increases with greater lifetime cannabis exposure.

(b) Dependent cannabis users show intact emotion evaluation while exhibiting increased OFC activation and OFC-striatal as well as OFC-amygdala functional connectivity in response to images of negative scenes. The observed changes in the striato-limibic-prefrontal pathways related to affective processing persist with prolonged abstinence.

(c) Regular cannabis users show lower emotion regulation capacity in conjunction with increased prefrontal activation and diminished prefrontal-amygdala connectivity during reappraisal of negative affect. Emotion regulation success decreases with stronger craving indicating that motivational urges can challenge cognitive resources at the expense of efficient regulatory control.

Changes in reward and affective processing in cannabis dependent users are comparable to other substance dependences and adaptations to the striato-limbic-prefrontal circuitry thus may represent a common pathway across addictive disorders rather than be specific to the pharmacological interaction related to drug class. Neuroadaptations may be initiated through distinct pharmacological mechanisms of each drug but share a common downstream path which is reflected in analogous behavioral patterns and clinical symptoms. Adaptations of the frontal-subcortical network may impact appetitive and aversive processing irrespective of the valence, consequently challenging behavioral response selection related to diminished reward functioning and imbalanced processing of negative affect.

We could show that differences in neural activation patterns don't normalize with 28 days of abstinence. As such, lasting dysfunctional reward and affective functioning, even after symptoms of withdrawal and residual drug effects have ceased, may be central to relapse risks associated with cannabis dependence. Moreover, a pilot study with regular recreational cannabis users displaying attenuated emotion regulation success combined with potentially compensatory prefrontal activation and weaker prefrontal-amygdala coupling indicates that prefrontal control deficits may additionally shift behavior in favor of limbic responses. Yet, whether these neural alterations occur in cannabis dependence remains to be investigated. We here provide a sensitive tool for this future investigation. Together, the findings presented in this dissertation bring forth evidence supporting an imbalanced prefrontal-subcortical communication in cannabis users.

The low differential reward perception in dependent cannabis users shows that pleasantness of natural rewards in form of social interaction is blunted, which corresponds to the lower reward threshold hypothesis in drug addictions (Blum et al., 1996). Interestingly, BOLD activation in response to both pleasant social interaction as well as negative events revealed changes in the dorsal striatum. The dorsal striatum has been implicated in habit formation and learning of stimulus-outcome contingencies while the ventral striatum codes reward prediction (O'Doherty et al., 2004). In the progression of addictive disorders a shift from the ventral to dorsal striatal control of behavior has been proposed (Everitt and Robbins, 2013) that accompanies the transition from voluntary to compulsive habitual drug use. Additionally, heightened activations in the dorsal striatum have been observed during cue-exposure in addicted individuals as compared to a heightened ventral striatal response in initial drug users (Vollstädt-Klein et al., 2010) and changes to the dopamine system as measured by PET pharmacological challenge have also been mapped to the dorsal striatum (Volkow et al., 2006; Leroy et al., 2012). Together, functional dysregulations of the dorsal striatum reflect a domain general neurobiological substrate of addictive disorders. This idea is further supported by the altered OFC-striatal functional connectivity in the absence of task challenge. Changes in striatal activation and the communication of this brain structure may functionally impact a range of processes, including processing of natural rewards and negative emotions, but also cue-induced craving (Volkow et al., 2006), habitual behavior (Schiltz, 2006) and the reinstatement of drug use (Yager et al., 2015), all involved in the pathology of substance addictions.

Moreover, we report a change in OFC-amygdala connectivity during processing of negative affect in dependent cannabis users and in PFC-amygdala functional coupling during regulation of negative affect in regular non-dependent users. OFC-amygdala coupling has been implicated in automatic control of affect (Ochsner and Gross, 2005; Phillips et al., 2008). Therefore, both automatic and volitional prefrontal regulation of the amygdala may be compromised in

cannabis users indicating that regular cannabis exposure can be linked to compromised frontal control of affective responses. Dynamic communication in prefrontal-limbic networks may be shifted in favor of subcortical signaling as reflected in lower regulatory success (i.e. higher amygdala signals during reappraisal and higher negative affect in reappraisal conditions in cannabis users). The experience of greater emotional distress as the result of regulatory failure that increases with stronger craving together with a diminished reward response that increases with greater cannabis exposure, may in concert contribute to a higher incentive for continued drug use, especially for individuals with stronger disorder symptomology (i.e. strong craving and greater use).

Most noteworthy is the observed persistence of neuroadaptations with prolonged abstinence in this cortico-striatal network that may place cannabis dependent users at a higher risk of relapse. However, the minimum abstinence phase in the presented studies aligns with the minimum of days necessary for reliable bodily elimination of cannabinoids and cannabinoid metabolites. It is fathomable that neural functioning in these domains may normalize with longer abstinence phases.

6. Outlook

Although the findings of this dissertation extend our knowledge on cannabis-associated changes in neural and behavioral functioning, limitations restrict the interpretation regarding the following aspects. The present studies revealed functional alterations in cannabis dependent individuals in the domains of reward and emotion. The association of striatal activation with cumulative lifetime amount argues that neuroadaptations may be linked to the regular exogenous stimulation of the ECBs and lasting changes thereof. However, future studies including both non-dependent and dependent cannabis users would allow to more precisely distinguish effects directly related to cannabis exposure compared to neurobiological basis of

a developed dependence. It is conceivable that the extent of impairments may be mirrored on a continuous scale as in relation to the severity of this disorder (Cousijn et al., 2012; Vingerhoets et al., 2016).

As our findings pertaining to striatal alterations indicate that context matters, following investigations may include different types of natural rewards as well as drug cues. Collectively, this would allow to assess whether changes to the brain's reward system in dependent cannabis users align with the incentive salience theory of addiction.

Finally, neural alterations to the OFC and OFC-subcortical circuits was not reflected in a shift in negative emotions. A potential explanation may be that negative emotions are either impacted at a subliminal level or with more evocative negative or stressful content. Future studies addressing the emotional response to negative events in cannabis users may employ stimuli based on personal experience, such as Sinha and colleagues (2005).

7. Concluding remarks

With rising prevalence rates of problematic cannabis use and current legalization debates, it is becoming more crucial to comprehensively understand neurobiological mechanisms underlying cannabis dependence - for better prevention and intervention programs. Together, the presented findings provide evidence that cannabis use and dependence are linked to motivational and affective dysregulations that compare to other substances dependences.

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9. Acknowledgements

I would like to use this space to express my gratitude toward the people who supported me and helped me so very much throughout this process.

First and foremost I wish to thank Prof. Dr. Benjamin Becker for entrusting me with this interesting project and giving me the opportunity to complete this dissertation under his supervision. I highly appreciate his valuable guidance and constant support.

I would like to thank my colleagues at the Medizinische Psychologie in Bonn who brightened up each and every single day in the lab and were always willing to help and offer insightful comments. A special thanks to Ann-Kathrin, Ayline and Laura - the reason I could look forward to making the trip to Bonn every day. Not only did they encourage me to keep going and help my work advance, but they became true friends who I cannot thank enough for being so wonderful.

