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Brain alterations related to tobacco smoking: target points for prevention and intervention

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i. Keywords

Tobacco use disorder, addiction, cue-reactivity, reward processing, threat processing, brain morphology, grey matter volume, craving, cognitive control, down-regulation of craving, neuroimaging, fMRI, smoking cessation, prevention

ii. Abbreviations

ACC	anterior cingulate cortex
ADS-K	Allgemeine Depressionsskala (short version)
BOLD	blood-oxygen-level-dependent
dlPFC	dorsolateral prefrontal cortex
dmPFC	dorsomedial prefrontal cortex
DSM	diagnostic and statistical manual of mental disorders
fMRI	functional magnetic resonance imaging
fNIRS	functional near-infrared spectroscopy
FTND	Fagerström Test for nicotine dependence
GMV	grey matter volume
ITI	inter trial interval
MNI	Montreal Neurological Institute
MTG	middle temporal gyrus
NAcc	nucleus accumbens
NF	neurofeedback
NS	never-smoker
OFC	orbitofrontal gyrus
PD	panic disorder
ROI	region of interest
SMA	supplementary motor area
STAI	state-trait anxiety inventory
SUD	substance use disorder
TUD	tobacco use disorder
VBM	voxel-based morphometry
vlPFC	ventrolateral prefrontal cortex
VTA	ventral tegmental area

iii. Original publications

This dissertation is based on the following research articles:

Experiment I

Kunas, S.L., Stuke, H., Heinz, A., Ströhle, A., & Bermpohl, F. (2021). Evidence for a "hi-jacked" brain reward system but no desensitized threat system in quitting motivated smokers: An fMRI study. *Addiction*, 2021;1-12.

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Experiment II

Kunas, S.L., Hilbert, K., Yang, Y., Richter, J., Hamm, A., Wittmann, A., ... & Lueken, U. (2020). The modulating impact of cigarette smoking on brain structure in panic disorder: a voxel-based morphometry study. *Social cognitive and affective neuroscience*, *15*(8), 849-859.

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Experiment III

Kunas, S.L., Stuke, H., Plank, I.S., Laing, E.M., Bermpohl, F., & Ströhle, A. (submitted). Neurofunctional alterations of cognitive down-regulation of craving in quitting motivated smokers.

Experiment IV

Kunas, S.L., Bermpohl, F., Plank, I.S., Ströhle, A., & Stuke, H. (2021). Aversive drug cues reduce cigarette craving and increase prefrontal cortex activation during processing of cigarette cues in quitting motivated smokers. *Addiction Biology,* 2021;e13091.

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iv. Abstract

Addiction, as a chronic and relapsing condition, is defined as a disorder of the brain, characterized by the continuation of drug use despite the knowledge of its negative consequences. Several theoretical models propose a framework for the development and maintenance of addictive behaviors in general. These models address different processes of brain structure and function. First, incentive sensitization theory proposes a disruption of the brain reward network. According to this theory, reward processes are altered in form of a hypersensitivity to drug-associated rewards and a desensitization to non-drug-related rewards. Second, threat and punishment processing may be disrupted in the aversion related brain network in the case of an addictive disorder. Addicted individuals may present a desensitization to aversive drug-related cues as proposed by threat/ punishment desensitization theory. Third, dual systems theory proposes a "lack of control" to resist temptations to take drugs. This lack occurs in form of weaker prefrontal control network activations in response to drug-related cues. The assumptions made by these models are not contradictory, but rather address different aspects of drug-related processes in the brain. In tobacco use disorder (TUD), empirical evidence addressing particularly or simultaneously altered reward, threat/ punishment and cognitive control processes is lacking. As TUD represents one of the most common substance use disorders (SUDs) in general, a better understanding of the neurobiological underpinnings could help to inform prevention strategies and improve cessation rates.

The overall aim of this thesis is to investigate and integrate hypotheses on neural alterations in TUD. Therefore, alterations in reward, threat and cognitive control processes in TUD subjects compared to never-smokers (NS) and within the group of TUD subjects were investigated in form of: (1) altered reward processing in the brain reward network; (2) altered threat processing and neurostructural changes in the aversion related brain network; (3) alterations in cognitive control processes in the (prefrontal) control network and (4) the influence of aversive drug-related cues on appetitive cue-reactivity within TUD subjects.

For this purpose, four empirical studies were conducted. First, in accordance with incentive sensitization theory, alterations in the brain reward network in TUD subjects, compared to NS and alternative rewards were investigated (experiment I). Second, concerning threat/ punishment desensitization theory, structural alterations in the aversion related brain network were investigated in smokers and a potential alteration of brain structure related to smoking

behaviour was investigated in panic disorder (PD) patients (experiment II). A disruption of threat/ punishment processes in the aversion related brain network was further studied between TUD subjects and NS (experiment I). Third, regarding dual systems theory, aberrations in cognitive down-regulation of craving for appetitive stimuli in the (prefrontal) control network were assessed (experiment III). Fourth, the influence of aversive drug-related cues on the processing of appetitive drug-related cues in TUD subjects was part of the investigation (experiment IV). For experiment IV an interplay between the different brain networks within TUD subjects was examined.

The following key results were obtained: first, quitting motivated TUD subjects showed stronger activations in brain regions belonging to the reward network (e.g., thalamus, hippocampus, midbrain) compared to NS and alternative rewards. Brain alterations showed no significant association with smoking severity. Second, compared to non-smokers, smokers presented structural changes in brain regions belonging to the aversion related brain network (e.g., anterior cingulate cortex and insula) and these changes modulated morphological abnormalities commonly observed in PD patients. TUD subjects showed no desensitization of brain regions associated with the aversion related brain network compared with NS. Within the group of TUD subjects, activations in brain regions belonging to the aversion related brain network (e.g., anterior cingulate cortex and insula) were negatively related to the numbers of cigarettes smoked per day, suggesting that heavier smokers may present a desensitization of these brain regions during the processing of aversive drug-related cues. Third, TUD subjects presented alterations in cognitive down-regulation of craving in the middle temporal gyrus compared to NS on a neurofunctional level. Fourth, TUD subjects activated prefrontal control brain regions (e.g., dorsolateral prefrontal cortex) and exerted a down-regulation of brain regions belonging to reward areas (e.g., putamen, brain stem) through these control areas during the processing of appetitive drug-related cues, preceded by aversive drug-related cues.

Results gained by this thesis can establish an empirical basis for different neural alterations in TUD. Findings support an integrative model of TUD in form of alterations in the brain reward network that can be described as hypersensitivity to drug-related cues compared to alternative rewards and NS. Furthermore, aberrations in the aversion related brain network, in form of morphological changes compared to non-smokers, but no neurofunctional aberrations compared to NS can be assumed. General alterations in down-regulation of craving processes in TUD subjects, compared to NS, are suggested on a neurofunctional level. Moreover, TUD subjects can exert conscious cognitive control over craving (by applying cognitive

reappraisal) and implicit cognitive control over reward processing (when confronted with long-term consequences of consumption). Now it is of great importance to test and replicate assumptions of this integrative model in larger, independent samples. The results gained by this thesis offer new insights in neurobiological underpinnings of TUD and allow to derive clinical implications for prevention and intervention strategies.

v. Zusammenfassung

Abhängigkeitserkrankungen werden als chronische und wiederkehrende Erkrankungen des Gehirns beschrieben. Es existieren verschiedene theoretische Annahmen über die Entstehung und Aufrechterhaltung von Abhängigkeitserkrankungen, welche sich in unterschiedlichen Modellen zusammenfassen lassen. Diese Modelle basieren auf unterschiedlichen Prozessen des Gehirns sowie auf Annahmen über strukturelle Veränderungen in definierten Hirnregionen, welche durch den chronischen Konsum von Nikotin beeinflusst werden. Die „*incentive sensitization*“ Theorie postuliert dabei eine Veränderung des Belohnungssystems in der Form, dass an einer Abhängigkeit erkrankte Menschen hauptsächlich die Droge als Belohnung verarbeiten, nicht aber alternative Belohnungen wie z.B. gutes Essen. Zudem scheint die Verarbeitung von bedrohlichen und bestrafenden Reizen beeinträchtigt zu sein. Menschen mit einer Abhängigkeitserkrankung zeigen in bestimmten Hirnbereichen möglicherweise eine schwächere Rektion auf aversive drogenassoziierte Reize. Diese Annahme entspricht der „*threat/ punishment desensitization*“ Theorie. Zudem postuliert die sogenannte „*dual systems*“ Theorie, dass eine Abhängigkeitserkrankung sich durch eine Schwäche in kognitiven Kontrollfunktionen manifestiert. Hier nimmt bei der Konfrontation mit einem drogenassoziierten Reiz die kognitive Kontrolle ab und gleichzeitig steigt die motivationale Annährung an diesen. Diese beschriebenen Theorien widersprechen sich nicht, sondern adressieren vielmehr unterschiedliche parallele Prozesse. Für spezifische Abhängigkeitserkrankungen wie z.B. die Nikotinabhängigkeit (NA) fehlt bisher Evidenz dafür, welche spezifischen oder parallelen Veränderungen in Übereinstimmung mit den theoretischen Modellen beschreiben werden können. NA konstituiert nach wie vor eines der häufigsten Abhängigkeitsphänomene und ist für weitreichende Gesundheitsschäden der Bevölkerung verantwortlich. Ein besseres Verständnis neurobiologischer Grundlagen scheint unabdingbar, um Prävention und Abstinenz zu unterstützen.

Das Ziel der vorliegenden Arbeit ist es, bestimmte Hypothesen zu neuronalen Veränderungen bei NA Menschen zu untersuchen und miteinander zu integrieren. Um dies zu erreichen werden Veränderungen der Belohnungsverarbeitung, Bedrohungsverarbeitung und kognitiven Kontrollprozesse bei Menschen mit NA untersucht. Dabei sollen sowohl Prozesse innerhalb der NA-Gruppe als auch im Vergleich zu niemals-RaucherInnen unter folgenden Gesichtspunkten untersucht werden: (1) Veränderungen der Belohnungsverarbeitung im Belohnungszentrum; (2) funktionelle und strukturelle Unterschiede in Regionen der Bedrohungsverarbeitung; (3) Änderungen in kognitiven Kontrollprozessen im

Kontrollzentrum des Gehirns und (4) der Einfluss von aversiven drogenassoziierten Reizen auf die Verarbeitung von angenehmen drogenassoziierten Reizen.

Mit diesem Ziel wurden vier empirische Studien durchgeführt. Zunächst wurde in einem ersten Experiment (I) die Frage eines veränderten Belohnungszentrums bei Menschen mit NA im Vergleich zu niemals-RaucherInnen untersucht. In einem zweiten Experiment (II) wurden strukturelle Unterschiede in Gehirnarealen zwischen RaucherInnen und nicht-RaucherInnen beleuchtet sowie eine potentielle Modulation dieser strukturellen Veränderungen bei PatientInnen, die an einer Panikstörung (PS) leiden. Folgend wurde eine veränderte Bedrohungsverarbeitung in Bezug auf aversive drogenassoziierte Reize zwischen Menschen mit NA und niemals-RaucherInnen untersucht (Experiment I). Veränderte kognitive Kontrollprozesse wurden in einem weiteren Experiment (III) beleuchtet. Zusätzlich wurde der Einfluss von aversiven drogenassoziierten Reizen auf die Verarbeitung von angenehmen drogenassoziierten Reizen innerhalb der Gruppe der NA erforscht (Experiment IV).

Dabei konnten die folgenden Ergebnisse gefunden werden: Abstinenzmotivierte Menschen mit NA zeigen eine stärkere Aktivität in Regionen des Belohnungszentrums des Gehirns (z.B. im Thalamus und Hippocampus) während der Belohnungsverarbeitung von drogenassoziierten Reizen im Vergleich zu niemals-RaucherInnen und alternativen Belohnungsreizen. Diese Veränderungen zeigen keinen Zusammenhang mit der Schwere der NA. Im Vergleich zu nicht-RaucherInnen zeigen RaucherInnen strukturelle Veränderungen in Hirnregionen die mit der Bedrohungsverarbeitung zusammenhängen (z.B. im Inselkortex). Diese Veränderungen modulieren dabei morphologische Abweichungen in Hirnregionen die auch bei PatientInnen mit PS beobachtet werden können. Menschen mit NA zeigen keine funktionellen Unterschiede in Hirnregionen des Bedrohungssystems während der Verarbeitung von Bedrohungsreizen im Vergleich zu niemals-RaucherInnen. Innerhalb der Gruppe der NA zeigt sich jedoch eine Aktivierung von Hirnregionen des Bedrohungszentrums (z.B., anteriorer cingulärer Kortex), welche wiederum negativ mit der Stärke der NA korrelieren. Stärker abhängige und mehr rauchende Menschen zeigen demnach eine Abschwächung der Bedrohungsverarbeitung. Menschen mit NA zeigen zudem Veränderungen im temporalen Gyrus bei kognitiven Neubewertungsprozessen zur Regulation von Verlangen im Vergleich zu niemals-RaucherInnen. Innerhalb der Gruppe der Menschen mit NA zeigt sich eine veränderte Verarbeitung von drogenassoziierten Stimuli, wenn zuvor aversive drogenassoziierte Stimuli präsentiert wurden. Menschen mit NA zeigen verstärkte Aktivität in (präfrontalen) Kontrollarealen (z.B. im dorsolateralen präfrontalen Kortex) sowie eine

Herunterregulation von Aktivität in mesolimbischen Arealen (z.B. im Putamen) durch Kontrollareale.

Die Ergebnisse der Experimente I-IV bilden eine empirische Basis für verschiedene neuronale Veränderungen im Rahmen der NA. Ein integratives Modell der NA impliziert Evidenz für Veränderungen im Belohnungssystem. RaucherInnen zeigen demnach eine stärkere Sensitivität in Bezug auf drogenassoziierte Reize im Vergleich zu niemals-RaucherInnen und alternativen Belohnungsreizen. Hinzu kommen Veränderungen in der Bedrohungsverarbeitung. Es zeigen sich strukturelle Veränderungen im Bedrohungssystem jedoch keine funktionellen Unterschiede zwischen NA Menschen und nicht-RaucherInnen. Menschen mit NA zeigen zudem generelle Veränderungen auf einer neurofunktionellen Ebene beim Herunterregulieren von alternativen Belohnungsreizen im Vergleich zu niemals-RaucherInnen. Implizite Kontrollprozesse führen hingegen zu einer Veränderung von Belohnungsverarbeitungsprozessen, wenn Menschen mit NA zuvor mit den Langzeitkonsequenzen des Konsums konfrontiert werden. Die vorliegenden Ergebnisse ermöglichen neue Einblicke in neurobiologische Eigenschaften der NA und können somit die Entwicklung präventiver Ansätze und Entwöhnungstherapien unterstützen. Wichtig erscheint es jetzt, die Annahmen dieses integrativen Modells in einer unabhängigen Stichprobe zu replizieren.

CHAPTER 1

INTRODUCTION

Tobacco smoking presents the world´s leading cause of avoidable premature mortality (Samet, 2013), reflecting the potent toxicity of tobacco smoke. Although smoking in general has declined in many countries (Samet, 2013), smoking rates remain considerably high. Approximately 27% of the adult population in Germany are regular smokers (Schaller & Pötschke-Langer, 2016). Cigarette smoking has been clearly and unambiguously identified as a direct cause of cancers of the oral cavity, esophagus, stomach, pancreas and other body systems (Boyle, 1997). Health problems related to regular smoking lead to tremendous costs for society (e.g., health care costs) and individuals, including losses of productivity with broader implications for the smoking individual, their family and society (Effertz, 2016). Addiction from tobacco is defined in the diagnostic and statistical manual of mental disorders (DSM-5) as the presence of a set of certain criteria like drug tolerance, withdrawal, out of control use and/ or the continuation of the drug despite its negative consequences within the last month (American Psychiatric Association, 2013). Although most smokers in the German population self-report to be motivated to quit at some point (Thyrian et al., 2008), only few are successful in doing so. Several reasons have been identified that contribute to unsuccessful cessation attempts: strong urges to consume the drug (craving), the experience of withdrawal symptoms or a lack of available coping strategies for upcoming negative emotions are consistently reported in the literature as factors that attenuate quitting success (West et al., 1989; Brown et al., 2009). Those who are successful in quitting, however, have a high risk of restarting within the first few weeks of abstinence (Piasecki, 2006), which is called relapse. Relapse rates are especially high in unguided smoking cessation attempts, which represent the majority of all cessation attempts (Hughes et al., 2004). Available evidence shows that smoking cessation can be supported by counseling (Lancaster & Stead, 2017), cognitive behavioral therapy (CBT; Vinci, 2020), nicotine replacement (Wadgave & Nagesh, 2016) and bupropion (Richmond & Zwar, 2003). These approaches target different aspects of tobacco use disorder (TUD) and the withdrawal process. For instance, bupropion can mitigate withdrawal, while the application of cognitive strategies to regulate cravings or emotions are part of CBT interventions. Other approaches such as presenting pictorial cues on cigarette packets that show the long-term consequences of smoking behavior are used to prevent smoking initiation. Although these efforts were made in the past to reduce nicotine smoking, smoking initiation and relapse rates remain high (Samet, 2013). This highlights the need to better understand essential alterations related to tobacco smoking on different body systems

where addiction develops, e.g., the brain (Leshner, 1997). A clearer understanding of tobacco related changes in brain structure and function could pave the way to enhance already existing treatment strategies and prevention programs. It could further contribute to the development of new, alternative therapy approaches.

The goal of this thesis is to examine and integrate different neural alterations related to tobacco consumption. Using different neuroimaging techniques, this thesis investigates:

- alterations in reward processing;
- alterations in threat/ punishment processing;
- alterations in cognitive down-regulation of craving processes;
- the influence of aversive drug-related cues on the processing of appetitive drug-related cues;

in TUD subjects. These processes are related to brain structure and function in dedicated brain networks (related to reward, aversion, cognitive control) as proposed by results of previous empirical studies that support general theoretical models of addiction. The previously gained empirical evidence and general models build the theoretical framework of this thesis and will be used to derive hypotheses specific for TUD. The overall aim of this thesis is to investigate and integrate these hypotheses to establish an empirical basis for an integrative model in TUD. Results of this thesis might allow to derive strategies for smoking cessation interventions and prevention methods.