I want to thank Paul for his great technical support and for time and again saving the day.

Last but not least, I want to thank my close friends and family – for always believing in me, for being there for me and for giving me the strength to complete this work.

Without all of you this dissertation would never have been possible. Therefore, I cannot thank you enough!

10. Publications and contribution statements

Title: Cannabis Dependence is Associated with Lasting Context-dependent Reductions in Reactivity to Social Reward

Authors: Zimmermann K, Kendrick KM, Scheele D, Dau W, Banger M, Maier W, Weber B, Ma Y, Hurlemann R, Becker B.

Own contribution to this work:

Zimmermann and Kendrick contributed equally to this work. The original study was part of the DFG-project "Neuronale Aktivierungsmuster bei Cannabis- und Amphetaminabhängigen Individuen: Veränderungen nach 28 Tagen Abstinenz und Zusammenhang mit dem Rückfallrisiko" (BE5465/2-1, HU1302/4-1). I conceptualized and implemented the study in coordination with Dr. Benjamin Becker. I collected and analyzed the data, and wrote and published the manuscript. Dr. Benjamin Becker supervised these processes and helped in optimizing the manuscript.

Publication status:

The presented manuscript has been resubmitted to NEUROPSYCHOPHARMACOLOGY and is currently under revision.

Cannabis Dependence is Associated with Lasting Context-dependent Reductions in Reactivity to Social Reward

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ABSTRACT

Public perception of cannabis as relatively harmless, alongside claimed medical benefits, have led to moves towards its legalization. However, long-term consequences of cannabis dependence, and whether they differ qualitatively from other drugs, are still poorly understood. A key feature of addictive drugs is that chronic use leads to adaptations in reward processing, blunting responsivity to the substance itself and other rewarding stimuli. Against this background, the present study investigated whether cannabis dependence is associated with reductions in hedonic representations by measuring behavioral and neural responses to social reward in 23 abstinent cannabis-dependent men and 24 matched non-using controls. In an interpersonal pleasant touch fMRI paradigm, participants were led to believe they were in physical closeness of or touched (CLOSE, TOUCH) by either a male or female experimenter (MALE, FEMALE), allowing the assessment of touch- and social context-dependent (i.e. female compared to male social interaction) reward dynamics.

Upon female compared to male touch, male dependent cannabis users displayed a significantly attenuated increase of reward experience compared to healthy controls. Controls responded to female as compared to male interaction with increased striatal activation whereas cannabis users displayed the opposite activation pattern, with stronger alterations being associated with a higher lifetime exposure to cannabis. In contrast, dependent cannabis users demonstrated intact processing of pleasant touch.

These findings demonstrate that cannabis dependence in men is linked to similar lasting neuroadaptations in striatal responsivity to hedonic stimuli as observed for other drugs of abuse. However, reward processing deficits seem to depend on the social context.

Clinical trial identifier: NCT02711371

INTRODUCTION

Together with claimed medical benefits, perception of cannabis as less harmful than other drugs (Anthony *et al*, 1994) has promoted recent moves towards legalization. With long-term regular use, however, dependence risks increase, and relapse rates are comparable to other drugs (Hall and Degenhardt, 2009). Although neuroadaptations associated with cannabis use have been examined extensively, most studies focused on recreational users, or dependent users during early abstinence, a period characterized by withdrawal (Budney *et al*, 2003), neural recovery (Hirvonen *et al*, 2012) and potential residual effects of cannabis metabolites for up to 28 days (McGilveray, 2005). Functional alterations have been reported to both normalize and persist (Sneider *et al*, 2008) 4 weeks following cessation of cannabis use. Whether persistent neurobiological changes related to cannabis dependence are similar to those observed following chronic exposure to other drugs thus remains a subject of debate.

Current conceptualizations of addiction propose dysregulations in reward circuits leading to lasting allostatic adaptations in hedonic processing (Koob, 2015; Volkow *et al*, 2012). Animal models have linked the mesolimbic system, particularly striatal nodes, to acute drug reward signaling and neuroadaptations thereof are thought to drive compulsive drug seeking (Di Chiara and Imperato, 1988). Studies in human users suggest that exaggerated striatal reactivity to drug-reward cues and concomitantly reduced sensitivity for natural (non-drug) rewards (Volkow *et al*, 2012) contribute to the addictive process during which drug seeking becomes the central motivational drive and promote relapse (Lubman *et al,* 2009). This imbalance at the core of the brain's reward circuit thus plays an important role in the behavioral maladaptations in dependent individuals.

Previous findings on non-drug reward processing in cannabis users following short abstinence remain inconsistent (Nestor *et al*, 2010; Jager *et al*, 2013; Martz *et al*, 2016). Residual effects of chronic cannabis use on striatal blood flow can be observed even after 72h of abstinence (Filbey *et al*, 2017) and, together with the use of monetary rewards, which associate with drug-cue properties, may have contributed to the inconsistencies. Moreover, recent evidence suggests that alterations across striatal subregions in cannabis users strongly depend on the social context, such as exposure to social information (Gilman *et al*, 2016).

Social factors such as peers considerably influence the addictive process and predict initiation and escalation of use, and treatment success (Nikmanesh *et al*, 2015). In return, drug use itself profoundly affects social behavior ranging from initially enhanced sociability to social withdrawal once a dependence has been developed (McGregor *et al*, 2008). Therefore, social interaction deficits are increasingly recognized as core characteristics of addictive disorders (DSM 5). In line with these observations, animal models indicate lasting social impairments and reduced social interactions following chronic drug exposure (O'Shea *et al*, 2006) possibly rooted in deficient striatal sensitivity for social rewards (Zernig and Pinheiro, 2015). Indeed, positive social interactions engage the striatal reward system (Izuma *et al*, 2008) and may represent an alternative natural reward to drug use.

Pleasant interpersonal touch is a vital instrument for conveying social reward and positive social interaction (Ellingsen *et al*, 2016). As a powerful natural reward, the affective experience of pleasant interpersonal touch elicits activations in the brain's reward network (Ellingsen *et al*, 2016). Both the hedonic experience and associated striatal response strongly depend on the social context (Kreuder *et al*, 2017). Specifically, increased pleasantness and striatal activity have been observed when male subjects believe touch is applied by a female as opposed to a male experimenter (Scheele *et al*, 2014).

The present study addressed whether cannabis dependence is associated with lasting impairments in processing of social rewards and whether these impairments depend on the social context. A pleasant interpersonal touch fMRI paradigm (Gazzola *et al*, 2012; Scheele *et al*, 2014) was employed allowing social context-dependent reward variation by making abstinent (≥28 days) cannabis-dependent men and controls believe that pleasant touch was applied by either a female or male experimenter.

Based on the proposed significance of blunted natural reward sensitivity and social impairments in drug dependence, we expected reduced hedonic experience of pleasant touch and its contextual modulation. In accordance with recent evidence for social context-dependent striatal alterations in cannabis users we furthermore expected blunted striatal coding of reward modulation induced by opposite sex as compared to same sex interaction.

MATERIALS and METHODS

Participants

For selection pipeline of study sample see **SI**. To control for confounding effects of hormonal fluctuations related to menstrual cycle or

contraceptives on the outcome parameters, including reward-related striatal activity (Dreher *et al*, 2016), and dependence symptoms such as craving (Franklin *et al*, 2015) the present study focused on male participants (similar approach see Zimmermann *et al*, 2017). After initial contact, 23 abstinent dependent cannabis users and 24 demographically-matched non-using controls were scheduled for a second assessment that included questionnaires, cognitive tests, drug urine screen and fMRI.

Inclusion criteria for all participants were: 1) Age 18-35, 2) right-handedness, 3) heterosexuality and 4) a negative urine toxicology for cannabis and other illicit drugs (Drug-Screen® Pipette test, Nal van Minden, Moers, Germany, Multi 7TF for amphetamines (cut-off: 500 ng/ml), cocaine (300 ng/ml), methamphetamine (500 ng/ml), THC (50 ng/ml), MDMA (300 ng/ml), opiate (300 ng/ml), methadone (300 ng/ml)) at the day of the fMRI assessment. Cannabis users were included if they fulfilled the DSM-IV criteria for cannabis dependence during the previous 18 months and agreed to abstain from cannabis in the 28 days before the assessment. At the time of enrollment, most users were still using cannabis or were in an early phase of abstinence. Cannabinoid metabolites remain in the body for up to 4 weeks after cessation (McGilveray, 2005) and withdrawal symptoms peak in the first week after last of use (Budney *et al*, 2003). Therefore, this time frame was selected to allow the assessment of lasting effects, in line with comparable MRI studies (Sneider *et al*, 2008). Abstinence was based on self-report and negative urine toxicology. Active cannabis users were included if they were willing to abstain for 28 days and currently abstinent users were asked to maintain abstinent for the 28 days prior to fMRI assessment. One user reported having used cannabis on one occasion 14 days before the experiment, but was included due to a negative urine toxicology. Control subjects were included if their cumulative lifetime cannabis use was below 10g. Exclusion criteria for all participants were: 1) any profound DSM-IV axis I or axis II disorder, e.g. psychotic or bipolar disorders, 2) Beck Depression Inventory score (BDI-II) $\geq$ 20 (maximum BDI in the final sample = 15, mean scores comparable for users and controls, $p > 0.05$), 3) medical disorder, 4) current/regular medication intake, and 5) MRI-contraindications. Attention, attitude toward interpersonal touch, social interaction anxiety, anxiety, mood and relationship status (y/n) were assessed as potential confounders (details **SI**). Experience with other licit and illicit drugs was documented. Given that the co-use of other illicit substances is common in cannabis users, users with > 75 lifetime occasions of other illicit drugs were excluded. Due to high co-occurrence of cannabis and tobacco use (Agrawal *et al*, 2012), groups were matched for the number of tobacco smokers and use patterns. As a trade-off between confounding effects of acute nicotine and nicotine craving on striatal reward processing, all smokers underwent 1.5h of supervised abstinence before the fMRI. Users were recruited in cooperation with the Department of Addiction and Psychotherapy of the LVR Clinics Bonn (Germany). Written informed consent was obtained from all participants. The study had full ethical approval by the University of Bonn and was registered as clinical trial (NCT02711371). Procedures were in accordance with the latest revision of the Declaration of Helsinki.

Interpersonal Touch Paradigm

An interpersonal touch fMRI paradigm with context-dependent reward variation was employed (Scheele *et al*, 2014; adapted from Gazzola *et al*, 2012). Before entering the scanner participants were introduced to a male and female experimenter that were the same throughout the study. The experiment consisted of two sessions (one male, one female), each with three conditions indicated by photographs depicting the experimenter: 'HOME', where the experimenter stands at 2 m distance, 'CLOSE', where the experimenter stands at the junction of the MRI table and opening, and 'TOUCH', where the experimenter administers repeated soft touch using downwards strokes to the shin of both legs (20 cm on the shin, velocity: 5 cm/s). This design allowed to vary rewarding properties and to assess two natural social reward dimensions ('TOUCH > CLOSE' as touch-associated reward, 'FEMALE > MALE' as context-dependent reward). To control for differences in physical properties of touch, only the male experimenter applied the soft strokes (details see **SI**). Following each 'CLOSE' and 'TOUCH' trial subjects rated the perceived pleasantness (1 (unhappy emoticon) 'very unpleasant' to 20 (happy emoticon) 'very pleasant', see also Scheele *et al*, 2014; Kreuder *et al*, 2017; based on the SAM non-verbal assessment for affective experience (Bradley and Lang, 1994)). All participants rated attractiveness and likeability of the experimenters on a scale from 0 (not likeable at all; not attractive at all) to 10 (very likeable; very attractive) after the experiment. Cannabis craving was assessed before and after fMRI (CCS-7; Schnell *et al*, 2011).

Behavioral Data Analysis

Data was analyzed in SPSS20 (SPSS Inc., Chicago, IL, USA). Demographic and questionnaire data were analyzed using independent t-tests (for non-normal distributed data corresponding non-parametric analyses were used) and results considered significant

at $p < .05$ (two-tailed). Median and range are reported for non-normal distributed data.

Pleasantness ratings were examined by mixed analysis of variance (ANOVA) with condition (touch vs close) and experimenter (male vs female) as within-subject factors and group (users vs controls) as between-subject factor. To more specifically address the hypothesized reduced reward dynamics in cannabis users an exploratory analysis focused on the comparison of the two conditions (female touch > male touch) that showed the strongest pleasantness increase in previous studies (Gazzola et al, 2012; Scheele et al, 2014). To this end between-group differences in the mean percent pleasantness increase between these conditions ([(pleasantness $rating_{FemaleTouch}$ − pleasantness $rating_{MaleTouch}$)/pleasantness $rating_{MaleTouch}$]*100) were compared using an independent t-test. Specifically, this targeted analysis allowed to address the strongest gain in reward value and therefore appears specifically sensitive to capture reduced reward dynamics. One cannabis user was excluded due to consistently rating male touch as very aversive (consistent $rating_{MaleTouch} = 1$) (details see SI), resulting in n = 22 cannabis users and n = 24 controls entering the final analyses.

fMRI Data Acquisition and Analysis

Data was acquired on a Siemens 3 Tesla system using established scanning and preprocessing procedures (**SI**). The first level model included four conditions: '$TOUCH_{Female}$', '$CLOSE_{Female}$', '$TOUCH_{Male}$', and '$CLOSE_{Male}$'. 'HOME' served as implicit baseline and motion parameters were included as additional regressors. Condition-specific regressors were convolved with the hemodynamic response function and estimated using a general linear model (GLM). In line with the pleasantness ratings, a mixed ANOVA including the within-subject factors touch *vs* close and male *vs* female, and the between-subject factor group (users *vs* controls) was performed. The ANOVA was implemented using a partitioned error-approach and first level contrasts assessing dynamic coding of touch-associated reward ('TOUCH > CLOSE'), context-dependent reward ('FEMALE > MALE'), and their interaction ('FEMALE $_{touch>close}$ > MALE $_{touch>close}$'). Groups were compared in SPM independent t-tests. Results were thresholded using a cluster-level FWE-correction of $p < .05$ (in line with recent recommendations an initial cluster-defining threshold of $p < .001$ was applied to data resampled at 3x3x3 mm^2, Slotnick, 2017).