In the following, the different general models, that form the theoretical framework of this thesis will be introduced.

1.1 General overview of theoretical models of addiction

Various psychobiological models have been proposed to describe the development and maintenance of addictive behaviors in general (e.g., Koob & Volkow, 2010; Campbell, 2003; Robbins & Everitt, 1999; Robinson & Berridge, 1993). These models can roughly be categorized according to the different processes of addiction that are addressed by them, i.e., either as models that describe a disruption of reward processing in form of hypersensitivity to drug-associated rewards and desensitization to non-drug-related rewards (Robinson & Berridge, 1993), models proposing decreased sensitivity to punishment and the negative aspects of consumption (Campbell, 2003) and models that propose a “lack of control” to resist

temptations to take drugs (Mcclure & Bickel, 2014). These models are not contradictory but rather address different processes that may be disrupted in addictive disorders.

According to incentive sensitization theory (Robinson & Berridge, 1993; Heinz, 2002), addiction and craving develop as a consequence of neuroadaptations induced by repeated consumption of drugs. It is proposed that the mesocorticolimbic brain system, which is involved in the assignment of incentive salience to rewarding stimuli, gradually becomes sensitized to drug-related stimuli and desensitized to non-drug-related alternative rewards (Robbinson & Berridge, 2008).

In addition to sensitized reward processes in favor of drug stimuli, addiction is marked by persistent drug use despite knowledge or experience of its negative consequences (American Psychiatric Association, 2013). Different explanations may account for this phenomenon. According to Campbell (2003) addiction can be defined as a disease of faulty volition, that is caused by a cognitive impairment, resulting in harmful behavior. This cognitive impairment minimalizes or negates the recall of, or the access to aversive memories of the negative effects or consequences of previous addictive behavior and can vary in strength. Once started, the addictive behavior reinforces this impairment (Campbell, 2003). Further cognitive deficits implicated in addiction include reduced error processing and impaired behavioral correction of errors (Franken et al., 2007). These impairments in error-processing can be related to less effective learning from punishments and thus implicate a lower punishment sensitivity in addicted individuals. This learning impairment may cause or contribute to the persistence of addictive behavior (Luijten et al., 2014; Luijten et al., 2011). Accordingly, it can be assumed that addicted individuals present a desensitization towards the negative consequences of addictive behavior, which will be referred to as threat/ punishment desensitization theory in this thesis.

Besides disrupted reward and threat/ punishment processing, addiction has been described as a disorder of disturbed self-control over automatically triggered impulses to use the drug (Baler & Volkow, 2006). The dual systems model of addiction proposes an imbalance of an overactive, motivational “approach” system and a second, less well functioning control system (Bechara 2005; Gladwin et al. 2011; Wiers et al. 2007). Together, dysfunctional processing in these two systems may explain the conflict that characterizes addiction: automatic drug intake, even when the individuum expresses an explicit wish to quit.

The following sections of this thesis will refer to empirical studies supporting the aforementioned theoretical models. At the end of each paragraph empirical evidence, gained by previous research, and theoretical assumptions will be used to formulate hypothetical models concerning neural alterations in TUD. Furthermore, remaining open questions in TUD subjects will be emphasised. At the end of this chapter the hypothetical models will be summarized. Afterwards the hypothetical models will be used to formulate more specific hypotheses in TUD subjects (chapter 2) that will be investigated by the empirical studies conducted for this thesis.

1.2 Reward processing and the mesolimbic brain network

The incentive sensitization theory of addiction (Robinson & Berridge, 1993) proposes that repeated exposure to addictive drugs can change brain cells and circuits that normally regulate the attribution of incentive salience to stimuli. The nature of these 'neuroadaptions' is to render these brain circuits hypersensitive (sensitized), which results in pathological levels of incentive salience being attributed to drugs and drug-associated stimuli. Persistence of incentive sensitization leads to pathological incentive motivation (wanting) for drugs (Robinson & Berridge, 2008). Sensitized incentive salience can then be manifested in behavior via implicit (e.g., as unconscious wanting) or explicit (e.g., as conscious craving) processes, depending on individual circumstances. When a neutral stimulus predicts drug delivery, it becomes attractive and desirable, and its presence is sufficient to motivate compulsive drug seeking behaviors (Robinson & Berridge, 2001). Brain dopamine systems can be permanently sensitized by drugs (Robinson & Becker, 1986). Mesolimbic sensitization occurs in particular if the drugs are taken repeatedly and at high doses (Robinson & Becker, 1986). It renders the brain's 'wanting' systems hyperactive to drug cues and contexts, thus conferring more intense incentive salience to drug cues and contexts (Robinson & Berridge, 2008). Consequently, addicted individuals have stronger cue-triggered urges and intensely 'want' to take drugs. In contrast, the response towards rewards unrelated to the drug is proposed to be blunted (Robinson & Berridge, 1993). The reason for this shift is presumably that only the drug itself, or drug-related cues, cause a dopamine release that is sufficient enough to 1) signal reward and 2) induce motivation for obtaining it. As a result, the motivation to obtain rewards that are unrelated to the drug diminishes.

1.2.1 Neural drug cue-reactivity

With the application of neuroimaging techniques, it has become possible to investigate functional processes of the human brain to study reward processing. The non-invasive technique of functional Magnetic Resonance Imaging (fMRI), has led to an accumulation of neurobiological findings that support theoretical models and can be used for applications in therapy and prevention programs. Changes in the blood-oxygen-level-dependent (BOLD) signal represents an (indirect) measure of brain activity. As a standard paradigm to investigate drug addiction the so-called cue-reactivity paradigm is used systematically to evaluate the motivational responses to drug-related cues that may elicit drug-seeking and consumption (Carter & Tiffany, 1999). Concerning TUD, several studies have used fMRI to investigate the neurofunctional substrates of cigarette cue-reactivity by comparing the BOLD signal between drug-related (cigarettes) and neutral cue conditions (for reviews, see London et al., 2009; Engelmann et al., 2012). Structures involved in the reward pathway include the amygdala, ventral tegmental area (VTA), hippocampus, ventral pallidum, nucleus accumbens (NAcc), medial dorsal thalamus, and medial prefrontal cortex (Koob, 2010) and have consistently been identified in neuroimaging studies applying cue-reactivity in smokers (e.g., Engelmann et al., 2021). Beside these brain structures, meta-analyses identified greater activations in response to smoking cues compared with neutral cues within the extended visual system of the occipital cortex, the inferior temporal and posterior parietal lobes, and in the cingulate gyrus and prefrontal cortex (Engelmann et al., 2012; Goldstein & Volkow, 2002). These results support the assumption of abnormally increased motivational relevance of drug and drug-related cues in smokers consistent with the incentive sensitization theory (Robinson & Berridge, 2008). However, purely observing differences between drug-related and neutral stimuli (the two categories typically investigated in cue-reactivity paradigms) seems not sufficient to conclude aberrant or exaggerated brain responses to drug-related rewards in smokers (Versace et al., 2017). To reach this conclusion, a more appropriate control condition might be necessary, as e.g., non-drug-related alternative rewards capable of engaging brain motivational systems (Versace et al., 2017).

1.2.2 Altered reward processing in smokers

Stronger reactivity to drug-related cues compared to non-drug-related alternative rewards in mesolimbic brain areas allow to assess the extent to which TUD subjects show blunted reactivity to alterative rewards. This represents another key feature hypothesized in incentive

sensitization theory (Robinson & Berridge, 2008). Recent meta-analytic evidence is available suggesting significant decrease in activation of mesolimbic (e.g., caudate, striatum) brain areas in smokers compared with healthy controls during alternative reward processing (Bühler et al., 2010; Versace et al., 2017; Oliver et al., 2016; for a meta-analysis see Lin et al., 2020). As mentioned before, in substance-dependent individuals the incentive values of non-drug-related rewards are lost, leading to a lower activation e.g., in the striatum, during the processing of other natural rewards (Sweitzer et al., 2016). In accordance with evidence on reward processing gained by previous studies and incentive sensitization theory the reward system appears to be "hijacked" by the initiation of nicotine dependence, expressed as greater activation (↑) of the mesolimbic brain reward system in response to drug-related cues and lower activation (↓) of the reward system in response to non-drug-related, alternative rewards (Figure 1). However, whether there is an association of these alterations with the severity of smoking behavior remains to be tested. Some studies identified positive relations between mesolimbic brain activation during drug cue processing and smoking severity (Courtney et al., 2014; Smolka et al., 2006; McClernon et al., 2008), while others found a stronger mesolimbic activation in light or moderate smokers (Vollstädt-Klein et al., 2011; Hogarth et al., 2003) and still others report mixed results (Lin et al., 2020). According to incentive sensitization theory, however, a greater sensitization of mesolimbic brain structures would be expected in heavy smokers. Besides the neurofunctional level, altered reward processes in smokers could also manifest in subjective craving ratings, which should be higher for drugs compared to alternative rewards in TUD subjects.

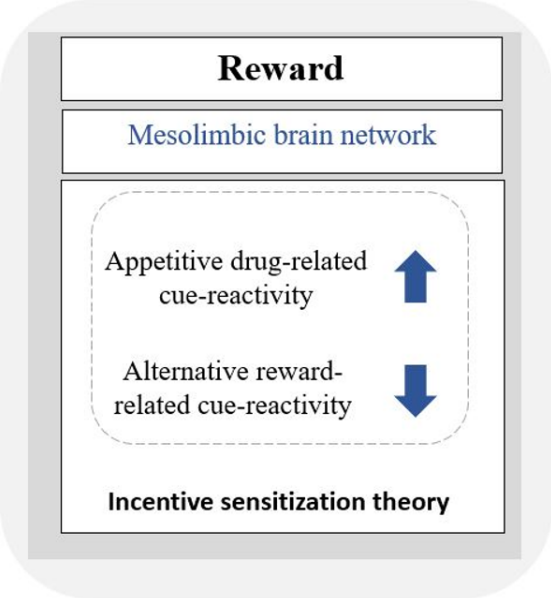

Figure 1: hypothetical model of proposed alterations in reward processing in TUD subjects. According to incentive sensitization theory, TUD subjects are expected to show an increased activation (↑) in the mesolimbic brain network to drug-related cues and decreased activation (↓) to alternative rewards as compared to never-smokers.

Currently, however, experimental investigations that could provide an empirical basis for the assumption of a "hijacked" brain reward system in TUD subjects according to incentive sensitization theory are lacking. Former studies typically compared drug-related or alternative reward cues directly to neutral cues or compared smokers with never-smokers (NS), while not investigating the function of smoking status (smokers versus NS) and type of reward (smoking related or not) together. This leaves the question if an interaction between both factors exists, which would present an empirical basis for a "hijacked" brain reward system in TUD. Moreover, mostly non-individualized monetary cues were used as alternative rewards (e.g., Gray et al., 2014; MacKillop et al., 2012; Wilson et al., 2014), thus limiting the external validity of previous studies. Additionally, it is unclear whether such changes are related to the severity of smoking behavior.

1.3 Threat and punishment processing and the aversion related brain network

An addictive disorder is defined by regular drug use despite the knowledge or even the experience of its negative consequences e.g., chronic cough in TUD subjects (American Psychiatric Association, 2013). Previous literature shows that addicted individuals present decreased punishment anticipation and sensitivity as well as diminished error processing

(Luijten et al., 2013; Luijten et al., 2010). That means that, a punishment is not perceived as aversive and therefore this punishment has no substantial influence on encoding and recall of a memory, resulting in low subsequent anticipation of the punishment (Duehlmeyer & Hester, 2019; Duehlmeyer et al., 2018; Luijten et al., 2011). According to threat/ punishment desensitization theory, an addictive disorder can be characterized by an altered processing of the negative consequences of the drug (e.g., health consequences). The processing of threat and punishment stimuli in general was investigated by Hayes & Northoff (2011) who identified a core aversion related brain network. This includes, beside other areas, the amygdala, anterior cingulate cortex (ACC), anterior insula, orbitofrontal cortex (OFC), hippocampus, parahippocampus and thalamus. This aversion related brain network seems to be common in humans and animals and its activity might largely be independent of sensory modalities of aversive cues (Hayes & Northoff, 2011). From a neurofunctional perspective, it can be assumed that this network is also involved in processing of punishment and aversive consequences of drug use and may thus be altered in subjects suffering from SUDs in general and in TUD subjects specifically. Potential alterations of the aversion related brain network associated with smoking behavior can be expected on a neurostructural and neurofunctional level.

1.3.1 Neurostructural alterations

Persistent smoking has been related to several structural brain changes, as demonstrated by previous cross-sectional studies (Brody et al., 2004; Gallinat et al., 2006; Zhang et al., 2011; Liao et al., 2012; Fritz et al., 2014; for a meta-analysis see Pan et al., 2013). More precisely, voxel-based morphometry (VBM) analyses found reduced gray matter volumes (GMV) in the ACC (Brody et al., 2004; Liao et al., 2012), the medial PFC (Brody et al., 2004; Gallinat et al., 2006; Liao et al., 2012; Fritz et al., 2014), the OFC (Kühn et al., 2010; Morales et al., 2012; Fritz et al., 2014) and in the left thalamus (Liao et al., 2012; Hanlon et al., 2016). Contradictory evidence is available for amygdala volume, where Durazzo et al. (2017) reported smaller GMV, whereas Shen et al. (2017) did not find any differences between smokers and non-smokers. Brain structures where abnormalities were identified in smokers, belong to the aversion related brain network. Thus, previous studies indicate structural abnormalities (in form of reduced GMV (↓)) in the aversion related brain network, related to smoking behavior (Figure 2). Such structural changes could possibly explain alterations in threat/ punishment processing or might be related to potential functional alterations in this network in smokers. Interestingly, similar brain regions were identified to be affected by other

mental disorders, such as anxiety disorders and specifically panic disorder (PD). Structural alterations in PD subjects have been reported for the amygdala, hippocampus, ACC, the brain stem (midbrain, pons), basal ganglia (caudate, putamen) and the thalamus (Massana et al., 2003; Uchida et al., 2008; Asami et al., 2009; Hayano et al., 2009; Del Casale et al., 2013; Dresler et al., 2013). It appears that brain structural abnormalities in the aversion related brain network in smokers substantially overlap with those associated with PD pathophysiology. As smoking behavior is overrepresented among various mental disorders (Lasser et al., 2000; Lê Cook et al., 2014; Johnson et al., 2000) and within PD, it may represent a potential modulator of structural brain changes. For areas of the aversion related brain network, which seems to be affected in both conditions (smoking and PD), the modulating impact of smoking in PD remains yet unresolved.

1.3.2 Neurofunctional correlates of threat and punishment processing

Cognitive failures described in addictive disorders are reduced error processing and impaired behavioral correction of errors (Franken et al., 2007). These implicate a lower punishment sensitivity and may contribute to the persistence of an addictive behavior (Campbell 2003; Luijten et al., 2013; Luijten et al., 2011). Previous studies examining error-related activity in response inhibition tasks identified error processing impairments in smokers compared to NS (De Ruiter et al., 2012). Errors, made by smokers were coupled with reduced brain activation in the superior frontal gyrus and superior temporal gyrus. Furthermore, they showed lower error-related activation in the dorsal ACC (dACC; De Ruiter et al., 2012). Lower sensitivity to punishment in smokers compared to NS could further be associated to a cluster, spanning the somatosensory cortex and motor cortex (Duehlmeyer et al., 2019). For most people, negative consequences (e.g., for their health) serve as punishments and lead to learning and acting differently in similar future situations (Wypych & Potenza, 2021). A specific, although passive, form of smoking related punishments are aversive drug-related cues, such as pictures showing the long-term consequences of smoking (e.g., on cigarette packets). According to previously gained evidence on punishment and error related processing and threat/ punishment desensitization theory, a decreased activation (↓) of the aversion related brain network to the negative consequences of smoking might be proposed in TUD subjects (Figure 2). Previous fMRI studies investigating aversive drug-related cue-reactivity within smokers found an involvement of the aversion related brain network, including the hippocampus, thalamus and amygdala (Dinh-Williams et al., 2014a; Dinh-Williams et al., 2014b; Chua et al., 2011). Alterations in drug cravings in relation to aversive drug-cue processing have been

sparsely investigated in previous studies. For instance, Do and Galván (2014) found a decrease in cigarette craving ratings during the presentation of graphic health warning labels within the scanner compared to pre-scan craving ratings.

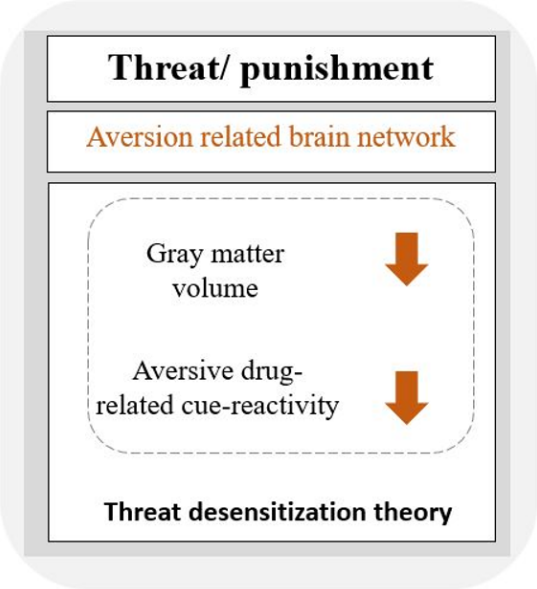

Figure 2: hypothetical model of proposed alterations in threat/ punishment processing in TUD subjects. In accordance to threat/ punishment desensitization theory, the aversion related brain network may be altered in different ways. Reduced GMV (↓) of structures belonging to the aversion related brain network can be assumed. Furthermore, a desensitization (↓) to aversive drug-related cues in TUD subjects (compared to NS) could present a brain alteration, related to smoking addiction.