Parameter estimates were extracted from significant clusters showing group differences (contrasts: 'FEMALE > MALE'; 'FEMALE > baseline', 'MALE > baseline'). Associations between use-based measures of dependence severity (cumulative lifetime amount [z-transformed]) and recovery (days since last use [z-transformed]), as well as measures of withdrawal (BDI-II, STAI and CCS-7) with behavioral and neural indices were examined using bivariate correlation ($p < .05$, two-tailed).

RESULTS

Group Characteristics

Groups were comparable in potential confounders, including alcohol/nicotine use (**Table 1**). Cannabis users reported comparable low craving before and after the experiment (scale 7-49; pre: 19.05 ±11.37; post: 18.68 ±10.72, $p = .67$, dependent t-test). **Table 2** shows cannabis use parameters. Examining mood scores using an ANOVA with the within-subject factor assessment time (pre- *vs* post-experiment) and the between subject factor group (users *vs* controls) did not reveal significant differences (all $p > .14$). Together, craving and mood data argue against confounding effects of acute cannabis withdrawal.

Perceived Attractiveness and Likability

Examination using repeated-measures ANOVAs including group (users *vs* controls) as between-subject factor and experimenter (male *vs* female) as within-subject factor revealed a main effect of experimenter for both, attractiveness ($F = 37.97$, $p < .001$) and likability ($F = 15.33$, $p < .001$), however no main or interaction effects with group (all $p > .12$), suggesting that the female experimenter was perceived as more attractive (female: 9.01 ±1.19; male: 5.05 ±1.95) and likable (female: 8.67 ±1.39; male: 7.68 ±1.21) across groups.

Behavioral Results

Examining the pleasantness ratings revealed a significant main effect of condition ($F_{(1,44)} = 11.61$, $p = .001$, $\eta^2 = .21$) and experimenter ($F_{(1,44)} = 4.84$, $p = .033$, $\eta^2 = .01$) as well as a significant interaction between these factors ($F_{(1,44)} = 32.40$, $p < .001$, $\eta^2 = .42$), however no effects involving the factor group reached significance (all $p > .17$, **Figure 1**). Across groups TOUCH (mean ±SD: 12.63 ±2.41) was rated as significantly more pleasant than CLOSE (11.41 ±2.74), and FEMALE presence (12.18 ±2.42) was rated as significantly more pleasant than MALE presence (11.87 ±2.22) (effect sizes comparable to Scheele *et al*, 2014). Post-hoc tests further revealed that female touch was rated as more pleasant than all other conditions (all $p < .001$). Comparing increased pleasantness experience for female relative to male touch revealed a significantly lower increase in cannabis users (mean % increase ± SD: 4.49 ±6.79)

relative to controls (10.79 ±12.27; $t_{(44)} = 2.13$, $p = .04$, Cohen's $d = .64$) (**Figure 2**).

fMRI Results

We initially replicated previous findings (Gazzola *et al*, 2012; Scheele *et al*, 2014). The application of soft touch ('TOUCH > CLOSE') elicited activity in a network encompassing primary somatosensory, striatal and insula regions in controls ($p < .05$; see **SI Figure S1, Table S1**) possibly reflecting the sensory and rewarding properties of pleasant soft touch. Cannabis users engaged a similar network (see **Figure S1, Table S1**). The contextual modulation of pleasant touch ('FEMALE $_{touch>close}$ > MALE $_{touch>close}$') in controls revealed significant interaction effects in the right somatosensory cortex (peak at MNI 30 / -37 / 37, $t_{(23)} = 5.54$, $k = 352$, $p < .001$), the right posterior insula (peak at 33 / -13 / 20, $t_{(23)} = 5.40$, $k = 72$, $p = .025$) and the left precentral gyrus (peak at -24 / -16 / 41, $t_{(23)} = 5.29$, $k = 223$, $p < .001$), which is in accordance with previous studies (Gazzola *et al*, 2012; Scheele *et al*, 2014) and meta-analyses (Morrison, 2016) on the involvement of these regions in affective modulation of touch. For cannabis users no significant interaction effects were observed.

Groups did not differ significantly in touch-related processing ('TOUCH > CLOSE') and its contextual modulation ('FEMALE $_{touch>close}$ > MALE $_{touch>close}$'). However, significant group differences in context-dependent reward variation related to the presence of the female or male experimenter ('FEMALE > MALE') revealed that cannabis users displayed altered activity in a cluster encompassing the right dorsal striatum (peak at 27 / 17 / -1, putamen, $t_{(44)} = 5.21$, $k = 87$, $p = .014$) (**Figure 3**). Extracted parameter estimates demonstrated that controls exhibited increased dorsal striatal activity during the presence of the female experimenter relative to the male experimenter ($t_{(23)} = 2.71$, $p = .01$, paired t-test), whereas cannabis users exhibited the opposite pattern ($t_{(21)} = -4.84$, $p < .001$, paired t-test). The striatal response dynamics mirrored the condition-specific pleasantness experience in the controls, but not in cannabis users (**Figure 3**).

Associations with Severity of Cannabis Use and Recovery with Abstinence

Measures of withdrawal showed no significant association with behavioral or neural indices (all $p > .05$). A higher cumulative lifetime use was significantly associated with a stronger decrease in dorsal striatal activity during the presence of the female experimenter relative to the male experimenter ('FEMALE > MALE') ($r = -.48$; $p = .024$, $R^2 = .23$) **(Figure 4),** suggesting an association between a higher cannabis exposure and stronger alterations. The duration of abstinence was not significantly associated with neural indices ($p > .24$) consistent with the notion that striatal alterations may be enduring rather than transient.

DISCUSSION

Conceptualizations of drug dependence emphasize the important role of exaggerated striatal responsivity to drug-related rewards and concomitantly blunted sensitivity to natural reinforcers in compulsive drug seeking (Koob, 2015; Volkow *et al*, 2012). To address whether processing of natural rewards is persistently disrupted in cannabis dependence, the present study examined behavioral and neural responses to social rewards and demonstrated social context-dependent alterations in abstinent cannabis dependent individuals. Specifically, upon female compared to male touch, cannabis users displayed a significantly attenuated increase of reward experience compared to healthy controls. Moreover, while control subjects responded to context-dependent reward variation during female as compared to male presence with an increased dorsal striatal activation, cannabis users displayed the opposite pattern. Examining condition-specific pleasantness ratings and striatal activity revealed a convergent pattern in the controls, whereas the pattern of striatal responses appeared to vary independent of pleasantness experience in users, possibly reflecting blunted striatal coding of reward. Alterations in dorsal striatal reward dynamics increased as a function of cannabis dependence severity. However, neural processing of pleasant touch did not differ between abstinent dependent cannabis users and controls.

The striatum codes both the anticipation and delivery of natural reward (Izuma *et al*, 2008), including the perception of opposite sex physical attractiveness (e.g. Hahn and Perrett, 2014), and show a high sensitivity to social information (King-Casas *et al*, 2005). Controls exhibited increasing dorsal striatal activity during the putative presence of the female experimenter and a marked increase in pleasantness experience when they believed the touch was applied by the female relative to the male experimenter. This pattern may reflect either direct natural reward processing associated with the higher perceived attractiveness of the female experimenter or an indirect modulation of the reward response via expectations of opposite sex interaction. Although attractiveness ratings did not differ between the groups, dependent cannabis users demonstrated the opposite dorsal striatal activation pattern and an attenuated increase in pleasantness experience reflecting blunted dynamic coding of context-dependent social reward processing.

The findings generally converge with previous reports on residual effects of chronic cannabis use on striatal processing of both, non-drug rewards (Nestor *et al*, 2010; Jager *et al*, 2013; Martz *et al*, 2016) as well as social context information (Gilman *et al*, 2016) and additionally extend the literature with regard to the following aspects.

First, in line with previous findings (Nestor *et al*, 2010; Martz *et al*, 2016), striatal reward processing deficits increased as a function of cannabis exposure indicating these maladaptations may be related to chronic use rather than be a predisposition for cannabis dependence. Furthermore, alterations were observed after prolonged abstinence and therefore may reflect lasting adaptations rather than residual effects of recent cannabis exposure. In the context of accumulating evidence on the relevance of intact striatal reward processing of non-drug rewards (for cannabis dependence see e.g. Yip *et al*, 2014) and social factors (Nikmanesh *et al*, 2015) for the long-term success of addiction treatment interventions, the present results appear particularly concerning.

Second, blunted dorsal striatal reward coding was specifically observed during context-dependent reward modulation whereas processing of touch remained intact. These findings argue against general natural reward processing deficits in cannabis users, and rather suggest that striatal processing may be impacted differentially depending on the type of natural reward stimulus, adding to previous reports that alterations across striatal subregions in cannabis users vary with social context (Gilman *et al*, 2016).

Third, there is ongoing controversy whether chronic cannabis use is associated with lasting striatal neuroadaptations as observed for other drugs of abuse (Curran *et al*, 2016). Initial findings suggest normal dopamine receptor availability in cannabis users (Urban *et al*, 2012), whereas more recent studies reported decreased striatal dopamine release capacity (van de Giessen *et al*, 2017). Moreover, the altered striatal dopaminergic response during early abstinence has been directly linked to anhedonia, and dependence severity (van de Giessen *et al*, 2017). Therefore, the present findings may be linked to dopaminergic striatal dysfunction, yet also argue for a more complex mechanisms.

Striatal dopaminergic neurotransmission is regulated by the endocannabinoid system (Silveira *et al*, 2016) and endocannabionoid-mediated adaptations in reward pathways have increasingly been associated with chronic drug dependence (Zlebnik and Cheer, 2016). Animal models suggest a direct association between endocannabinoid transmission in the striatum and hedonic experience of natural, sensory rewards (Mahler *et al*, 2007). Although homeostatic neuroadaptations in the endocannabinoid system rapidly recover with abstinence (Hirvonen *et al.,* 2012), the present findings may reflect sustained disruptions between subjective hedonic experience and striatal responses, or in the interaction of the endocannabinoid system with other transmitter systems. In the context of previous reports on the contribution of striatal dopamine and endocannabinoid neurotransmission to social reward (Parsons and Hurd, 2015), particularly social play/interaction (Manduca *et al*, 2016) and expectancy-related modulation of reward (Jubb and Bensing, 2013) the present findings may reflect disruptions in the interplay with the dopaminergic system.

Finally, the ventral striatum has been linked to anticipation of rewards (Schott *et al*, 2008) while the dorsal striatum encodes reward outcomes (Delgado *et al*, 2003). Previously, observations regarding reward processing alterations in cannabis users pertained to the ventral portion of the striatum (Nestor *et al*, 2010; Jager *et al*, 2013; Martz *et al*, 2016). However, these studies focused on anticipatory reward phases and non-dependent samples. A shift underlying the control of behavior from the ventral to dorsal part of the striatum has been postulated as a common denominator across substance addictions thought to reflect the transition from voluntary to compulsive behavior (Everitt and Robbins, 2013). As such, the current observation of altered dorsal striatal activation may reflect adaptations in neural mechanisms underlying cannabis dependence.

However, potential limitations should be considered. Abstinence was unsupervised and the cut-off of the immunoassays can only reliably detect cannabis use for a maximum of 15 days (Goodwin *et al*, 2008). Despite previous literature indicating high reliability of self-reported cannabis use (Martin *et al*, 1988), we therefore cannot entirely exclude sporadic cannabis use during the abstinence phase as small amounts below the cut-off would solely be detectable in quantitative analyses. To control for effects of tobacco the groups were matched with respect to tobacco use and underwent 1.5h of tobacco abstinence. However, confounding effects related to complex tobacco-cannabis interaction and differences in the time since last use cannot be completely ruled out. Cannabis-withdrawal associated sleep-disturbances may persist for up to 4 weeks, however, sleep disturbances have not been assessed in the present study.

Finally, findings are based on male users. Given the growing evidence for sex-differences in reward-processing in drug using populations future studies are needed to evaluate long-term effects of chronic cannabis use on social reward processing in females.

Taken together, cannabis dependence is associated with lasting adaptions in processing of social rewards. Striatal functioning may be affected differentially across different modalities of reward and future research may need to carefully evaluate different reward dimensions when addressing the striatal system in the context of drug dependence.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (NSFC, 91632117; 31530032), the German Research Foundation (DFG, grant: BE5465/2-1, HU1302/4-1), and an Open Research Fund of the State Key Laboratory of Cognitive Neuroscience and Learning.

FINANCIAL DISCLOSURES

All other authors report no biomedical financial interests or potential conflicts of interest.

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FIGURES and TABLES

Table 1. Group Characteristics and Drug Use Parameters.

[a]Mann-Whitney-U test, *Median(Range), ** Prescription medicinal use.

Measure	Cannabis Users M (SD)	Controls M (SD)	p
Age	23.86(3.36)	23.67(2.88)	.83
Years of education	15.00(11.00-22.00)*	14.50(12.00-19.00)*	.92[a]
d2 concentration performance	196.32(41.19)	201.75(52.95)	.70
STQ mean	1.15(0.80-1.85)*	1.20(0.55-2.40)	.86[a]
STAI state	33.95(8.08)	30.54(7.49)	.14
STAI trait	35.55(8.34)	32.46(6.92)	.18
SIAS	18.00(8.00-46.00)*	16.00(5.00-33.00)*	.32[a]
Relationship status (N) (y/n)	(13/9)	(12/12)	
Age of first nicotine use	14.62(1.93)	15.02(4.56)	.71
	N = 21	N = 22	
Years of nicotine use	9.25(1.00-18.00)*	7.00(2.00-17.00)*	.29[a]
Cigarettes per day	6.50(0-20.00)*	10.00(1-20.00)*	.24[a]
Age of first alcohol intake	14.00(11.00-16.00)*	14.00(8.00-16.00)*	.34[a]
Alcohol occasions per week	2.00(0-4.00)*	1.00(0-4.00)*	.18[a]
Alcohol units per week	6.00(0-46.00)*	4.90(0-18.00)*	.66[a]
Past ecstasy use	N = 13	N = 2	-
Lifetime occasions ecstasy	14.67(1-75)*	(1-8)*	
Past cocaine use	N = 10	N = 0	-
Lifetime occasions cocaine	5.98(1-70)*	-	
Past amphetamine use	N = 13	N = 1	-
Lifetime occasions amphetamine	20(1-75)*	6.00	
Past hallucinogen use	N = 10	N = 0	-
Lifetime amount hallucinogen	5.50(1-50)*	-	
Past opiate use	N = 3	N = 1	-
Lifetime occasions opiate	2.00(1.73)	30.00**	
Past Cannabis use	N = 22	N = 21	-
% Lifetime cannabis dependence	100%	0%	

Table 2. Cannabis Use Parameters (n = 22). *Median.