However, these hypothetical assumptions are primarily based on results of altered error and punishment processing. Therefore, empirical evidence is needed to elaborate on differences in aversive drug-related cue processing between TUD subjects and NS to test these hypotheses. It remains unclear, whether TUD subjects indeed present an aberrant processing of aversive drug-related cues, which could constitute an important brain alteration and may be targeted by smoking cessation programs.

1.4 Cognitive control and the prefrontal control network

According to dual systems theory of addiction, an addictive disorder can be described in the form of disturbed self-control over automatically triggered impulses to use the drug (Baler and Volkow 2006). This might explain, beside other variables, why individuals continue with a problematic progression after an initial experimental use (Bühringer et al., 2008; Le Moal &

Koob, 2007). The imbalance of a hyperactive motivational system and an impaired control system (Bechara, 2005; Gladwin et al., 2011; Wiers et al., 2007) may further explain the conflict to continue drug taking although being motivated to quit. Cues that automatically trigger such cravings can be situational stimuli like objects associated with the drug but also pictures or emotions. Central to the compulsive nature of addiction is the gradual reinforcement of stimulus-driven implicit processes, which overwhelm the progressively weaker executive control system (Copersino, 2017). With improved capacity for cognitive control, attention naturally shifts to cognitive strategies that may be particularly effective at promoting self-control (McClure & Bickel, 2014). One set of such strategies falls in the category of emotion regulation (ER). These refer to various strategies that may be used to change how rewards are assessed to support behavioral goals. Such strategies are applied in everyday life e.g., by explicitly suppressing attention to distract from stimuli (Ochsner & Gross, 2008). One ER strategy that has been investigated with respect to self-control is called reappraisal (Hutcherson et al., 2012). Reappraisal is defined as explicitly changing the way a situation is perceived and evaluated by an individual. In the light of addiction, reappraisal may be thought of as a cognitive control strategy that would promote far-sighted behavior.

The prefrontal cortex is involved in decision making and inhibitory control (Royall et al., 2002). Previous investigations demonstrate that different self-control tasks e.g., motor inhibition, affective and craving regulation (for different stimulus types) involve a common prefrontal neural network (Boswell & Kober, 2016; Demos McDermott et al., 2019; Giuliani et al., 2013; Giuliani & Pfeifer, 2015; Siep et al., 2012). Cognitive neuroscience research suggests that successful self-regulation is dependent on top-down control from the prefrontal cortex over subcortical regions involved in reward and emotion (Heatherton & Wagner, 2011). In addicted individuals, imaging studies consistently found abnormalities in the prefrontal cortex (Goldstein & Volkow, 2002). A disruption of prefrontal cortex areas could lead to inadequate decisions that favor immediate rewards over delayed but more favorable responses and could account for the impaired control over the intake of the drug (Volkow et al., 2003). Thus, substance-dependent individuals who show impaired control over drug consumption could be affected by disruption in prefrontal neural functioning that is associated with regulation (Volkow et al., 2003).

Moreover, personality and addiction specific characteristics could be related to a disruption in cognitive control processes (Copersi et al., 2017; Dalley et al., 2011; Azizian et al., 2008). Impulsivity in particular is often associated with cognitive control failure (Copersi et al., 2017). Impulsive traits, which can be defined as the tendency to act prematurely without foresight,

have in fact been reported in association with most forms of drug taking and abuse (Dalley et al., 2011). Impulsivity is often considered as a product of impaired cognitive control. Moreover, the severity of addiction was linked to a lack of control in SUD (Dong et al., 2021; Azizian et al., 2008). However, specific SUDs like TUD have been sparsely investigated concerning impairments in cognitive control processes.

1.4.1 Cognitive control of craving in smokers

In TUD, neuroimaging studies demonstrated abnormal PFC functions (for a review see Sutherland et al., 2012). Such alterations in the PFC could possibly represent the neurobiological basis of cognitive control impairments in TUD, which would be in accordance to dual systems theory. However, only few neuroimaging studies are available investigating cognitive control in form of down-regulation of craving processes within smokers. They found that cognitive down-regulation of craving for drug-related stimuli (e.g., through reappraisal or suppression) leads to increased activity in (prefrontal) control circuits and decreased activity in craving-related mesolimbic regions (Brody et al., 2007; Hartwell et al., 2011; Kober et al., 2010; Zhao et al., 2012). Specifically, the prefrontal cortex (dorsolateral PFC & ventrolateral PFC), but also the angular gyrus, supplementary motor area (SMA), middle temporal gyrus (MTG) and ACC have been shown to be activated during exertion of cognitive down-regulation compared to passive viewing (e.g., Brody et al., 2007; Hartwell et al., 2011). Impulsivity could further be linked to higher levels of craving for cigarettes and more rapid relapses in smokers (Doran et al., 2004), while the severity of abuse could be related to impairments in fronto-striatal circuits and general cognitive processes within smokers (Azizian et al., 2008; Yuan et al., 2016). Concerning empirical evidence on down-regulation of craving in SUD in general as well as dual systems theory, alterations in cognitive down-regulation of craving processes in TUD subjects might be observed in form of weaker top-down control (↓) from the (prefrontal) control network over hyperresponsive subcortical brain regions (↑) involved in reward processing (Figure 3).

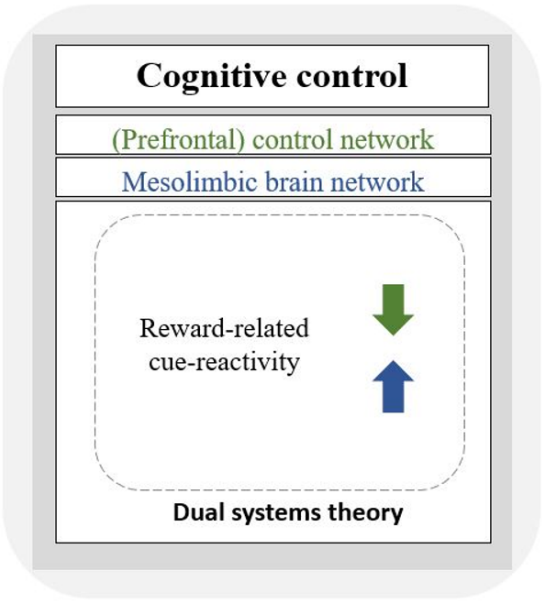

Figure 3: hypothetical model of proposed alterations in cognitive down-regulation of craving in TUD subjects. The (prefrontal) control network is supposed to be involved in different cognitive control strategies to regulate cravings (e.g., reappraisal). According to dual systems theory, (prefrontal) control processes might be impaired in TUD subjects in form of a dysregulation of (prefrontal) control activation (green arrow ↓) and an increased mesolimbic brain activation (blue arrow ↑) when reward-related cues are presented.

However, various questions remain unanswered by the previous literature. To the best knowledge of the author, no study is available investigating cognitive down-regulation of craving alterations in smokers compared to NS. This might be explained by the difficulty to investigate cognitive down-regulation of craving processes for cigarettes in non-smokers. However, this difficulty could be solved by using an alternative reward condition (e.g., food) that is efficient to induce craving in both groups. As studies comparing down-regulation of (alternative reward) craving between smokers and non-smokers are lacking, general (e.g., both drug-related and non-drug-related) down-regulation deficits in smokers remain unknown.

In addition to the question of general down-regulation deficits in smokers (between-subjects), it remains unclear whether the described changes in the down-regulation of coveted stimuli are specific for drug-related cues or represent a stimulus-independent change in smokers (within-subjects). This question might be of interest since it could be shown that smokers recruit similar brain circuits during regulation for different appetitive stimuli or emotional states, like for instance for cigarettes, food and negative affect (Tabibnia et al., 2014). Only one study directly investigated cognitive down-regulation of craving for drug specific cues

compared to alternative rewards and found a stronger dorsomedial PFC activation during reappraisal of craving for alternative reward compared to cigarette cues (Kober et al., 2010).

A possible association with trait impulsivity and smoking severity has not been investigated so far. Therefore, it is unclear if these characteristics, which were related to craving and relapse, show associations with cognitive control processes.

1.5 The influence of threat processing on appetitive drug cue-reactivity

The proposed alterations in reward, threat/ punishment and cognitive control processes in TUD subjects are based on empirical evidence of previous investigations and established theoretical frameworks. However, it is still unclear how aversive drug-related cues modulate the subsequent processing of appetitive drug-related cues and their related craving. Hence in contrast to the previous sections, where hypothetical models primarily propose differences between addicted and non-addicted individuals, this section focuses on changes within TUD subjects. Alterations of reward processing by preceding aversive drug-related cues will be elaborated. As outlined in the previous paragraphs of this thesis, neural correlates of resisting craving for tobacco have been linked to prefrontal cortex areas, associated with higher executive functioning and cognitive reward control (Brandl et al., 2019; Hartwell et al., 2011). These brain regions (e.g., PFC, ACC, anterior insula) possess a rich set of connections to cortical and subcortical areas that are key to emotional and reward processing as well as to craving. This connectivity is assumed to underlie craving regulation processes (Eippert et al., 2007; Wager et al., 2008). Specifically, Do and Galván (2014) showed negative functional connectivity patterns between prefrontal (dlPFC) and limbic (bilateral amygdala) brain regions in smokers while viewing graphic health warning labels. This was interpreted as improved regulatory control over emotionally responsive brain regions. A reduced activation of the mesolimbic brain network might additionally be expected during drug cue-reactivity preceded by aversive drug-related cues. Wang et al. (2015) found evidence for this assumption in non-treatment seeking smokers. Highly emotional graphic health warning labels strongly attenuated the electrophysiological response normally evoked by smoking cues in regular smokers. Furthermore, they found decreased cravings for cigarettes as response to drug-related cues preceded by emotionally aversive graphic health warning labels (Wang et al., 2015).

Based on the described prior findings of appetitive and aversive drug cue-reactivity as well as craving and its cognitive control, it might be suggested that aversive drug-related cues

influence the subsequent processing of rewarding drug stimuli through different ways. They might lead to: (i) a decreased activation of reward areas (e.g., ventral striatum, putamen) and perceived craving; (ii) an increased activation of control and self-regulation areas (PFC, ACC, anterior insula) and (iii) an increased down-regulation of reward areas by control areas (Figure 4). Consequently, the subjective craving for the drug itself might be reduced by preceding aversive drug-related cues.

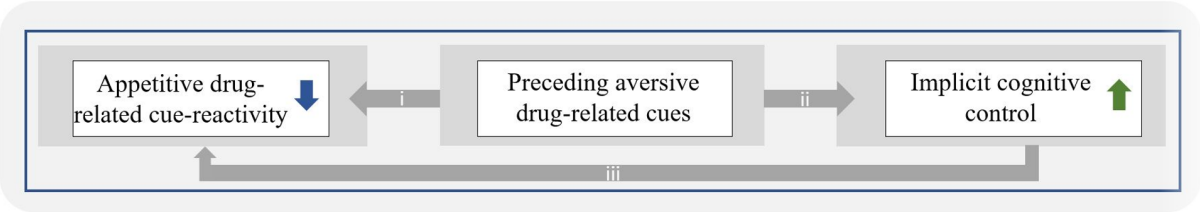

Figure 4: hypothetical model of the influence of aversive drug-related cues on the processing of appetitive drug-related cues within TUD subjects. The blue arrow describes a decrease of mesolimbic brain activation whereas the green arrow describes an increase in (prefrontal) control activation. Path (i) proposes a direct reduction of mesolimbic activation during the processing of appetitive drug-related cues; path (ii) proposes an activation of prefrontal control regions during drug cue-reactivity and path (iii) proposes a reduction of mesolimbic activation through inhibitory effects of prefrontal control areas.

Empirical evidence for this hypothesis is missing in TUD subjects. No study is available (to the best knowledge of the author) investigating an influence on appetitive drug cue-reactivity by aversive drug-related cues using fMRI in TUD.

1.6 Summarized hypothetical model of hypothesized alterations in TUD

In the previous sections, empirical findings in SUD and TUD subjects were presented, indicating that alterations in different processes (reward, threat, cognitive control) may be underlying SUD in general and TUD specifically. Based on these findings, potential alterations related to TUD (compared to non-addicted individuals) were hypothesized and specific hypothetical models were proposed within each section.

Additionally, a hypothesis was generated concerning an interplay of the processes within the group of TUD subjects. Based on previous empirical findings reviewed in the previous sections, Figure 5 presents a summarizing hypothetical model of hypothesized alterations in TUD. The summary of the different hypotheses focuses on within TUD subject alterations and between group comparisons of TUD subjects with non-addicted individuals.

Reward processing seems to be altered in TUD subjects in form of a sensitization to drug-related rewarding stimuli and a desensitization to alternative rewards in the mesolimbic brain network, compared to NS. This hypothesis is based on incentive sensitization theory (Robinson & Berridge, 1993). Investigations showing an interaction of smoking status (TUD subjects vs. NS) with reward type (alternative reward vs. drug rewards) are lacking. Therefore, empirical evidence for a "hijacked" brain reward system in TUD is missing and warrants investigation in experimental studies.

Threat and punishment processes might be altered in TUD subjects compared to non-addicted individuals. Aberrations might be linked to GMV changes in the aversion related brain network on a neurostructural level and to reduced activations during punishment processing on a neurofunctional level. These hypotheses were formulated in accordance with threat/ punishment desensitization theory (Campbell, 2003). However, this hypothetical model is primarily based on results of altered error and punishment processing. It remains unclear, whether TUD subjects indeed present an aberrant processing of aversive drug-related cues compared to NS. Studies are necessary investigating differences in brain structure and function of the aversion related brain network between TUD subjects and NS.

Cognitive control processes (in form of down-regulation of craving) might be weakened within TUD subjects and compared to non-addicted individuals. This might be reflected in impairments of the prefrontal control network and increased activation of mesolimbic brain areas, which is proposed by dual systems theory of addiction (McClure & Bickel, 2014). However, studies comparing down-regulation of craving deficits between TUD subjects and NS are absent. Furthermore, drug-specific impairments in cognitive down-regulation of craving processes in TUD subjects, compared to alternative rewards, are understudied. Therefore, investigations whether cognitive down-regulation of craving processes is altered in smokers and thus represent a general and/ or specific deficit, are warranted.

A within-subject hypothesis further suggests an influence of a prior presentation of aversive drug-related cues on the processing of appetitive drug-related cues within TUD subjects. It is anticipated that aversive drug-related cues apply direct and indirect influences on the reward and cognitive control network. Specifically, it is expected that preceding presentation of aversive drug-related cues: (i) exerts a direct inhibiting effect on mesolimbic brain activation while viewing appetitive drug-related cues and decreases the perceived craving for the drug; (ii) leads to a direct activation of (prefrontal) control areas through the application of implicit cognitive control strategies and (iii) exerts an inhibiting effect on the mesolimbic brain network through activation of the (prefrontal) control network and thereby indirectly weakens

reward processing through this route (Figure 5). Empirical evidence gained by previous studies suggest an influence of aversive drug-related cues on the processing of appetitive drug-related cues (Wang et al., 2015; Do & Galván, 2014). However, in TUD fMRI studies investigating such neural alterations are missing.

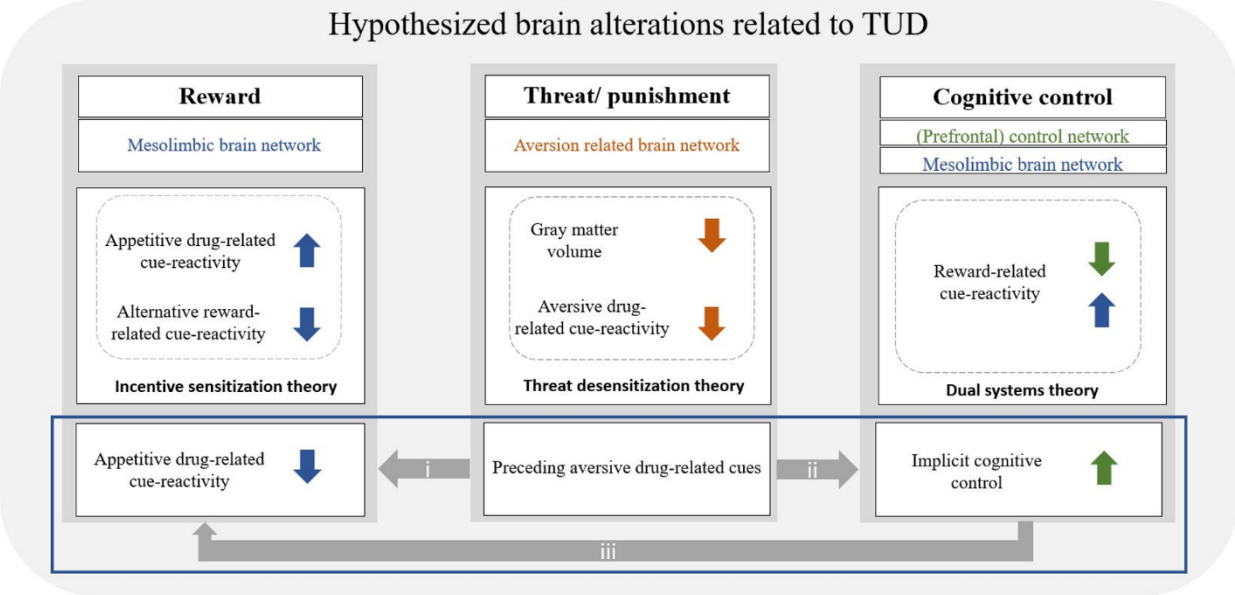

Figure 5: summary of hypothetical models of reward, threat and cognitive control processes in TUD. Arrows refer to increased or decreased brain activation or structure within the corresponding brain network (blue = mesolimbic brain network, orange = aversion related brain network, green = (prefrontal) control network). The top row of boxes labels the processes that are being addressed, respectively. The second row of boxes refer to the brain network involved in this process. The third row of boxes addresses the alterations related to TUD according to the three models outlined in the introduction of the thesis. These are the incentive sensitization theory for reward processing (left), the threat/ punishment desensitization theory for threat and punishment processing (middle) and dual systems theory for cognitive control processes (right). The blue frame around the last row of boxes represents an interplay of the three different processes in form of an influence of preceding aversive drug-related cues on drug cue-reactivity within the group of TUD subjects. The letters correspond to the three routes described in section (1.5).