Cannabis Use Parameter	Mean ± SD (range)
Age of first cannabis use	15.14 ± 1.27 (13 - 17)
Days since last cannabis use	30.00* (14 - 500)
Frequency of cannabis use (days per month)	27.91 ± 4.68 (14 - 30)
Duration of regular cannabis use (months)	77.05 ± 36.56 (19 - 144)
Lifetime amount of cannabis in grams	1503.50* (62 - 5786)

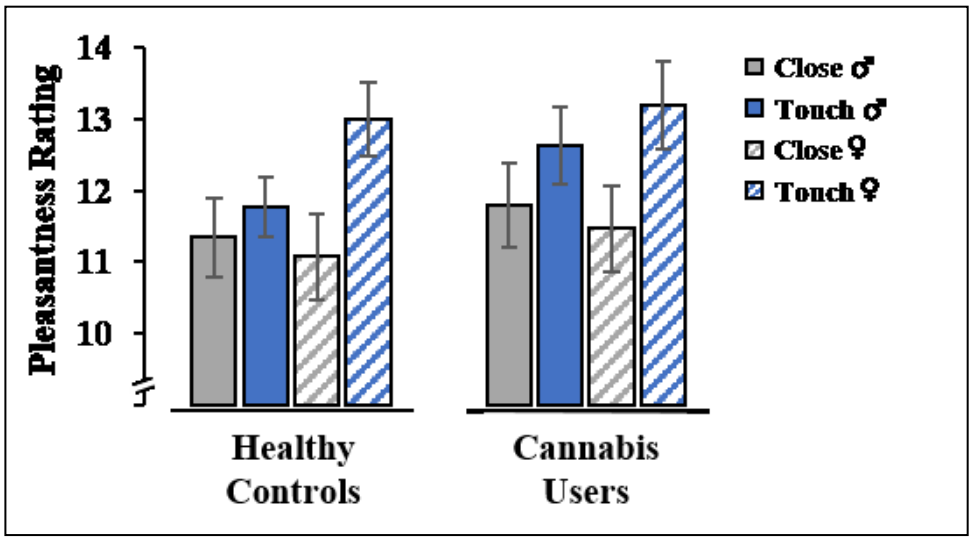

Figure 1. Pleasantness Ratings per Condition and Group (Healthy Controls n = 24, Cannabis Users n = 22). In both groups, pleasantness ratings are higher under TOUCH as compared to CLOSE and under FEMALE as compared to MALE. ♂: Male, ♀: Female. Error bars indicate SEM.

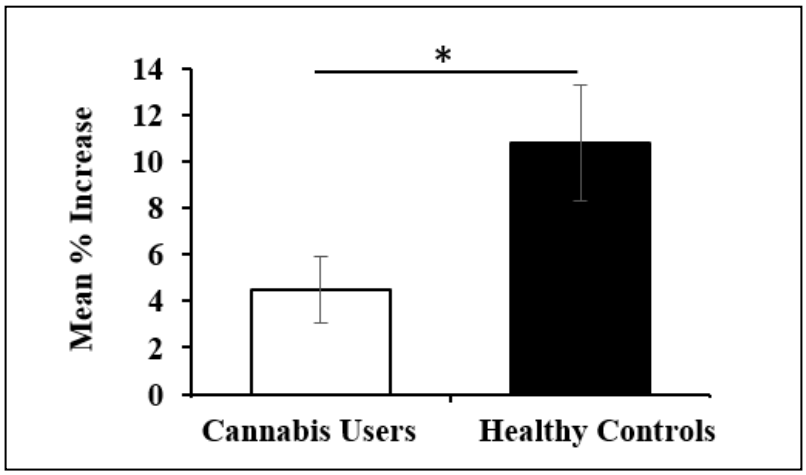

Figure 2. Group Differences in Mean % Increase of Pleasantness. Relative to controls, cannabis users show a significantly lower increase in pleasantness to female touch compared to male touch. Mean % increase = [(pleasantness rating$_{FemaleTouch}$ – pleasantness rating$_{MaleTouch}$)/pleasantness rating$_{MaleTouch}$]*100. Error bars indicate SEM. * $p < .05$.