1.7 Aims of the thesis

The overall aim of this thesis is to provide empirical evidence for specific hypotheses in TUD subjects. This aim will be realized by investigating and integrating alterations in reward, threat and cognitive control processes in TUD subjects compared to NS and within the group of TUD subjects in form of:

(1) altered reward processing in the mesolimbic brain network;
(2) altered threat processing and neurostructural changes in the aversion related brain network;
(3) alterations in cognitive control processes in the (prefrontal) control network;
(4) an influence of aversive drug-related cues on appetitive cue-reactivity.

In the next chapter, research questions will be formulated in line with unanswered questions mentioned after each paragraph. Specific hypotheses will be outlined after each research question consistent with the hypothetical models introduced previously. Chapter 3 will summarize the methodology of the four empirical studies that were conducted to investigate these hypotheses. The empirical studies are described afterwards in chapter 4. Chapter 5 discusses the main findings of the studies in light of an integrative reward, threat and cognitive control processing model of TUD. Clinical implications for prevention strategies and cessation interventions will be highlighted before study limitations and future research directions will be outlined.

CHAPTER 2

RESEARCH QUESTIONS AND HYPOTHESES

This chapter will introduce the general research questions of this thesis and the appropriate hypotheses. Hypotheses were derived from results of previous empirical studies and theoretical assumptions on reward, threat and cognitive control processes in TUD. The four experiments that form the main body of this thesis (Kunas et al., 2021; Kunas et al., 2020; Kunas et al., under review; Kunas et al., 2021) aimed to answer the general research questions by investigating the appropriate hypotheses.

2.1 Research questions

The following research questions were investigated in this thesis:

First question: Do quitting motivated TUD subjects show a "hijacked" brain reward system?

Experiment I

Second question: Do smokers present neurostructural alterations of the aversion related brain network and do these smoking-related alterations modulate structural brain changes reported in panic disorder?

Experiment II

Third question: Do quitting motivated TUD subjects show a desensitization towards aversive drug-related cues in the aversion related brain network?

Experiment I

Fourth question: Do quitting motivated TUD subjects show alterations in the application of cognitive down-regulation of craving for appetitive stimuli in the (prefrontal) control network?

Experiment III

Fifth question: Does the processing of aversive drug-related cues have an influence on the subsequent processing of appetitive drug-related cues within TUD subjects?

Experiment IV

2.2 Hypotheses

First question: Do quitting motivated TUD subjects show a "hijacked" brain reward system?

Experiment I

As described in the previous chapter, there is empirical evidence for an altered processing in favour of drug-related cues compared to alternative rewards in the mesolimbic brain system in TUD subjects (Lin et al., 2020). However, as the interaction effect between smoking status and reward cue type has not been investigated so far, several aspects of how quitting motivated TUD subjects and NS differ in terms of reward processing remain unknown.

Following incentive sensitization theory and results on drug and alternative reward cue-reactivity in smokers (see paragraph 1.2 and Figure 1), an increased activation elicited by drug-related cues in mesolimbic brain areas in quitting motivated TUD subjects compared to NS and alternative rewards is hypothesized.

Results on the association between smoking severity and reward processing in TUD subjects are inconclusive so far. Some studies suggest a negative or no association between smoking severity and drug cue-reactivity, while others propose a positive relationship (see paragraph 1.2.2). In accordance with incentive sensitization theory, a positive association is hypothesized between smoking severity and greater activations in mesolimbic brain areas during altered reward processing.

Second question: Do smokers present neurostructural alterations of the aversion related brain network and do these smoking-related alterations modulate structural brain changes reported in panic disorder?

Experiment II

Persistent smoking could be associated with structural brain changes (Pan et al., 2013). These GMV changes in smokers are also found in regions of the aversion related brain network, e.g., in the ACC (Pan et al., 2013). As outlined in paragraph 1.3.1 structural brain changes of the

aversion related brain network were also identified in subjects suffering from PD. Smoking is highly overrepresented in patients with PD, but the modulating impact of smoking on structural brain alterations in PD remains to be investigated.

According to threat desensitization theory, it was first hypothesized that smokers present morphological alterations in the aversion related brain network, compared with non-smokers.

Secondly, it was hypothesized that GMV reductions in fronto-limbic circuits, which are frequently observed as a feature of PD pathophysiology, may be partly driven by differential rates in smoking behavior.

Third question: Do quitting motivated TUD subjects show a desensitization towards aversive drug-related cues in the aversion related brain network?

Experiment I

Threat/ punishment desensitization theory proposes that smokers are not that sensitive to the negative aspects of smoking (Figure 2). TUD can be defined as continuous drug use despite the knowledge of its negative consequences (American Psychiatric Association, 2013). Previous work showed that smokers activate an aversion related brain network, when confronted with the negative aspects of consumption (Dinh-Williams et al., 2014 a,b). As summarized in paragraph 1.3.2, it remains to be tested if TUD subjects present a desensitization towards aversive drug-related cues in comparison to NS. This could be expected based on results on lower punishment sensitivity and reduced error processing in smokers.

Specifically, it is hypothesized that compared to quitting motivated TUD subjects, NS show stronger activations in the aversion related brain network, which is characteristic for threat processing, in response to aversive drug-related cues (e.g., lung cancer).

Further, associations between alterations of the aversion related brain network and smoking severity remain to be tested (paragraph 1.3.2). It is hypothesized that heavier and more dependent TUD subjects show stronger reductions of activation in the aversion related brain network to aversive drug-related cues. Furthermore, it is expected that these brain responses will be negatively correlated to cigarette craving reduction elicited by threat processing.

Fourth question: Do quitting motivated TUD subjects show alterations in the application of cognitive down-regulation of craving for appetitive stimuli in the (prefrontal) control network?

Experiment III

According to dual systems theory of addiction, it is proposed that down-regulation of craving over automatically triggered impulses is disrupted in addicted individuals in general (Baler and Volkow, 2006) and in TUD subjects specifically. Furthermore, specific clinical and psychological characteristics such as severity of addiction or trait impulsivity could be related to cognitive down-regulation deficits (Dalley et al., 2011). As described in chapter 1, the prefrontal cortex, especially the dlPFC and vlPFC seem to play a crucial role in down-regulation of appetitive states (Brandl et al., 2020). One previous study found evidence for a difference in prefrontal cortex activation during reappraisal for drug-related cues vs. alternative rewards (food) within the dmPFC (Kober et al., 2010). However, the investigation of altered cognitive down-regulation of craving remains largely understudied in TUD, as to the knowledge of the author no neuroimaging study yet has compared smokers directly to NS.

By studying the behavioral and neural correlates of down-regulation of craving in TUD subjects as a function of smoking status (compared to NS) and reward cue-type (drug cues vs. alternative rewards), alterations in TUD subjects are hypothesized in various ways.

On a behavioral level, it is hypothesized that TUD subjects show lower reductions in ratings after down-regulation of craving for alternative rewards, compared to NS (between-subjects) which would reflect a general (e.g., both drug-related and non-drug-related) deficit in cognitive down-regulation of craving in TUD. In contrast to drug-related cues, it is expected that TUD subjects present higher reductions in craving ratings for alternative rewards (within-subjects) which would reflect an additional drug-specific deficit in cognitive down-regulation of craving in TUD.

On a neurofunctional level, decreased activations in the (prefrontal) control network and decreased deactivation of mesolimbic brain regions in TUD subjects, compared to NS, during down-regulation for alternative rewards (food) are hypothesized (between-subjects), reflecting a general deficit in down-regulation in TUD. Within TUD subjects, stronger (prefrontal) activations and mesolimbic deactivations are expected during down-regulation for alternative

rewards compared to drug-related cues (within subjects), indicating an additional drug-specific impairment.

Furthermore, an association between behavioral and neurofunctional correlates of down-regulation of craving with smoking severity and trait impulsivity is hypothesized (within subjects).

Fifth question: Does the processing of aversive drug-related cues has an influence on the subsequent processing of appetitive drug-related cues in quitting motivated TUD subjects?

Experiment IV

Resisting tobacco craving has been linked to prefrontal cortex activation and deactivation of mesolimbic reward areas (see paragraph 1.4.1). Furthermore, prefrontal control areas show connections to cortical and subcortical areas that are key to emotion and reward processing (see paragraph 1.5.). Do et al (2014) found a negative functional connectivity between prefrontal (dlPFC) and limbic (amygdala) brain regions during the processing of graphic health warning labels in smokers. This was interpreted as an improved regulatory control over emotionally responsive brain regions. The influence of aversive drug-related cues on the processing of appetitive drug-related cues has not been investigated using fMRI. According to the hypothetical model presented in Figure 4, aversive drug-related cues are expected to exert direct and indirect effects on the (prefrontal) control network and the mesolimbic reward network during drug cue-exposure.

On a behavioral level, a reduction of cigarette cue induced craving in TUD subjects by prior presentation of aversive drug-related cues, reflected in subjective craving ratings is hypothesized.

On a neurofunctional level, reduced activation of the mesolimbic brain network (e.g., ventral striatum, putamen) during the processing of appetitive drug-related cues after presentation of aversive drug-related cues is expected. On the other hand, a greater activation in craving-regulating control areas (e.g., PFC, ACC, anterior insula) is hypothesized.

Finally, examining functional connectivity patterns, a negative functional connectivity between control (e.g., dlPFC) and reward (e.g., putamen) related brain areas is assumed.

CHAPTER 3
MATERIALS AND METHODS

In this chapter, the general methodology of the four empirical studies that constitute the main body of this thesis (Kunas et al., 2021; Kunas et al., 2020; Kunas et al., under review; Kunas et al., 2021) is briefly described.

First, the samples included in the experiments will be defined, then the two fMRI tasks will be introduced in more detail including the timings, pictures and craving rating scales. Thereafter, details of fMRI parameters are provided as well as a brief overview of the analysis pathway of the different studies.

3.1 Participants

Experiments I, III and IV were conducted within the framework of the German Collaborative Research Center (TRR 265: Losing and regaining control over drug intake), funded by the German research foundation (Heinz et al., 2020). The included sample represents a sub-sample of a larger longitudinal study on smoking cessation. The longitudinal study design was preregistered at ClinicalTrails.gov (*NCT04251936*). Participants were recruited in the Berlin metropolitan area through advertising and flyers. In total, 82 participants (39 TUD subjects and 43 NS) underwent fMRI scanning. Due to technical issues and results of the quality control a few participants had to be excluded from particular experiments which is described in the methods section of each study. Inclusion criteria for TUD subjects were a current DSM-5-TR diagnosis of TUD proven by a structured clinical interview for DSM-5-TR (First et al., 2016) and an age between 18 and 65 years. Exclusion criteria were a comorbid DSM-5-TR mental disorder (other than TUD) within the last 12 months; a lifetime history of any substance-use disorder other than TUD, bipolar or psychotic disorders; current suicidal intent; concurrent psychopharmacological or psychotherapeutic/ psychiatric treatment; a history of brain injury or pregnancy. Participants were classified as NS if they smoked less than 10 cigarettes during their lifetime. The NS group was free of current or past medical, neurological or mental illness. NS as well as TUD subjects received a financial compensation of 50 euros for their participation in the study. After the examination all TUD subjects took part in a free, 6-week smoking cessation intervention, as all of them were highly quitting motivated**.** Furthermore, half of the participants were randomized to an additional sport intervention. The study was approved by the local ethics committee and all subjects gave

written, informed consent prior to participating in the study. *A priori* sample size and power analyses for the cross-sectional design used G-Power (Faul et al., 2007). In order to detect an effect of partial eta squared = .06 with 80% power in a two-way between-subjects ANOVA with repeated measures (two groups, alpha = .05), G-Power suggested a sample size of 85 participants. For a within-subjects repeated measures ANOVA (using the same parameters) a sample size of 35 participants was suggested. In order to detect an effect size of Cohen´s *d* = 0.5 with 80% power (alpha = .05), G-Power suggested a sample size of 42 subjects per group for an independent sample t-test and of 28 participants for a paired samples t-test.

Experiment II was part of the German national research network PANIC-NET (second funding period; for more information see also Kircher et al., 2013). MRI measurements were conducted in Marburg, Berlin, Dresden, Greifswald and Muenster. Inclusion criteria for the subjects were: a current DSM-IV-TR primary diagnosis of PD (American Psychiatric Association, 2000) evidenced by the Composite International Diagnostic Interview (CAPI-WHO-CIDI; DIAX-CIDI) and validated by clinical experts; a score ≥ 3 on the Clinical Global Impressions Scale and an age between 18 and 65 years. Exclusion criteria were: a comorbid DSM-IV-TR psychotic or bipolar I disorder; current alcohol dependence/current abuse or dependence on benzodiazepine and other psychoactive substances; current suicidal intent; borderline personality disorder; concurrent ongoing psychopharmacological treatment. The healthy control group was free of current or past medical, neurological or mental illness as evidenced by a clinical interview. Smoking status was assessed on a categorical level (yes/no) by self-report and compared with 12-month DSM-IV-TR diagnosis of TUD in the PD group, as assessed in the clinical interview. The study was approved by all ethic committees of the participating centers and all subjects gave written, informed consent prior to participating in the study.

3.2 fMRI paradigms

3.2.1 Extended cue-reactivity paradigm

A novel extended cue-reactivity paradigm was established for experiments I and IV, which was performed during fMRI. The task was designed to study drug-related positive (appetitive drug-related cues), drug-related negative (aversive drug-related cues) and alternative reward cue-reactivity at the psychological and neurofunctional level. The experimental paradigm contains four conditions: established photographs showing cigarette items (appetitive drug-

related cues), pictures of healthy, low-fat, attractive food (alternative reward cues), pictures showing long term consequences of smoking (e.g., bronchial carcinoma, aversive drug-related cues) and pictures displaying neutrally valenced items (neutral control condition). Before the session, participants were asked to rate a set of 144 appetitive drug-related cues, alternative reward cues and aversive drug-related cues each. The question used for appetitive drug-related cues and alternative reward cues was “how strong is your desire to consume this now?”. The question for aversive drug-related cues was “how deterrent do you experience this picture?”. An eight-point Likert-scale from “not at all” to “very much” was used. For the final experiment, the 50% most rewarding / threatening stimuli were selected in an automated manner to maximize effects.

Four pictures of one category were presented per block during fMRI. Each block lasted 16 seconds and ended with the presentation of a fixation cross with an inter trial interval (ITI), jittered around 2.5 seconds. In each run, two blocks of each of the four categories were presented. Subjects were instructed to attend to all stimuli and to rate their current desire to consume the shown items (cigarette or food) twice per run, by pressing one of eight buttons covering an eight-point Likert scale ranging from “not at all” to “very strongly”. At the end of each run, participants were additionally asked to rate how strongly they desire to smoke a cigarette, using the same rating scale as described above. In total, the task consisted of 9 runs, between the runs participants had the possibility to ask questions and to have a short break, so that the task altogether lasted 38 minutes at the maximum (Figure 6).

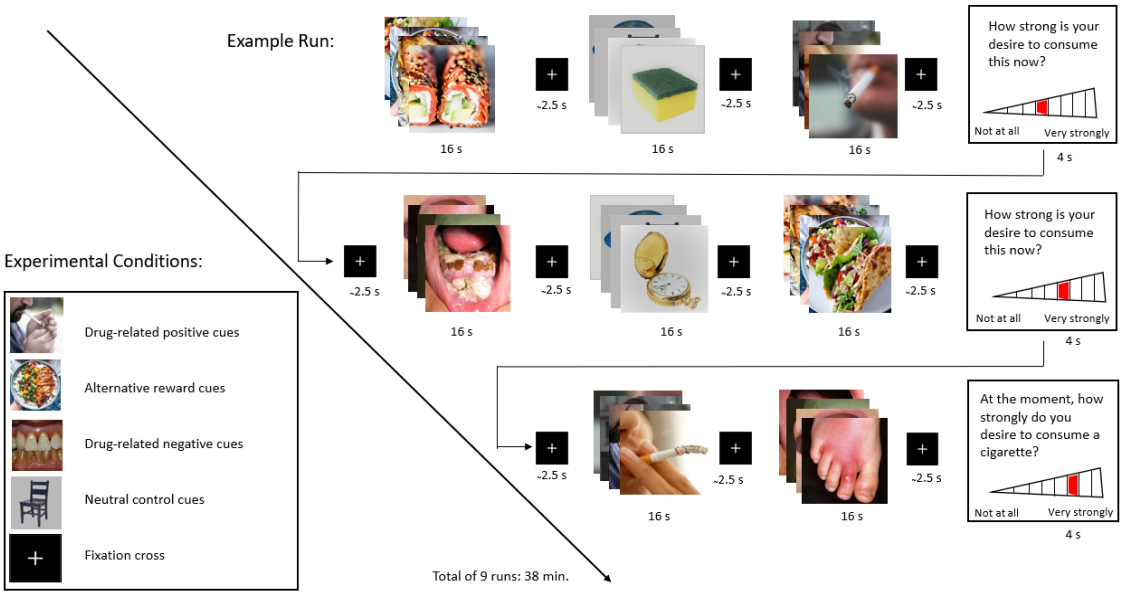

Figure 6: example run of the novel extended cue-reactivity paradigm used in experiments I and IV.

3.2.2 Cognitive down-regulation of craving paradigm

This paradigm was used for experiment III. During the task, drug-related (cigarettes), alternative reward (high-caloric food) as well as neutral (glass of water) cues were presented. Prior to each cue, an instruction (NOW or LATER) was given for 2 seconds. NOW cues instructed participants to consider the positive feelings of consuming the depicted cue at that moment. LATER cues instructed participants to down-regulate their craving for the item by thinking of the negative long-term consequences (reappraisal). The neutral category (glass of water) was included only for the NOW condition to constitute a neutral baseline. Each stimulus (drug-related, alternative reward, neutral) was presented for 6 seconds followed by a rating (3s) where participants indicated how much they want to consume the item now using an eight-point Likert scale from “not at all” to “very much”. Afterwards an ITI jittered around 2.5 seconds followed (see Figure 7). One-hundred trials (20 NOW drug-related, 20 NOW alternative reward, 20 LATER drug-related, 20 LATER alternative reward, 20 NOW neutral) were presented. The total task duration was approximately 25 minutes and consisted of five runs. Before the fMRI session, participants received an extensive description of the task and the possibility to practice the LATER condition, furthermore, they rated a set of 50 alternative reward (food) pictures with two questions each in sequence: “how strong is your desire to consume this now?” and afterwards “how deterrent do you expect this in the long-term?”, using an eight-point Likert scale from “not at all” to “very strong”. For the experiment, the

50% most rewarding respectively most detrimental stimuli were automatically selected to maximize effects.

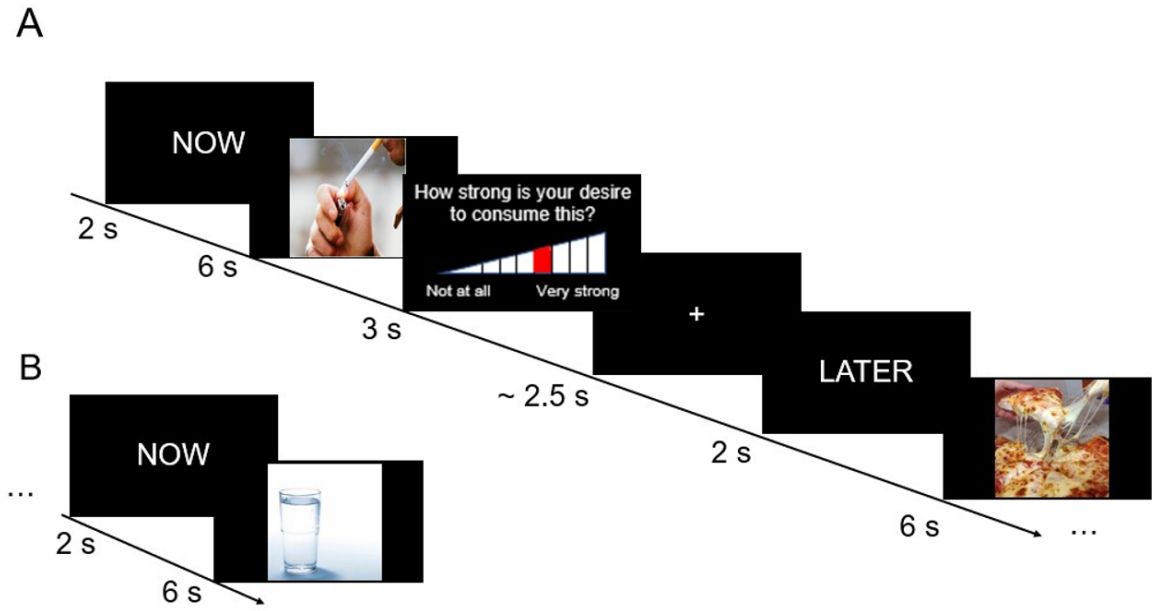

Figure 7: A) example of a full cigarette NOW trial followed by a food LATER condition; B) example of the neutral NOW condition where a similar cue was used across all neutral trials.

3.3 Scanning protocol

For experiments I, III and IV an identical scanning protocol was used:

A 3 Tesla whole-body scanner (MAGNETOM Prisma, Siemens, Erlangen, Germany) was used, equipped with a 64-channel head coil. Functional images were acquired using a Siemens simultaneous multi-slice T2*-weighted gradient-echoplanar imaging (EPI) sequence (TR = 869 ms, TE = 38 ms, 60 slices, slice thickness = 2.4 mm, voxel size 2.4 × 2.4 × 2.4 mm, no inter-slice gap, field of view (FoV) = 210 mm, matrix size 88 x 88, acquisition orientation T > C, interleaved slice order, acceleration factor slice = 6, flip angle = 58°, bandwidth = 1832 Hz/Px, prescan normalize, weak raw data filter, fat sat).
Field map images were obtained using a Siemens dual gradient-echo sequence (TR = 698 ms, TE1 = 5.19 ms, TE2 = 7.65 ms, 64 slices, slice thickness = 2.4 mm, no slice gap, voxel size 2.4 × 2.4 × 2.4 mm, field of view (FoV) = 210 mm, matrix size 88 x 88, acquisition orientation T > C, interleaved slice order, flip angle = 54°, bandwidth = 279 Hz/Px).
High-resolution anatomical images were acquired using a T1-weighted MPRAGE sequence (TR = 2000 ms, TE= 2.01 ms, TI = 880ms, FoV = 256 mm, 208 sagittal slices, voxel size 1 ×

1 × 1 mm, flip angle = 8°, GRAPPA factor 2 (PE), 24 ref. lines, prescan normalize, 23.1% slice oversampling, bandwidth = 240 Hz/Px).

For experiment II the following scanning protocol was used:
MRI data were acquired using 3 Tesla scanners. The following scanners were used: a 3T Philips Achieva scanner (Philips Medical Systems, Best, The Netherlands) in Muenster; a 3T Siemens Trio scanner (Siemens AG, Erlangen, Germany) in Dresden and Marburg; a 3T Siemens Verio scanner (Siemens AG, Erlangen, Germany) in Greifswald; a 3T General Electric Healthcare scanner (General Electric Healthcare, Milwaukee, WI) and a 3T Siemens Trio scanner in Berlin. MP-RAGE T1-weighted images were acquired with the following param- eters: voxel size = 1×1×1 mm3; repetition time (TR) = 1900 ms; inversion time (TI) = 900ms; field of view (FOV) = 256 × 256mm2; slices per slab = 176; thickness = 1 mm; f lip angle = 9, echo time (TE) = 2.26 ms.

3.4 Statistical analyses

3.4.1 fMRI analysis pathway

Image preprocessing for the functional images (experiments I, III and IV) was performed using statistical parametric mapping (SPM12; http://www.fil.ion.ucl.ac.uk/spm/software/spm12) and MATLAB R2020a (Mathworks, Sherborn, Massachusetts) based scripts. It comprised slice timing with reference to the middle slice, SPM12 standard realignment and unwarping including correction for field deformations based on a previously acquired field map, co-registration, normalization to MNI stereotactic space using unified segmentation based on the SPM tissue probability map for six tissue classes, and spatial smoothing with 8 mm full-width at half-maximum isotropic Gaussian kernel.

Image preprocessing for experiment II was performed with SPM12 (www.fil.ion.ucl.ac.uk) and the CAT12 toolbox (http://www.neuro.uni-jena.de/cat/) implemented in MATLAB R2016a (MathWorks, Sherborn, MA). Brain scans were segmented in gray matter, white matter and cerebrospinal fluid and normalized to the Montreal Neurological Institute reference (MNI) brain in CAT12. The voxel size was re-sampled to 1.5 × 1.5 × 1.5 mm^3 during this step. The resulting images were quality controlled with a visual inspection and CAT12-based outlier checks (homogeneity analysis). To exploratively examine structural

differences between TUD subjects and NS included in experiments I, III and IV, a VBM analysis was conducted in this sample, identical to the described procedure for experiment II.

3.4.2 Region of interest analyses

A region of interest (ROI) analysis of the *a priori* defined brain networks was conducted. Based on the available literature, the corresponding brain networks were defined as:

- Mesolimbic brain reward network (Koob & Le Moal, 2001; Koob & Volkow, 2010): NAcc, amygdalae, hippocampi, caudate, thalamus, pallidum and midbrain (including VTA)
- Aversion related brain network (Hayes & Northoff, 2011): amygdalae, hippocampi, insulae, ACC, OFC and thalamus
- (Prefrontal) control network (Brandl et al., 2019): prefrontal cortex (dlPFC, vlPFC), SMA, dACC, anterior insula, MTG and angular gyrus

Within the experiments (I-IV), parts of the networks were selected based on previous literature on the specific topic. This was done to keep the number of selected regions low and to avoid multiple testing. The *a priori* defined anatomical ROIs were built combining the definitions from the Automated Anatomical Labeling Atlas (AAL; Maldjian et al., 2003). Further, to define regions, not specified in the AAL atlas, the corresponding Brodmann areas were used. The bilateral ROIs were investigated using one single mask. Small volume correction on this single mask was applied using a family wise error corrected threshold of $p_{fwe} < 0.05$ with a minimum cluster size of $k = 10$ contiguous voxels.

CHAPTER 4

SUMMARY OF EMPIRICAL STUDIES

In this chapter I briefly summarize the four empirical studies (Kunas et al., 2021; Kunas et al., 2020; Kunas et al., under review; Kunas et al., 2021) that form the main body of the thesis.

4.1 Experiment I: Evidence for a "hijacked" brain reward system but no desensitized threat system in quitting motivated smokers: An fMRI study

A "hijacked" brain reward system in form of increased responsivity to appetitive drug-related cues and decreased responsivity to alternative rewards compared to NS may provide an aberrant process involved in the maintenance of TUD. This assumption is in accordance with incentive sensitization theory (Robinson and Berridge, 1993). A desensitization of the aversion related brain network to the negative consequences of smoking can further be proposed as a process in TUD preservation. However, only few studies are available addressing these processes in TUD subjects compared to NS, leaving it unclear if alterations in TUD subjects exist.

To address these points, quitting motivated TUD subjects and NS were examined using a novel extended cue-reactivity paradigm during fMRI recordings. The experiment aimed to gain evidence for a "hijacked" brain reward network and a desensitized aversion related brain network in quitting motivated TUD subjects. Furthermore, associations between neurofunctional alterations in reward and threat processing and behavioral smoking characteristics were of interest.

In total, 82 participants (39 TUD subjects and 43 NS) underwent fMRI scanning. Due to technical issues, 38 TUD subjects (55.26% female) and 42 NS (73.81% female) were included in the present analysis. The groups were matched regarding gender, age, handedness and education. All participants completed the Mehrfachwahl Wortschatztest (MWT), a questionnaire to assess their global level of intelligence (Lehrl et al., 1995), the trait part of the State-Trait-Anxiety-Inventory (STAI-T; Spielberger et al., 1970) and the short version of the German General Depression Scale (ADS-K; Hautzinger et al., 2012). The Fagerstroem Test of Nicotine Dependence (FTND; Heatherton et al., 1991) was used to assess severity of

nicotine dependence in the smoker group only. Furthermore, frequency information regarding alcohol use was acquired. All participants performed the extended cue-reactivity task (see paragraph 3.2.1) during fMRI. ROI analysis of *a priori* defined regions belonging to the mesolimbic brain reward network and the aversion related brain network (see paragraph 3.4.2) were conducted, followed by whole brain analyses. Craving ratings for drug-related cues and alternative rewards were compared between TUD subjects and NS and within the group of TUD subjects. Furthermore, an association between smoking severity and functional alterations was assessed.

According to the first research question formulated in paragraph 2.2, TUD subjects showed stronger activations in brain regions belonging to the reward network (bilateral hippocampi, thalamus and left midbrain) as response to appetitive drug-related cues in comparison to alternative rewards and compared to NS (Figure 8), supporting the first hypothesis. Contrary to the second hypothesis, no significant association between neurofunctional alterations and smoking severity was obtained. Concerning the third research question, hypothesis one could not be supported. Specifically, NS showed no significantly increased activation of brain regions belonging to the aversion related brain network as response to aversive drug-related cues compared to TUD subjects. However, within the TUD group, a significant negative association was identified between activations of brain regions belonging to the aversion related brain network (ACC and bilateral insulae) with the number of cigarettes smoked per day (Figure 9). A significant main effect of group as well as an interaction of group-by-stimulus-type was obtained in behavioral craving ratings within the task (Figure 10, A). Final craving ratings for drug-related cues at the end of each run were significantly higher in TUD subjects compared to NS (Figure 10, B). TUD subjects rated their craving for drug-related cues significantly lower at the end of each run, when aversive drug-related cues were presented before (Figure 10, C).

These results provide evidence that TUD involves a “hijacked” brain reward network in favor for appetitive drug-related cues, but no generally desensitized aversion related brain network to aversive drug-related cues, at least not in rather moderate smokers. Associations of significantly activated brain regions belonging to the aversion related brain network with cigarettes smoked per day might suggest that heavier TUD subjects show a desensitization to aversive drug-related cues. In chapter 5 the results will be discussed in light of the incentive sensitization (altered reward processing) and threat/ punishment desensitization (threat processing) theory.

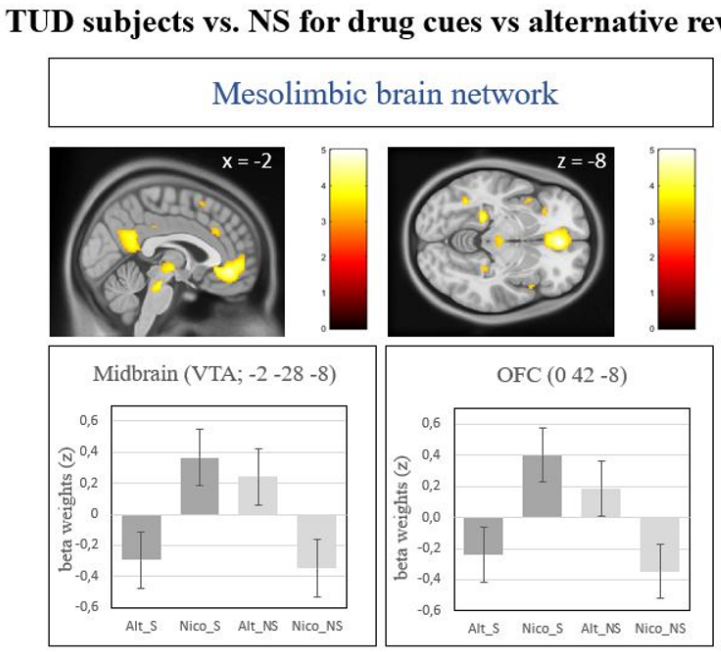

Figure 8: stronger activations in brain regions belonging to the mesolimbic brain reward network for drug-related cues in TUD subjects compared to NS and alternative rewards. Results provide evidence for a "hijacked" brain reward network. Bars represent the estimated, standardized beta values of the corresponding brain region. Error bars represent the standard error of the mean.

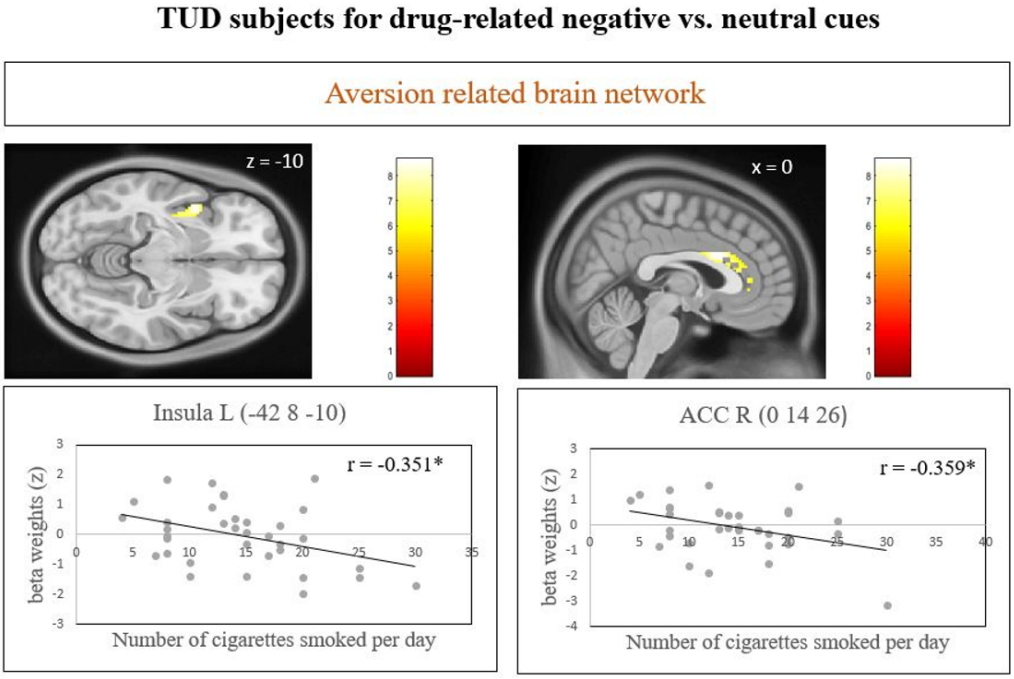

Figure 9: significant activations in brain regions belonging to the aversion related brain network in TUD subjects during processing of aversive drug-related cues compared to neutral cues. Scatter-plots show the (medium) negative correlations between extracted beta weights and the number of cigarettes smoked per day. Beta weights were estimated and standardized for the corresponding brain region. * $p < 0.05$

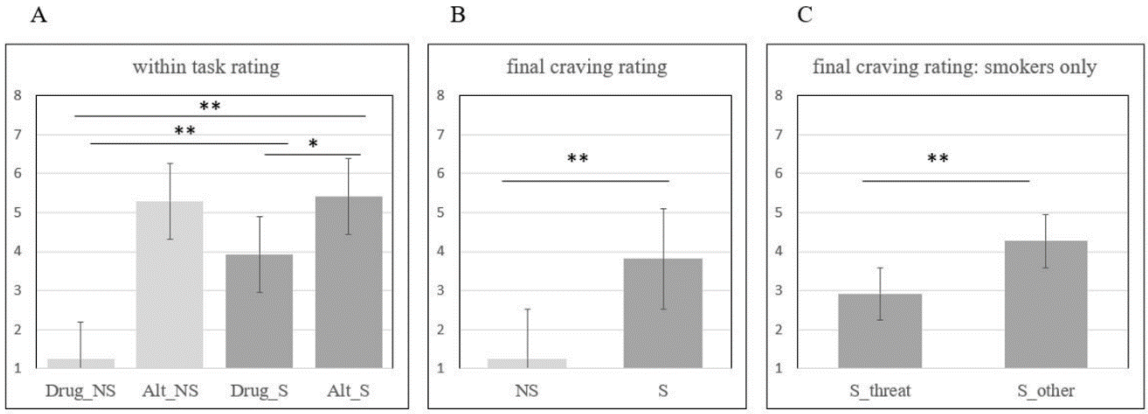

Figure 10: subjective craving ratings within the extended cue-reactivity task. S = TUD subjects; NS = never-smoker; Drug = rating following appetitive drug-related cues; Alt = ratings following alternative reward cues. **A)** Ratings separated for the two groups, results show a significant main effect of group ($H_{(1/156)}$ = 33.115, p < 0.001, η^2 = 0.179), stimulus ($H_{(1/156)}$ = 128.579, p < 0.001, η^2 = 0.458) and group-by-stimulus interaction ($H_{(1/156)}$ = 27.851, p < 0.001; η^2 = 0.155); **B)** final craving ratings that were presented at the end of each run refer to craving for cigarettes at the moment, separated for the two groups; results show a significant difference between the groups of -2.583(95%CI -3.119,-2.047), ($t_{(76)}$ = -9.60, p < 0.001, d = -2.192; 95% CI [-2.756, -1.619]); **C)** final craving ratings in the group of TUD-subjects only, separated for ratings preceded by drug-related negative cues (threat) vs. alternative reward, appetitive drug cues and neutral cues (other), results show a significant difference of -1.340 (95% CI -1.802, -0.878), (t(36) = -6.09, p < 0.001, d = 1.348; 95% CI [-1.380, -0.580]).
* p < 0.05, ** p < 0.001.