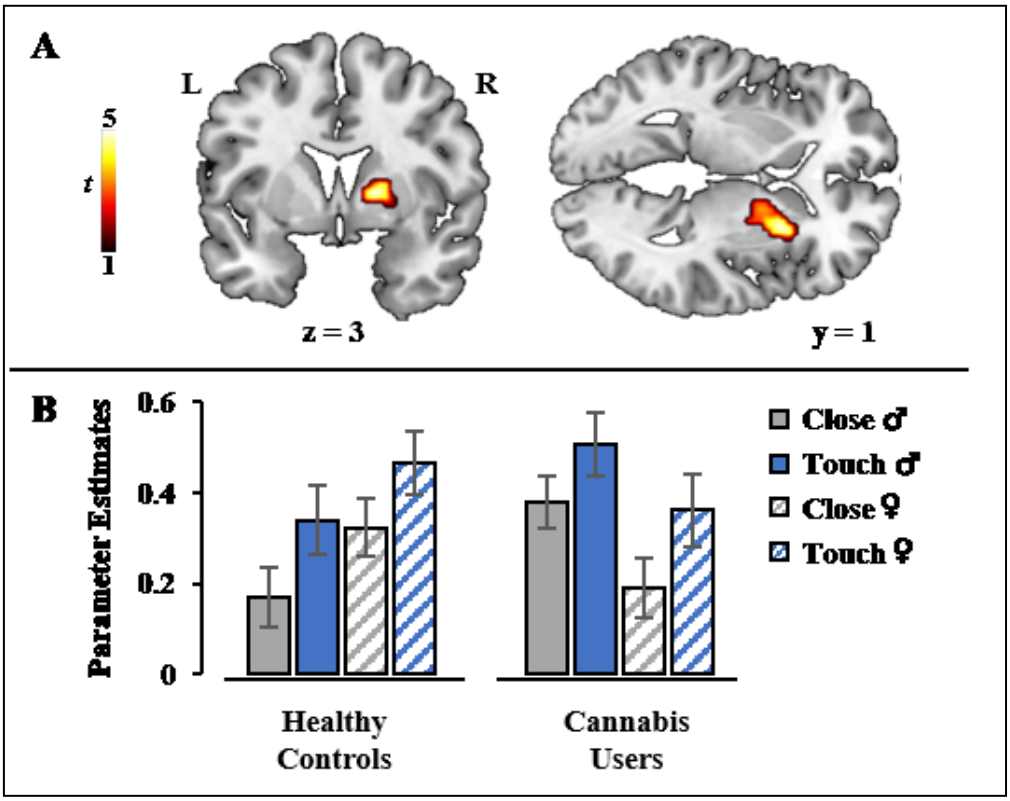

Figure 3. Striatal Response to Rewarding Female Interaction between groups.
A: Difference in striatal activation at MNI-coordinates x = 27 / y = 17 / z = -1 in contrast 'FEMALE > MALE' between cannabis users (n = 22) and controls (n = 24) displayed at $p_{FWE\text{-}corrected} < .05$, cluster level. **B:** Extracted parameter estimates from significant cluster from contrasts '$CLOSE_{Male}$ > Baseline', '$TOUCH_{Male}$ > Baseline', '$CLOSE_{Female}$ > Baseline' and '$TOUCH_{Female}$ > Baseline' per group. In controls, the striatal response increases significantly upon female interaction. In users, striatal activity decreases. Error bars indicate SEM.

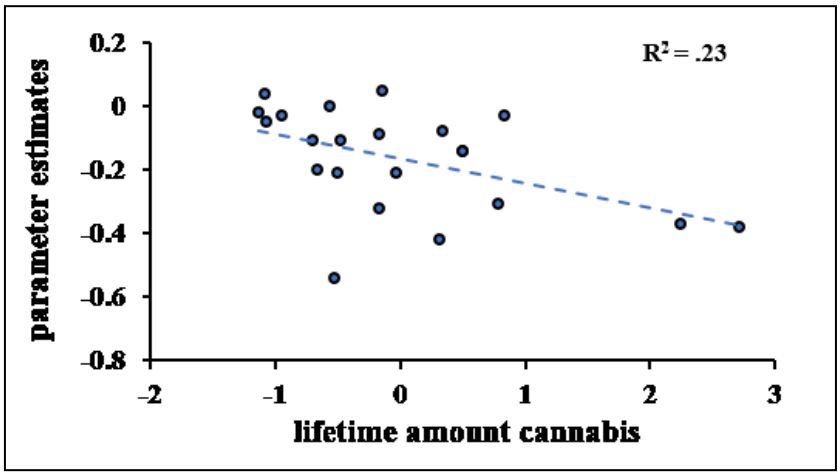

Figure 4. Hedonic Activity and Severity of Cannabis Use. Activation of the dorsal striatum upon 'FEMALE > MALE' associates inversely with the cumulative lifetime amount of cannabis use in gram. **(x)** z-transformed cumulative lifetime amount of cannabis use, **(y)** parameter estimates from significant cluster from contrast 'FEMALE > MALE', r = -.48, p = .024.

SUPPLEMENTARY INFORMATION

Participants and Study Protocols

Assessment of Potential Confounders

To control for potential confounding effects of depressive symptom load, attention, attitude towards interpersonal touch and social anxiety all subjects completed the Beck Depression Inventory (BDI-II) (Beck *et al*, 1996), the d2 test of attention (Brickenkamp and Zilmer, 1998), the social touch questionnaire (STQ) (Wilhelm *et al*, 2001), the social interaction anxiety scale (SIAS) (Mattick and Clarke, 1998) and the state-trait-anxiety inventory (STAI) (Spielberger, 1989). The positive and negative affect schedule (PANAS) (Crawford and Henry, 2004) was completed before and after the MRI session to control for differences in mood.

Sample Selection

Following a telephone-based assessment of general study eligibility n = 26 cannabis users and n = 24 male controls were invited for a detailed screening appointment. N = 3 users were excluded due to too high co-use of other illicit drugs in accordance with the exclusion criteria.

23 abstinent male subjects with cannabis dependence and 24 non-using male controls participated in the study. An initial quality check of the pleasantness ratings revealed one cannabis user consistently rated male touch with 1 – corresponding to 'very unpleasant'. A comparably negative perception of male touch was not observed in the other participants (minimum – maximum pleasantness ratings for male touch: cannabis users, 9.55-18.85; controls 8.65-17.6). The unusual negative reaction of this participant to male touch was further confirmed by an outlier analysis (z-value = -3.21, male ratings, within the group of cannabis users). Consequently data from this subject was excluded from all subsequent analyses. This exclusion resulted in a sample size of n = 22 cannabis users and n = 24 control subjects for the final behavioral and fMRI data analysis.

Group Characteristics

Cannabis users reported greater lifetime experiences with illicit drugs (**Table 1**) than controls. Cannabis, however, was the primary drug of abuse. Cannabis users had abstained from cannabis for a minimum of 28 days; one user reported having used marijuana on one occasion 14 days before the experiment, but was included in the analysis due to his negative urine drug screen on the day of the fMRI examination.

Groups were comparable in age, years of education, basal attention, attitude towards interpersonal touch, social anxiety measures, and nicotine and alcohol use (all $p > .05$, **Table 1**).

Interpersonal Touch Paradigm Parameters

Standardized tactile stimulation was facilitated through thorough training of the male experimenter prior to the onset of the study, and by signaling the duration of the stimulation to the experimenter via visual cues. The order of the 4 s 'CLOSE' and 'TOUCH' (20 trials each) conditions was randomized and interleaved with a 'HOME' period (4-6 s; mean jitter-time 5 s, 40 trials).

MRI Data Acquisition

MRI data were acquired on a Siemens 3.0 T TRIO scanner (Siemens, Erlangen, Germany) with a T2*-weighted echo-planar imaging (EPI) sequence (TR = 2500 ms, TE = 30 ms, FoV = 192 mm, flip angle = 90°, voxel size = 2.0 x 2.0 x 3.0 mm^3, matrix size = 64 x 64, slice thickness = 3.0 mm, 37 axial slices with no gap, 224 whole brain acquisitions oriented along the AC-PC axis for each the male and female session. A high-resolution anatomical reference image was acquired using a T1-weighted mprage sequence (TR = 1660 ms, TE = 2.54 ms, FoV = 256 mm, flip angle = 9°, voxel size = 0.8 x 0.8 x 0.8 mm^3, matrix size = 256 x 256, slice thickness = 0.8 mm, 208 sagittal slices).

fMRI Data Preprocessing and Analysis

MRI data was processed and analyzed with Statistical Parametric Mapping12 software (SPM 12, Wellcome Trust Centre for Neuroimaging, London, UK; http://www/fil.ion.ucl.ac.uk/spm) implemented in Matlab (The MathWorks Inc., Natick, MA). The first five volumes of each time-series were discarded to assure T1 equilibration. During realignment, affine transformation was applied to correct for head motion between volumes. In a two-pass procedure images were initially aligned to the first image of the time-series and subsequently realigned to the mean image. Normalization parameters were determined using the T1 image and the segmentation algorithm that combines image registration, tissue classification, and bias correction within the same generative model.