These data have been published as: Kunas, S.L., Stuke, H., Heinz, A., Ströhle, A., & Bermpohl, F. (2021). Evidence for a “hijacked” brain reward system but no desensitized threat system in quitting motivated smokers: An fMRI study. *Addiction*, 2021;1-12.

https://doi.org/10.1111/add.15651

4.2 Experiment II: The modulating impact of cigarette smoking on brain structure in panic disorder: a voxel-based morphometry study

Structural brain alterations in smokers were identified within cortical and subcortical brain regions encompassing the aversion related brain network including the ACC, thalamus, prefrontal cortex and the hippocampus (Bordy et al., 2004; Gallinat et al., 2006; Zhang et al., 2011; Liao et al., 2012; Fritz et al., 2014). Noteworthy, smoking behavior is overrepresented in patients with mental disorders in general (Lasser et al., 2000) and prospective epidemiological studies link smoking to an increased risk of anxiety disorders including PD. Neural system models of PD emphasize that structural brain changes in limbic structures (e.g., amygdala, hippocampus), cortical areas (ACC) and the thalamus (Massana et al., 2003; Uchida et al., 2003; Asami et al., 2009; Hayana et al., 2009) are highly overlapping with those identified in smokers. Although brain structures altered by smoking partly overlap with morphological changes identified in PD, the modulating impact of smoking on structural alterations in PD has not yet been addressed.

In total, 143 PD patients (71 smokers) and 178 healthy controls (62 smokers) participated in an MRI study. For in- and exclusion criteria see paragraph 3.1. T1-weighted images were used to examine structural brain alterations within smokers and non-smokers (with and without a diagnosis of PD) using VBM in *a priori* defined regions of the aversion related brain network. Age, gender, education, total intracranial volume, study center and Beck depression inventory (BDI-II; Beck et al., 1996) scores were included as covariates of no interest. To examine an additive effect of smoking and PD, a linear regression model using three groups with increasing health burden as independent variable was conducted.

Smokers (without a diagnosis of PD) showed reduced GMV in the right insula and ACC, which supports hypothesis one (second research question) of reduced GMV in parts of the aversion related brain network. Independent of the diagnosis of a PD, smokers showed reduced GMV in the right insula, left ACC and in the OFC (all regions defined as parts of the aversion related brain network). Concerning hypothesis two of the second research question, PD was associated with GMV reductions in the bilateral amygdalae and hippocampi. This difference was significant only in non-smokers and absent when smoking subjects were included. Furthermore, bilateral amygdalae volumes were reduced with increasing health burden (neither PD nor smoking > either PD or smoking > both PD and smoking, see Figure

11). These results support hypothesis two and provide evidence for a modulating impact of smoking on brain structure in PD patients.

To replicate structural brain alterations within TUD subjects included in experiments I, III and IV, an explorative VBM analysis was conducted. Significant GMV reductions in TUD subjects compared to NS were found in the left parahippocampal area (MNI: -23, -33, -14; t = 4.76; $p < 0.001$; k = 924) and in the right superior frontal gyrus (MNI: 27, 39, 36, t = 4.09; $p <$ 0.001; k = 387) in a whole brain approach. Investigating the aversion related brain network, GMV reductions in TUD subjects were identified in the right hippocampus (MNI: 23, -30, -11, t = 4.01; p = 0.021, k = 49). GMV of the right hippocampus was negatively correlated with pack years in TUD subjects (r = -0.313; p = 0.028; see Figure 12).

Results suggest structural alterations of the aversion related brain network in smokers (with and without the diagnosis of a PD). Moreover, smoking behavior can narrow or diminish commonly observed structural abnormalities in PD, pointing to the fact of shared structural brain changes in both conditions. Thus, the effect of smoking should be considered in MRI studies focusing on patients with pathological forms of fear and anxiety. Future studies are needed to determine if smoking may increase the risk for subsequent PD psychopathology via brain functional or structural alterations. Additionally, an explorative VBM analysis of TUD subjects included in experiments I, III and IV partly replicates the findings of experiment II. However, results gained by this explorative analysis are highly limited by the small sample size of 78 subjects, which needs to be considered when interpreting the findings. Chapter 5 discusses the current data in light of the threat/ punishment desensitization theory and provides future research directions as well as limitations of experiment II.

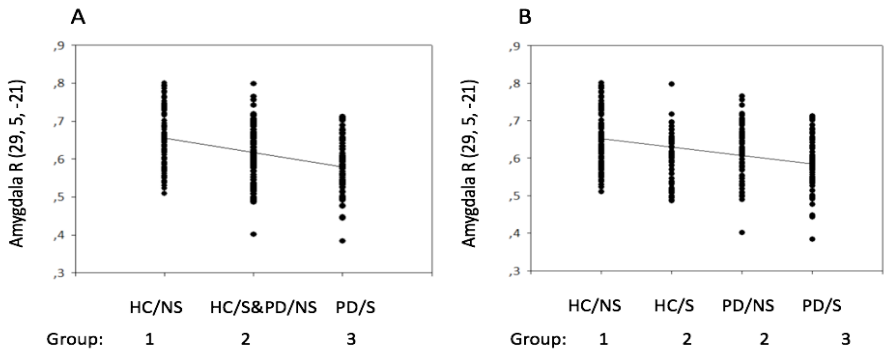

Figure 11: scatterplot of right amygdala volumes (extracted from a sphere with 5mm diameter around the peak identified in the group analysis). Plot **A** pictures the three groups of the regression analysis and plot **B** additionally separates group 2 in healthy smokers and PD non-smokers. Results provide evidence for reduced amygdala volumes with increasing health burden.
HC = healthy controls; NS = non-smokers, PD = panic disorder; S = smoker

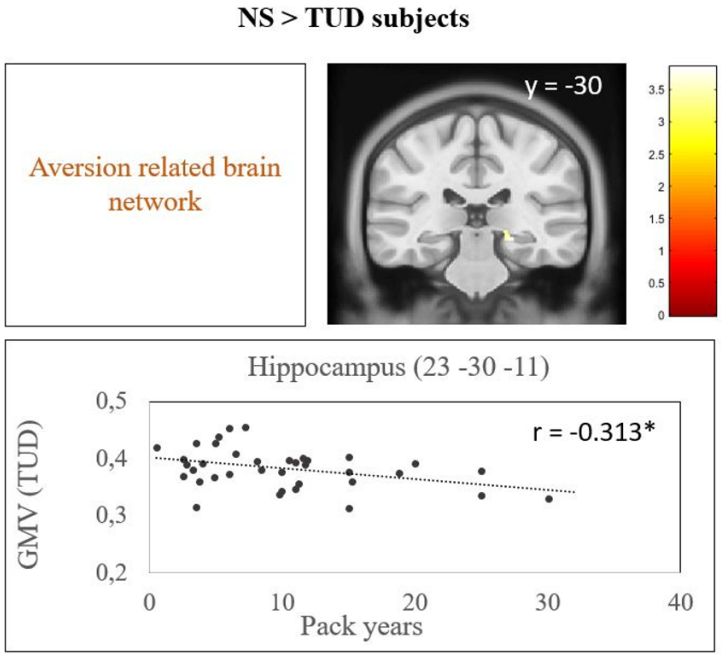

Figure 12: explorative VBM analysis in TUD subjects and NS. Significantly reduced GMV in the right hippocampus in TUD subjects compared to NS in the ROI analysis. GMVs were extracted using the toolbox marsbar with a sphere of 5 mm around the peak voxel (MNI: 23, -30, -11). A spearman correlation between extracted GMV and pack years was conducted as the saphiro wilk test showed a significant result ($p < 0.001$), indicating that the normality assumption was violated. Four subjects (one TUD subject and three NS) had to be excluded from the VBM analysis based on the results gained in the quality control procedure (see paragraph 3.4.1), resulting in 38 TUD subjects and 40 NS. TIV values were introduced as covariates of no interest. No other covariates were included as subjects did not differ in any other characteristics. Right hippocampus volumes showed a medium negative correlation with pack years, indicating that stronger smokers showed smaller GMVs in the hippocampus.
*$p < 0.05$

Parts of the presented data have been published as: Kunas, SL, Hilbert, K., Yang, Y., Richter, J., Hamm, A., Wittmann, A., Ströhle, A., Pfleiderer, B., Herrmann, M.J., Lang, T., Lotze, M., Deckert, J., Arolt, V., Wittchen, H.U., Straube, B., Kircher, T., Gerlach, A.L., Lueken, U. (2020). The modulating impact of cigarette smoking on brain structure in panic disorder: a voxel-based morphometry study, *Social Cognitive and Affective Neuroscience*, *15*(8); 849–859.

https://doi.org/10.1093/scan/nsaa103

The VBM analysis between TUD subjects and NS included in experiments I, III and IV has not been published.

4.3 Experiment III: Neurofunctional alterations of cognitive down-regulation of craving in quitting motivated smokers

Dual systems theory of addiction proposes an imbalance of a hyperactive motivational system and a less well functioning control system in SUDs in general (Baler and Volkow, 2006). This deficit could explain the conflict to continue drug taking despite the motivation to quit. Cognitive neuroscience research suggest that successful down-regulation of craving is dependent on top-down control from the prefrontal cortex over subcortical regions involved in reward and emotion (Heatherton & Wagner, 2011). Previous studies identified functional PFC abnormalities in TUD subjects (Sutherland et al., 2012), which could constitute the neurobiological basis of down-regulation deficits. Only few studies investigated cognitive down-regulation of craving within TUD subjects. These identified a neural network within the prefrontal cortex (dlPFC, vlPFC) and related areas (angular gyrus, SMA, MTG and ACC; e.g., Kober et al., 2010; Hartwell et al., 2011; Brody et a., 2007) during down-regulation compared to passive viewing. Furthermore, trait impulsivity and smoking severity were associated with craving, prefrontal cortex activation and failures in cognitive control in smokers (Copersi et al., 2017; Dong et al., 2021; Dalley et al., 2011).

However, various aspects of down-regulation of craving processes in TUD subjects remain unresolved. To the best knowledge of the author no study is available comparing down-regulation of craving effects for alternative rewards between TUD subjects and NS. This could constitute evidence for a general (e.g., both drug-related and non-drug-related) down-regulation deficit in smokers. Furthermore, it is unclear whether TUD subjects present specific drug-related down-regulation deficits, compared to alternative rewards. A possible association between smoking severity and trait impulsivity remains unclear so far.

In total, 82 participants (39 TUD subjects and 43 NS) underwent fMRI scanning. Due to technical issues two TUD subjects and two NS had to be excluded, resulting in 37 TUD subjects (56.80% female) and 41 NS (73.17% female). All TUD subjects were highly quitting motivated and took part in a free, 6-week smoking cessation program after the assessment. All participants completed the MWT, the STAI-T and the ADS-K (see 4.1 for more details). The FTND was used to assess severity of nicotine dependence in the smoker group only and the Baratt Impulsiveness Scale short version to measure trait impulsivity (BIS-11; Meule et al., 2011). All participants performed a cognitive down-regulation of craving task (see paragraph 3.2.2). Subjective craving ratings were obtained after each cue (drug-related, alternative

reward and neutral). The neutral control condition was excluded in this analysis as cue-reactivity effects were not of main interest. Craving ratings for appetitive drug cues and alternative rewards for the LATER vs. NOW condition were compared between and within the group of TUD subjects and NS. ROI analysis of *a priori* defined (prefrontal) control and mesolimbic reward areas (see paragraph 3.4.2) were conducted for the LATER vs. NOW condition. Furthermore, the association between smoking severity and trait impulsivity with functional and behavioral correlates of down-regulation of craving was assessed.

Concerning the first hypothesis of the fourth research question, TUD subjects showed no differences in behavioral down-regulation of craving ratings, neither compared with NS (for alternative rewards), nor in comparison to alternative rewards (for drug-related cues; Figure 13). Concerning the second hypothesis, evidence was found for differences in down-regulation (LATER > NOW) compared to NS during an alternative reward (food) condition on a neurofunctional level. Contrary to our hypothesis, TUD subjects showed a stronger BOLD response of the middle temporal gyrus (Figure 14). No evidence was obtained for differences in cognitive down-regulation between different reward cue-types within TUD subjects. TUD subjects showed prefrontal cortex activation (e.g., vlPFC, dlPFC) as well as a decreased activation of mesolimbic brain regions (e.g., putamen) during down-regulation of craving (LATER vs. NOW) collapsed for both reward cue-types on a neurofunctional level. Significant negative associations between smoking severity (pack years, FTND) and trait impulsivity with BOLD responses (e.g., vlPFC, SMA) elicited during down-regulation across both reward cue-types were found in TUD subjects (Figure 14). The significant correlations implicate a role of these characteristics in down-regulation of craving, at least on a neurofunctional level and support hypothesis three.

This study extends research on cognitive down-regulation of craving in quitting motivated TUD subjects and offers starting points to better understand general and specific alterations in this group. The behavioral results can be interpreted as indicator for neither general nor specific deficits in cognitive down-regulation of craving in highly quitting motivated TUD subjects. Neurofunctional results may indicate a general alteration in cognitive down-regulation of craving in TUD. This alteration is present in form of a compensatory activation of the middle temporal gyrus in TUD subjects, compared to NS, to reach similar outcomes on a behavioral level. No specific differences were found for cognitive down-regulation between different reward cue-types within TUD subjects. Moreover, smoking severity showed a negative association with BOLD responses of the prefrontal cortex (e.g., vlPFC, SMA) during

down-regulation (LATER > NOW, pooled across both reward cue-types) in TUD, indicating stronger activations in less severe smokers. Chapter 5 provides a more detailed discussion of the results in light of the dual systems theory of addiction.

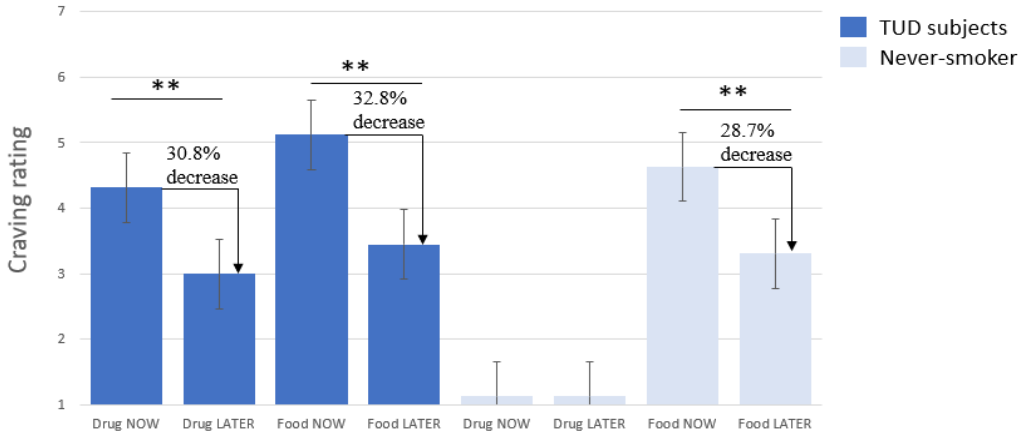

Figure 13: behavioral craving ratings within the cognitive down-regulation of craving task. Analyses of craving ratings reached a significant main effect for LATER vs. NOW for the alternative reward condition ($F_{(146,1)} = 26.31$; $p < 0.001$, $\eta^2 = 0.149$). Between-subjects analyses showed no significant main effect of smoking status ($F_{(146,1)} = 3.668$, $p = 0.057$, $\eta^2 = 0.021$) and no significant interaction effect of smoking status-by-reappraisal ($F_{(146,1)} = 0.353$, $p = 0.553$, $\eta^2 = 0.002$). Analyses within TUD subjects showed a significant main effect for LATER vs. NOW ($F_{(34,1)} = 60.74$, $p < .001$, $\eta^2 = 0.640$) but no significant main effect for reward cue-type ($F_{(34,1)} = 3.527$, $p = .069$, $\eta^2 = 0.090$) and no significant interaction between reward cue-type and reappraisal ($F_{(34,1)} = 2.70$, $p = .110$, $\eta^2 = .070$). Error bars represent the standard error of the mean. Drug = drug-related cues, FOOD = alternative rewards.