Next, normalization parameters were used to spatially normalize the functional time-series to the standard stereotaxic Montreal Neurological Institute (MNI) space template resampled at 3.0 x 3.0 x 3.0 mm^3. Normalized time-series were smoothed with an 8 mm FWHM Gaussian kernel. The 'HOME' condition served as implicit baseline in the first level analysis. To control for movement the head motion parameters were included in the first level matrix.

Evaluation of the Paradigm and Group-specific Activity in the Touch Network

Examination of the interpersonal touch network in healthy controls on the whole-brain level using the contrast 'TOUCH > CLOSE' revealed significant (cluster level FWE-correction, $p < .05$) activity in the bilateral somatosensory cortex, the bilateral insula, the bilateral dorsal striatum, the left anterior and middle cingulate cortex, and left middle temporal gyrus (**Figure S1, Table S1**) in the control subjects. Marijuana users showed activation in a similar functional network including the bilateral somatosensory cortex, the bilateral insula, the right dorsal striatum, the right anterior cingulate gyrus, the bilateral middle temporal gyrus, and the left precuneus **(Figure S1, Table S1)**.

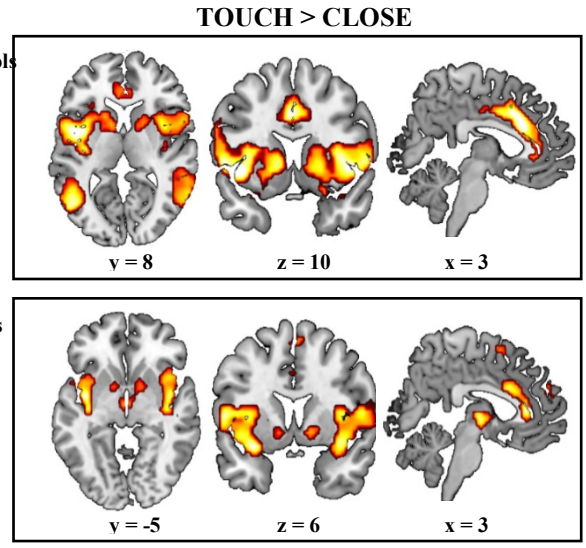

Figure S1. Whole-brain Random Effects Analysis for Contrast 'TOUCH>CLOSE' in Controls (n = 24) and Cannabis Users (n = 24). Cluster level FWE-corrected at $p < .05$, $k > 70$, MNI-coordinates: x / y / z.

Table S1. Whole-brain Random Effects Analysis for Contrast 'TOUCH>CLOSE' in Controls (n = 24) and Cannabis Users (n = 24). Cluster level FWE-corrected at $p < .05$, $k > 70$, MNI-coordinates: x / y / z.

x	y	z	t	k	Region
Control Subjects					
-54	-22	20	10.09	1671	Postcentral Gyrus L
-42	-22	20	8.41		Insula L
-39	-1	11	8.17		Insula L
-66	-22	26	7.71		Postcentral Gyrus L
-48	2	5	7.70		Rolandic Operculum L
-42	8	-4	7.07		Insula L
-12	5	-1	6.20		Pallidum L
-3	23	23	8.00	525	Anterior Cingulum L
-3	14	35	7.06		Middle Cingulum L
57	-19	20	7.43	810	Postcentral Gyrus R
57	-40	14	6.23		Superior Temporal Gyrus R
54	-34	26	5.88		Supramarginal Gyrus R
-48	-64	8	6.92	206	Middle Temporal Gyrus L
45	11	-1	6.29	786	Insula R
39	8	5	6.03		Insula R
15	5	-4	5.73		Pallidum R
Cannabis Users					

-63	-22	20	10.93	1274	Postcentral Gyrus L
-54	-34	23	10.46		Supramarginal Gyrus L
-42	-4	-4	7.77		Insula L
-57	-25	17	9.37		Postcentral Gyrus L
-42	2	11	7.23		Rolandic Operculum L
-39	-13	5	7.11		Insula L
-36	-16	-1	6.53		Insula L
-33	-19	17	6.38		Insula L
54	-28	23	9.46	591	Supramarginal Gyrus R
63	-40	17	7.96		Temporal Superior Gyrus R
60	-58	11	4.55		Middle Temporal Gyrus R
39	-4	-7	6.85	793	Insula R
39	-16	-7	6.68		Insula R
-12	-49	68	6.17	74	Precuneus L
3	32	8	6.14	193	Anterior Cingulum R
3	14	26	5.45		Anterior Cingulum R
-51	-64	11	5.99	72	Middle Temporal Gyrus L
12	5	-4	4.22	125	Pallidum R
21	-4	-10	3.69		Pallidum R

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Title: Altered orbitofrontal activity and dorsal striatal connectivity during emotion processing in dependent marijuana users after 28 days of abstinence.

Authors: Zimmermann K, Yao S, Heinz M, Zhou F, Dau W, Banger M, Weber B, Hurlemann R, Becker B

Own contribution to this work:

Zimmermann and Yao contributed equally to this manuscript. The original study was part of the DFG-project "Neuronale Aktivierungsmuster bei Cannabis- und Amphetaminabhängigen Individuen: Veränderungen nach 28 Tagen Abstinenz und Zusammenhang mit dem Rückfallrisiko" (BE5465/2-1, HU1302/4-1). I planned the implementation of the study in coordination with Dr. Benjamin Becker. I collected the data, and wrote and published the manuscript. Dr. Benjamin Becker supervised these processes and helped in optimizing the manuscript.

Publication status:

The presented manuscript has been published in PSYCHOPHARMACOLOGY on December 2nd 2017 (doi: 10.1007/s00213-017-4803-6).

Title: Emotion Regulation Deficits in Regular Marijuana Users

Authors:

Zimmermann K, Walz C, Derckx RT, Kendrick KM, Weber B, Dore B, Ochsner KN, Hurlemann R, Becker B

Own contribution to this work:

Zimmermann and Walz contributed equally to this work. The investigation was part of the DFG-project "Neuronale Aktivierungsmuster bei Cannabis- und Amphetamin-abhängigen Individuen: Veränderungen nach 28 Tagen Abstinenz und Zusammenhang mit dem Rückfallrisiko" (BE5465/2-1, HU1302/4-1). I planned the implementation of the study in coordination with Dr. Benjamin Becker and Christina Walz. I collected and analyzed the data, and wrote and published the manuscript in collaboration with Christina Walz. Dr. Benjamin Becker supervised these processes and helped in optimizing the manuscript.

Publication status:

The presented manuscript was published in Human Brain Mapping in August 2017 (38: 4270-4279, doi: 10.1002/hbm.23671).

Eidesstattliche Versicherung

Ich versichere an Eides Statt, dass die Dissertation von mir selbstständig und ohne unzulässige fremde Hilfe unter Beachtung der „Grundsätze zur Sicherung guter wissenschaftlicher Praxis an der Heinrich-Heine-Universität Düsseldorf“ erstellt worden ist.

30.01.2018

Kaeli Yvonne Zimmermann