** $p < 0.001$

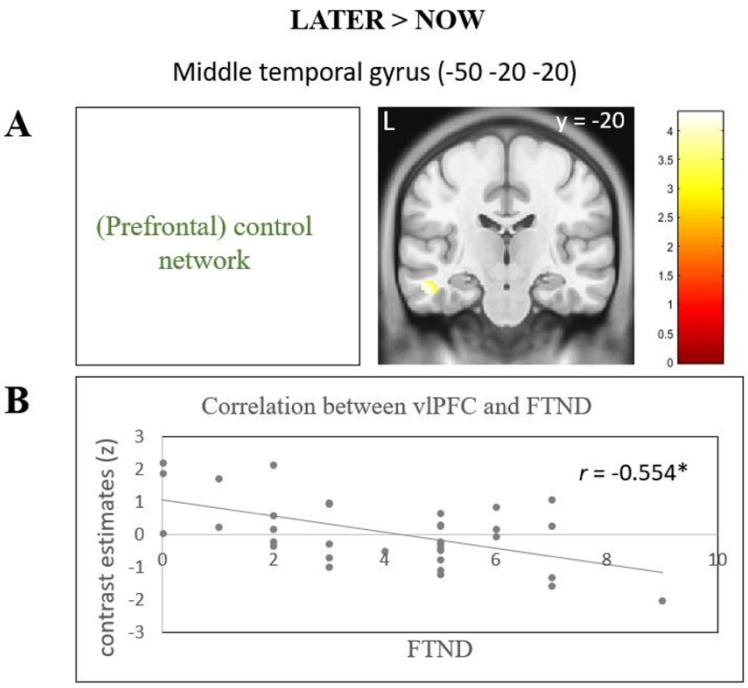

Figure 14: A) significant stronger activation of the middle temporal gyrus in TUD subjects compared to NS (TUD subjects > NS) during down-regulation of craving (LATER > NOW) for alternative rewards (upper row). **B)** The lower row presents the significant medium negative association between FTND scores and BOLD responses in the left vlPFC (LATER > NOW, collapsed for both rearward cue-types) within TUD subjects. Beta weights were estimated and standardized. * $p > 0.05$

At the time of writing, these data have been submitted as: Kunas, S.L., Stuke, H., Plank, I.S., Laing, E.M., Bermpohl, F., & Ströhle, A. (submitted). Neurofunctional alterations of cognitive down-regulation of craving in quitting motivated smokers.

4.4 Experiment IV: Aversive drug cues reduce cigarette craving and increase prefrontal cortex activation during processing of cigarette cues in quitting motivated smokers

Aversive drug-related cues (e.g., pictures showing lung cancer) can be used to support smoking cessation and create awareness of negative health consequences of smoking. Previous results suggest that such cues can effectively reduce craving in smokers (Partos et al., 2013) curtail the number of smoking initiators (Villanti et al., 2014) and augment quitting rates. Wang and colleagues (2013) found that highly emotional graphic health warning labels, presented directly prior to smoking cues, reduced the electrophysiological correlate (P300) elicited by smoking cues. However, to the best knowledge of the author, no fMRI study is available that has investigated the processing of appetitive drug-related cues when they are directly preceded by aversive drug-related cues. A better understanding of the effects of aversive drug-related cues on craving and the processing of appetitive drug-related cues in abstinence motivated smokers is important to further improve their usage in cessation therapy and smoking-related public health measures.

The aim of this study was to test specific hypotheses derived from previous investigations and theoretical assumptions (see paragraph 1.5) within quitting motivated TUD subjects.

Thirty-nine quitting motivated TUD subjects underwent fMRI scanning. One participant had to be excluded due to technical issues, resulting in 38 (55.26% female) analyzed data sets. The FTND, MWT, and AUDIT were completed by TUD subjects prior to the scanning session (see 4.1 for more details). Participants underwent fMRI scanning while performing a novel extended cue-reactivity paradigm (see paragraph 3.2.1). Processing of appetitive drug-related cues preceded by aversive drug-related cues ($Nico^{+}$) vs. other cues ($Nico^{-}$, appetitive drug-related, alternative reward and neutral cues) was investigated. *A priori* selected ROIs of the mesolimbic reward network and the (prefrontal) control network (paragraph 3.2.4) were examined. Additionally, a whole brain analysis was conducted. A gPPI analysis was further calculated using the significant ROIs of the group level analysis as seed regions. As a negative connectivity was expected, the gPPI analysis was limited to negative connectivity patterns.

Concerning the first hypothesis of research question five, behavioral craving ratings for drug-related cues were significantly reduced in TUD subjects when aversive drug-related cues preceded the presentation of appetitive drug-related cues ($Nico^{+}$; Figure 15). Hypothesis two could only partly be supported. Contrary to the hypothesis no evidence could be obtained for reduced activation in mesolimbic brain regions during processing of appetitive drug-related

cues preceded by aversive drug-related cues. However, greater activations in prefrontal (dlPFC) and paralimbic (dACC and anterior insulae) brain areas in the $Nico^{+} > Nico^{-}$ condition support hypothesis two (Figure 16). A positive association between behavioral craving reduction and neurofunctional activation could be shown for the right dACC. Stronger negative functional connectivity of activated prefrontal control areas to parts of the mesolimbic reward network (putamen, caudate, brainstem; Figure 16) could be identified in the gPPI analysis, supporting the third hypothesis.

Results suggest that aversive drug-related cues have an impact on the processing of appetitive drug-related cues, both on a neurofunctional and a behavioral level. Aversive drug-related cues can activate control-associated brain areas (e.g., dACC) during the processing of appetitive drug-related cues. These activations may lead to increased inhibitory control on reward-associated brain areas (e.g., putamen) and a reduction in subjective cravings. Thus, results suggest implicit cognitive control processes during the processing of appetitive drug-related cues preceded by aversive drug-related cues in TUD subjects. In chapter 5 these results are discussed in light of an interplay between reward, threat and cognitive control processes in TUD.

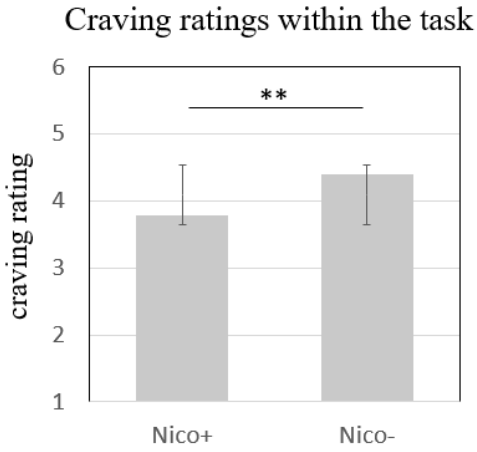

Figure 15: within the task, TUD subjects rated their craving significantly higher for appetitive drug-related cues preceded by other cues ($Nico^{-}$), compared to appetitive drug-related cues preceded by aversive drug-related cues ($Nico^{+}$), difference = – 0.60 (95% CI -0.919, -0.82), ($t_{(34)}$ = -3.827; $p < 0.001$; d = -0.647; [95%CI -1.008, -0.278]).

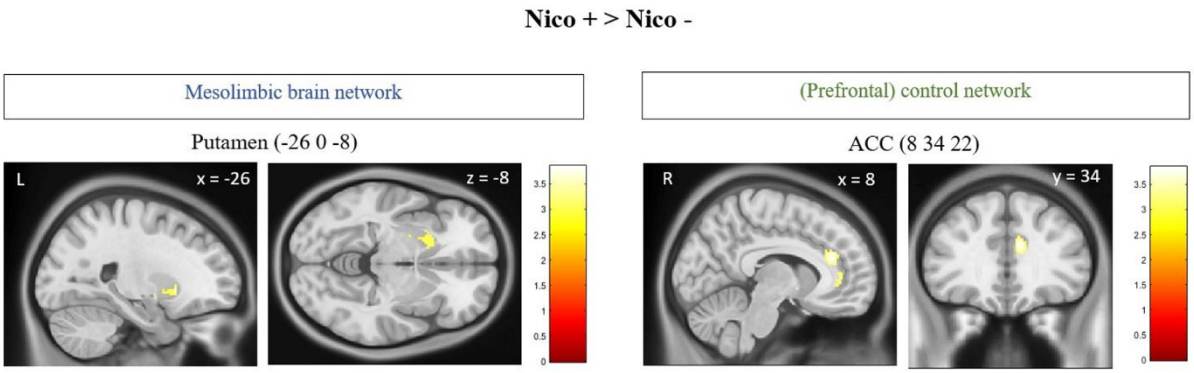

Figure 16: significant brain activations during the processing of appetitive drug-related cues preceded by aversive drug-related cues ($Nico^{+}$) in TUD subjects. Stronger activation in control areas are interpreted as increased implicit cognitive control (right). Significant inverse functional connectivity between control and mesolimbic brain areas propose a down-regulation of brain regions belonging to the reward network by brain regions belonging to the control network (left).

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CHAPTER 5

GENERAL DISCUSSION

In this chapter I discuss how the four studies can build an empirical basis for neural alterations in TUD. First, the results gained by the four experiments will be integrated in form of an integrative reward, threat and cognitive control processing model of TUD. Second the five research questions, introduced in chapter 2, will be discussed separately. Afterwards, clinical implications for prevention strategies and smoking cessation programs will be outlined. At the end of this chapter the limitations of the studies included in this thesis will be discussed and future directions will be defined before the conclusions are introduced.

The overall aim of this thesis was to test and integrate specific hypotheses derived from evidence of previous empirical studies and theoretical assumptions addressing reward, threat/ punishment and cognitive control processes in TUD subjects. Based on the results of the four empirical studies an integrative model might be suggested for TUD. Such an integrative model includes evidence for: (1) alterations in the brain reward network in TUD subjects in form of a hypersensitivity to drug-related cues compared to alternative rewards and NS; (2) aberrations in the aversion related brain network in TUD subjects, in form of morphological changes compared to non-smokers but no neurofunctional aberrations compared to NS; (3) general alterations in down-regulation of craving processes in TUD subjects, compared to NS, but no drug-specific alterations on a neurofunctional level; (4) implicit cognitive control over reward processes in TUD subjects, when confronted with long-term consequences of consumption. It is important to mention that the four studies provide only a first empirical basis for an integrative reward, threat/ punishment and cognitive control processing model in TUD. It is now highly relevant to replicate these findings in a large, independent sample to validate the model.

5.1 Discussion of research questions

First question: Do quitting motivated TUD subjects show a “hijacked” brain reward system?

Experiment I

TUD subjects showed stronger activations in mesolimbic brain regions (e.g., midbrain, thalamus) during processing of drug-related cues compared to alternative rewards and NS. This significant interaction presents an indicator for a “hijacked” brain reward system in

smokers. According to incentive sensitization theory (Robinson & Berridge, 1993), it can be assumed that brain areas that belong to the mesolimbic reward pathway of TUD subjects gradually became sensitized to drug-related cues and desensitized to alternative rewards. To the best of the knowledge of the author, previous studies did not directly investigate the interaction effect of smoking status and stimulus type. These results therefore provide first evidence for alterations in processing of appetitive drug-related cues vs. alternative rewards in TUD subjects compared to NS. However, functional activation of the mesolimbic reward network was not related to the number of cigarettes smokes per day. It can thus be speculated that alterations in brain reward processes are independent of the number of cigarettes an individuum is consuming. Therefore, these alterations may not be linked to heavier smoking behavior and might also occur in light smokers. Results in behavioral craving ratings were inconsistent with the reported fMRI findings. TUD subjects rated their craving for alternative reward (food) cues significantly higher than for drug-related cues. This phenomenon may be possibly explained by the high abstinence motivation of the investigated TUD subjects. It remains plausible that the motivation to quit has an impact on the craving ratings given during the task. However, craving ratings were found to be not prognostic for smoking behavior, which might have a strong habitual component (Tiffany & Carter, 1998).

As proposed by the hypothetical model of reward processing, results provide evidence for a sensitization towards drug-related cues and a desensitization towards alternative rewards in TUD subjects, consistent with incentive sensitization theory.

Second question: Do smokers present neurostructural alterations of the aversion related brain network and do these smoking-related alterations modulate structural brain changes reported in panic disorder?

Experiment II

Smoking behavior is highly prevalent in mental disorders in general and in PD patients in particular (Johnson et al., 2000; Lasser et al., 2000). Structural abnormalities characterizing the neurobiology of smoking were found in the aversion related brain network and do, to some extent, overlap with brain circuits involved in the pathophysiology of PD. Findings reported in experiment II provide evidence for structural abnormalities in the aversion related brain network in smokers. Moreover, results indicate that GMV reductions in brain structures commonly associated with PD are mainly driven by non-smokers. These structural differences diminish in smokers which may be attributed to already reduced GMV in smoking subjects.

Furthermore, bilateral amygdala volumes show a linear decrease with increasing health burden, indicating a possible additive effect of smoking behavior and PD. Additionally, an exploratively conducted VBM analysis of subjects included in experiments I, III and IV partly replicates the findings of experiment II. Compared to NS, quitting motivated TUD subjects showed structural abnormalities within parts of the aversion related brain network. Further, reduced GMV in the right hippocampus was negatively associated with pack years.

Regular smokers without a diagnosis of PD showed GMV reductions in the right insula and ACC, which is consistent with results from previous VBM investigations (Gallinat et al., 2006). Independent of any mental disorder, smokers showed additionally significant GMV reductions in the left ACC, a region that belongs to the aversion related brain network (Hayes & Northoff, 2011). These brain regions have previously been related to the neurobiology of addictive disorders, including TUD (Goldstein & Volkow, 2002). These abnormalities could constitute the structural basis of functional aberrations that have been reported in the neuroimaging literature in smokers (e.g., Duehlmeyer et al., 2019; Duehlmeyer et al., 2018; Luijten et al., 2011). Additionally, smoking behavior exerts a modulating impact on brain morphological correlates in PD patients, potentially blurring group differences associated with PD psychopathology. It can thus be concluded that current smoking behavior in PD patients and healthy control subjects can decline or diminish generally observed structural abnormalities in PD patients.

Considering three groups with increasing health burden (healthy control subjects, PD patients or regular smokers and PD patients who are regular smokers), a linear negative effect of decreasing GMV was observed for bilateral amygdalae volumes. This result suggests that the combined impact of health burden (smoking and PD) may be reflected in the structure of the amygdalae. Previous research linked structural alterations of these brain regions to both conditions, smoking and PD (Durazzo et al., 2017; Dresler et al., 2013). Regular smokers and non-smoking PD patients showed similar amygdalae volumes. One possible interpretation of this finding is that the combination of smoking and PD supports structural abnormalities in the amygdalae. Alternatively, GMV reductions in the amygdalae may have existed before subjects began smoking and developing panic symptoms and PD. Reduced GMV in brain structures belonging to the aversion related brain network could therefore also represent a potentially predisposing factor to smoking behavior or PD, or a shared factor for the development of both conditions.

As smoking behavior most commonly precedes the onset of an anxiety disorder (Breslau & Klein, 1999; Johnson et al., 2020), the effects caused by cigarette smoking may, at least partially, underpin the biological mechanisms through which smoking might contribute to the development of PD. Hence, it may be speculated that smoking can contribute to structural brain alterations which may serve as a vulnerability factor for the development of PD.

Quitting motivated TUD subjects showed structural abnormalities in parts of the aversion related brain network (e.g., parahippocampal area, hippocampus). Structural brain changes in the parahippocampal area were also found between non-smoking PD patients and healthy control non-smokers and diminished when smokers were included in the analysis. Furthermore, results suggest that some of the observed structural brain alterations were mainly driven by strong smokers, as a negative association between hippocampal volume and pack years was observed.

According to threat/ punishment desensitization theory, this study provides evidence for structural alterations in parts of the aversion related brain network. Structural brain changes in the aversion related brain network, that are associated with smoking behavior may present a vulnerability factor for the development of other mental conditions. As the pathophysiology of anxiety disorders in general and PD specifically could be linked to brain structures belonging to the aversion related brain network (e.g., Dresler et al., 2013), these may be influenced by morphological alterations related to smoking behavior.

Third question: Do quitting motivated TUD subjects show a desensitization towards aversive drug-related cues in the aversion related brain network?

Experiment I

The interaction between TUD subjects and NS for aversive drug-related cues vs. neutral cues [NS (aversive cues > neutral) > TUD subjects (aversive cues > neutral)] reached no significant results. The assumption of attenuated responses towards aversive drug-related cues in TUD subjects compared to NS, as proposed by threat/ punishment desensitization theory, could not be confirmed. This result points to the fact that there may be no desensitization of the aversion related brain network in moderately dependent quitting motivated TUD subjects. This thereby contradicts findings on altered punishment and error related processing in smokers (e.g., Duehlmeyer et al., 2019; Duehlmeyer et al., 2018; Luijten et al., 2011). It might be speculated that aversive drug-related cues, which present a passive form of punishments,

do not lead to similar effects as more active forms of punishments (e.g., losing money). It might further be presumed that passive forms of punishment may need a larger power to detect an effect.

However, when TUD subjects were investigated separately from NS they showed an activation of the aversion related brain network in response to aversive drug-related cues. This finding is consistent with results from previous investigations (Dinh-Williams et al., 2014a; Dinh-Williams, et al., 2014b). Thus, it is possible that quitting motivated TUD subjects process aversive cues as unpleasant and engage structures associated with negative emotions when they are exposed to the negative value of smoking (Lane et al., 1997; Taylor et al., 2000). This interpretation is further supported by the behavioral finding that prior presentation of aversive drug-related cues reduced subjective cigarette craving.

It is important to mention, though, that the observed activations in the aversion related brain network were driven by light smokers, as a negative association between brain activation and the number of cigarettes smoked per day was observed. This result suggests that, relative to light smokers, heavy smokers do present a desensitization of the aversion related brain network. This observation may imply that heavy smokers are not responding as strongly anymore to the negative aspects of smoking. Behavioral analyses of craving ratings that were preceded by aversive drug-related cues vs. other cues support this interpretation. Specifically, the reported negative association between the difference in craving ratings and smoking behavior suggests that more dependent smokers were less influenced in their craving when aversive drug-related cues were presented beforehand.

Previous studies investigating alterations in punishment and error related processing (e.g., Duehlmeyer et al., 2019; Duehlmeyer et al., 2018) did not evaluate associations between neurofunctional aberrations and smoking severity, nor did they include quitting motivated smokers. Therefore, it may be possible that the observed alterations, which were used to formulate the assumptions, are based on more dependent and heavier, non-quitting motivated smokers and cannot directly be transferred to light or moderate quitting motivated smokers. Another alternative explanation could be that TUD subjects who are less sensitive to aversive drug-related cues tend to consume more cigarettes per day. As the study is limited by a cross-sectional design, these alternative interpretations cannot be disentangled.

Presented findings and interpretations are partly based on analysis design decisions with regards to the conducted ROI analyses and the rationale for some choices shall be explained in this paragraph. The decision to include specific ROIs was based on previous literature that

described these as part of either the reward or threat network. Noteworthy, however, some ROIs belong to both, the brain reward and aversion related brain network (e.g., amygdala & hippocampus). Both reward- and threat-related stimuli are highly emotional, a fact that might be reflected in the shared activation of brain regions (Hayes & Northoff, 2011). Additionally, some areas code for multiple, even adversary processes (e.g., aversion and reward). There are several cell types with various response characteristics (e.g., throughout the amygdala) which may respond to the occurrence of rewarding, aversive or both types of stimuli (Hayes & Northoff, 2011). Further, some areas that were previously reported in the context of processing of appetitive drug-related cues (e.g., insula & ACC) were in this thesis assigned to the aversion related brain network and not included as part of the reward system. This approach was chosen because previous research linked these brain regions not primarily to reward processes, but rather to control, conflict and interoceptive processes during the processing of appetitive drug-related cues (Taylor et al., 2000; Naqvi et al., 2014).

Presented findings do not support the hypothesis that TUD subjects show a desensitization of the aversion related brain network towards aversive drug-related cues compared to NS per se, according to threat/ punishment desensitization theory. This result may be mainly driven by light smokers. Further, it may be that heavy and more dependent smokers may present functional aberrations in the aversion related brain network, as proposed by threat/ punishment desensitization theory.

Fourth question: Do quitting motivated TUD subjects show alterations in the application of cognitive down-regulation of craving for appetitive stimuli in the (prefrontal) control network?

Experiment III

Behavioral craving ratings showed no significant interaction between TUD subjects and NS in the NOW vs. LATER contrast for alternative rewards. Furthermore, no interaction was obtained between the two reward cue-types in the NOW vs. LATER contrast within TUD subjects. These findings are contrary to the first hypothesis of the fourth research question. However, as expected, craving ratings were significantly reduced in LATER compared to NOW trials independent of reward cue-type and smoking status. This result is in line with previous research investigating intentional down-regulation strategies to target craving for food (Wolz et al., 2020), cigarettes (e.g., Kober et al., 2010; Zhao et al., 2012) or emotions in general (Morawetz et al., 2017). Experiment III adds to these studies in that it provides a

direct comparison of down-regulation of craving between NS und TUD subjects. Compared to NS, TUD subjects showed no significant differences in craving regulation for food on a behavioral level. This result is well-suited with findings by Wu et al. (2015) in emotion regulation and suggests that TUD subjects may not exhibit general deficits in the cognitive down-regulation of craving for alternative rewards. Within the TUD group, the reduction of perceived levels of craving was similar for alternative rewards and drug-related cues. Thus, results suggest that TUD subjects do not present a specific form of cognitive down-regulation of craving deficits exclusively associated to drug-related cues. Findings of the behavioral analysis can be interpreted in form of neither general (between-subjects) nor specific (within-subjects) deficits in cognitive down-regulation of craving in highly quitting motivated TUD subjects.

Interestingly, TUD subjects showed a stronger activation in the middle temporal gyrus in the interaction analysis with NS in the alternative reward condition during down-regulation of craving. This brain region is in experiment III considered as part of the control network and may in particular play a role for the reenacting of an emotional scene (together with SMA and angular gyrus) which potentially represents the execution of down-regulation (Johnson-Frey et al., 2005). While decreased activations in control areas (representing regulation deficits) were hypothesized in TUD, the observed stronger activation in the middle temporal gyrus might be understood as a compensatory process, reflecting higher down-regulation efforts in TUD subjects compared to NS. On a behavioral level both groups showed comparable craving reductions for alternative rewards, thus different explanations may be plausible for a compensatory process in TUD subjects on a neurofunctional level. First, it might be speculated that TUD subjects need to compensatory down-regulate stronger mesolimbic cue-reactivity, elicited during the NOW condition, to reach similar craving ratings. Previous studies found stronger mesolimbic bottom-up processes, characterizing smoking behavior (Nestor et al., 2011). Second, it may be possible that a stronger middle temporal gyrus activation represents a compensation of a dysfunction in other cognitive control areas (e.g., vlPFC) to reach similar outcomes compared to NS on a behavioral level. It is important to note, that no differences in mesolimbic cue-reactivity for alternative rewards between TUD subjects and NS were found in this study. This speaks in favor of the second explanation (increased activation in smokers as a compensation of possible dysfunctions in other cognitive control areas), which would point to a general smoking-associated neurofunctional alteration during down-regulation of craving.

No evidence for a greater activation in (prefrontal) control areas during down-regulation of craving for alternative rewards compared to drug-related cues in TUD subjects was found.

Due to the on average moderate nicotine dependency, it might be possible that down-regulation of craving for both stimulus types was similarly challenging for TUD subjects. Results thus suggest no specific down-regulation impairment in TUD subjects, at least not in light to moderate smokers.

Significant relations between smoking severity and BOLD responses in control areas (vlPFC and SMA) during cognitive down-regulation of craving (collapsed across both reward cue-types) indicate a stronger activation in less severe smokers. Two explanations of this finding are possible: first, stronger down-regulation efforts (as reflected in stronger activation of control areas) may be present in more light smokers which may decline when individuals develop more severe smoking behavior. Second, it is also well possible that some individuals exhibit reduced down-regulation efforts (as reflected in lower activation in control areas) form the very beginning and consequently develop more severe smoking behavior. The cross-sectional findings do now allow conclusions on which explanation might be correct. Reduced down-regulation efforts, reflected in lower activation in parts of the prefrontal control network (e.g., vlPFC, SMA) might correspond with a possible deficit in down-regulation processes within TUD subjects, which might be driven especially by strong and heavy smokers.

As assumed in the hypothetical model on cognitive control processing, it can be proposed that TUD subjects show general cognitive down-regulation of craving alterations compared to NS on a neurofunctional level. No evidence for drug-specific alterations compared to other alternative rewards could be identified within TUD subjects. The general alterations in cognitive down-regulation of craving are in accordance to dual systems theory of addiction.

Fifth question: Does the processing of aversive drug-related cues has an influence on the subsequent processing of appetitive drug-related cues in quitting motivated TUD subjects?

Experiment IV

TUD subjects showed (i) reduced cigarette craving, but no reduced reactivity in mesolimbic reward areas, (ii) enhanced activation of prefrontal and paralimbic control areas (dlPFC, dACC and anterior insulae), with a positive association between aversion-related reduction of craving and prefrontal activation, and (iii) a negative connectivity between prefrontal and paralimbic control areas (dACC, anterior insula) with mesolimbic reward areas (putamen, caudate) during the processing of appetitive drug-related cues preceded by aversive drug-related cues.

The observed craving reduction induced by aversive drug-related cues was hypothesized *a priori* and is consistent with findings from a previous investigation that used graphic health warning labels with different emotional contents (Wang et al., 2015). However, contrary to another postulated hypothesis (pathway i in Figure 4), TUD subjects showed no significantly reduced activation of reward-associated brain areas. This result suggests that activation of the brain reward system through appetitive drug-related cues is not directly weakened by previous presentation of the negative consequences of smoking. One possible explanation could be that aversive drug-related cues do not exert a modulating impact on mesolimbic reward areas during processing of appetitive drug-related cues. It might be assumed that bottom-up reward processes in the mesolimbic brain network proceed automatically and are thus difficult to be influenced (Nestor et al., 2011). Another explanation might address the quitting interest of TUD subjects. Previous studies found that a quit interest can modulate smoking cue-reactivity responses in brain reward areas (Veilleux et al., 2016; Wilson et al., 2012). It might be presumed that an effect of aversive drug-related cues in the mesolimbic reward circuit is more difficult to detect in quitting motivated smokers and may require a lager sample.

Pathway ii of the hypothetical model proposed an (implicit) activation of prefrontal control areas. The prefrontal control network can be activated through different cognitive (implicit and explicit) strategies. Previous studies found that the usage of explicit instructions, to exert control over appetitive stimuli (e.g., reappraisal), activates the prefrontal control network (e.g., Kober et al., 2010). Results gained by experiment IV demonstrate an activation of prefrontal control areas during the processing of appetitive drug-related cues without the instruction to actively apply any strategies. This might suggest an indirect, implicit route of cognitive control through preceding aversive drug-related cues.

An increased negative functional connectivity between prefrontal control areas and parts of the mesolimbic reward system was proposed by the hypothetical model in pathway iii (Figure 4). This pathway suggests down-regulation processes from prefrontal control areas to mesolimbic brain areas (Do et al., 2015). A significant negative functional connectivity between parts of the prefrontal control network and mesolimbic brain areas could be identified. This result is complemented by the negative association between right dACC activation and craving reduction, induced by aversive drug-related cues. A reduction of the overall motivational appeal of smoking may be achieved through balancing the value of cigarettes with the value of the anticipated reward. The value of anticipated reward in mesolimbic brain regions may be down-regulated by prefrontal/ paralimbic control areas when aversive drug-related cues are presented before.

Overall, the findings gained by experiment IV provide a first empirical evidence for different hypotheses of an influenced drug-cue reactivity within TUD subjects. Whereas pathway i could not be confirmed on a neurofunctional level, evidence for pathway ii and iii could be obtained.

5.2 Clinical implications

The results gained by the experiments of this thesis can be translated into clinical applications, informing smoking prevention strategies and cessation interventions. As mentioned in chapter 1, pharmacotherapies such as bupropion or the substitution of nicotine show effects mainly in reducing withdrawal symptoms experienced during cessation. Besides withdrawal, relapse in abstinent smokers has, however, various causes. Therefore, additional efforts are needed to support nicotine abstinence (see paragraph 1.1). Non-pharmacological treatment is important to address the full spectrum of neurobiological alterations that underlie TUD. In this context, the results outlined in this thesis are clinically relevant as they offer suggestions to enhance already existing prevention strategies and smoking cessation interventions. Moreover, they may also provide starting points for new, alternative treatment approaches.

First, it can be suggested that smoking cessation interventions should address strategies to enhance the value and processing of alternative rewards, as for instance intended by psychoeducation and enjoyment trainings. This approach should be implemented from the beginning of TUD and should be addressed in every state of the disorder, because alterations in the brain reward network may develop rather fast. For instance, CBT methods could include an individualized training session to identify personally significant rewards and activate alternative rewards in the treatment of quitting motivated TUD subjects. Methods to train the perception and enjoyment of alternative rewards could present a separate treatment option which could be supported by virtual programs (e.g., in form of online applications). Further, individual reward reinforcement plans can be developed to guide the process of abstinence. For instance, by creating a list of individualized rewards that can be achieved after a specific period of abstinence (e.g., after one day, five weeks and so on). Furthermore, targeted prevention strategies could include the strengthening of alternative rewards to prevent an alteration of the brain reward network in subjects who are susceptible of becoming addicted (e.g., those who show a harmful use of nicotine).

Second, the confrontation with long-term consequences of chronic smoking behavior (independent of the subsequent presentation of appetitive drug-related cues) seems to be especially efficient for light smokers. Approaches for smoking cessation in light to moderate smokers may include the presentation of pictures showing long-term consequences to raise awareness in this group which seems more susceptible for the negative consequences.

Third, especially smoking individuals who are vulnerable of developing an anxiety disorder should be identified as early as possible (e.g., using specific questionnaires or clinical interviews) and specific smoking cessation options should be offered to them. If successful, such specific treatment approaches may ameliorate the adverse effects of smoking on the pathogenesis of a PD (and possibly other anxiety disorders) on a neural systems level.

Fourth, quitting motivated TUD subjects activate (prefrontal) control networks while applying cognitive down-regulation of craving for appetitive stimuli. The application of such cognitive down-regulation strategies is also part of clinical smoking cessation programs (e.g., CBT interventions). However, in recent years, a reduction in abstinence rates in treatment-seeking smokers has been observed (Irvin & Brandon, 2000; Leyro et al., 2016). One possibility to encounter these effects may be to enhance (e.g., using more intensive trainings) or augment (e.g., using passive and active neurofeedback methods) specific therapeutic strategies as e.g., cognitive down-regulation of craving. Especially heavy smokers might profit from an enhancement or augmentation of cessation techniques as results by this thesis suggest that (prefrontal) control activations are negatively associated with smoking severity. Results gained by this thesis can provide target regions (e.g., vlPFC) for an augmentation of down-regulation of craving processes in TUD subjects using e.g., neurofeedback (NF) techniques (see also point six for a more extensive explanation on NF methods).

Fifth, aversive drug-related cues do have an influence on the processing of appetitive drug-related cues. An indirect and automatic activation of control processes through prior presentation of (unknown) aversive drug-related cues can be suggested. Such strategies could complement explicit cognitive approaches through different forms of application. As a novel part of smoking cessation therapy, unknown aversive drug-related cues could be paired with individualized appetitive drug-related cues of quitting motivated smokers in a conditioning paradigm. Such strategies may induce decreased craving for these favorite drug cues through enhanced cognitive control. Furthermore, it could be beneficial to make aversive drug-related cues more visible in different places where smokers are used to consume cigarettes (e.g.,

smoking areas in public places). It can be assumed that the down-regulation effect of the contingent presented aversive cue is rather short in duration, evidenced by the connectivity analysis in experiment IV. This underlines that prevention strategies or cessation interventions may benefit from the immediate and contingent presence of aversive drug-related cues during drug consumption (e.g., on cigarette packets or in novel conditioning paradigms).

Sixth, the results can be used to inform alternative and innovative intervention strategies, which for example directly target the brain. Repetitive transcranial magnetic stimulation was for example used to modulate frontal brain activity to improve smoking cessation (Chang et al., 2018). Real-time (fMRI or fNIRS) NF techniques provide a more active form of neuromodulation by enabling the immediate visualization of brain activations. Real-time fMRI NF was for example successfully applied in TUD subjects as an appropriate method to attenuate craving (Hartwell et al., 2013; Kim et al., 2015). Thus, these approaches represent useful and clinically meaningful treatment modalities for TUD (Hanlon et al., 2013; for reviews see Martz et al., 2020 & Wing et al., 2013). However, for the successful application of such strategies, further research is needed to detect appropriate brain regions that can be targeted and environments that can be used for trainings. Results gained by this thesis can provide suggestions for both points. First, new target regions for such interventions might include e.g., the vlPFC, OFC or hippocampus, as these brain regions showed alterations in different tasks between TUD subjects and NS as well as within TUD subjects. Second, new surroundings, where these techniques might be applied include alternative reward learning paradigms and cognitive down-regulation of craving approaches. As an example, real-time fMRI NF approaches could use brain regions belonging to the prefrontal control network (e.g., vlPFC, dlPFC) as regions of interest in a paradigm where smokers are instructed to up regulate these ROIs during down-regulation of craving, thereby receiving a direct feedback from these target ROIs.

5.3 Limitations and future directions

Several limitations have to be considered when interpreting and translating the presented results.

First, all experiments have a cross-sectional design, and hence causal interactions between smoking behavior and neurofunctional and -structural findings cannot be inferred. It cannot be concluded if the observed alterations in different brain networks present primarily vulnerability factors for the initiation of smoking behavior or are caused secondarily by

smoking. Although it seems plausible that alterations in the brain reward system, concerning drug-related cues, develop after smoking onset, it remains possible that TUD subjects already present aberrant reward processes before starting to smoke. Vulnerability for aberrant reward processing could in fact constitute a risk factor itself for smoking initiation, which has to be elaborated in longitudinal designs**.** Furthermore, it remains possible that heavy smokers were more used to pictures showing drug-related negative consequences as they are more often confronted with pictures used for health campaigns on cigarette packets. However, to avoid this potential confounder, pictures were presented that are neither used by health campaigns and nor already used on cigarette packets. Additionally, the possibility of any preexisting structural differences between smokers and non-smokers cannot be excluded.

Second, while individualized cues are a strength of experiment I, this requires additional consideration as any differences in the types of cues each group selected could (conceivably) create a bias. However, no systematic preference for a specific food or threat category in any of the two groups could be shown and the groups did not differ in their ratings for single cues. Thus, the risk of bias seems rather low and does not account for the findings.

Third the inclusion of a younger age group in experiments I, III and IV (e.g., 18 years) may represent a potential bias, as smoking may not have been established yet and the brain is still in maturation at this age. However, there were only three participants between the age of 20–25 included in the analysis, assuming this level of bias rather low.

Fourth, the included sample of TUD subjects was limited to only moderately nicotine dependent individuals. Including TUD subjects who show stronger dependency could impact results, a possibility that has to be elaborated in future studies.

Fifth, it is important to keep in mind that the use of food cues as alternative rewards may have a different effect on the reward system compared to other alternative reward categories (e.g., money). Previous studies showed that nicotine can act as an appetite suppressant (Jo et al., 2002) or increased appetite can represent a withdrawal symptom (al'Absi et al., 2014). This needs to be considered when interpreting the results and comparing them to studies that used other categories of alternative rewards (e.g., money).

Sixth, concerning experiment II, the study was not primarily designed to examine the effect of smoking. Therefore, only limited data was available on smoking behavior. A more detailed assessment of smoking severity, history and dependence could however shed more light on the observed differences. Additionally, significantly more PD smokers suffered from a

comorbid depressive disorder which seems plausible, considering the link between smoking and symptom severity (Covey et al., 1998; Zvolensky et al., 2005). The effects, though remained stable after controlling for depressive symptom scores using the BDI-II. Concerning the explorative VBM analysis between TUD subjects and NS, results are limited by the small sample size that could create a bias (Ioannidis, 2011).

Seventh, the measured BOLD signal is dependent on neurovascular couplings (Mathias et al., 2017) and perfusion (London et al., 2009). Smoking has an impact on the neurovascular system (Durazzo et al., 2015; Paulson & Vigdis, 2020). Nicotine and tobacco smoke have been shown to have both vasoconstrictive and vasodilatory effects on the cerebrovasculature (Iida et al., 1998). Few studies have investigated smoking and cerebral blood flow (CBF) and found that chronic smoking was associated with reduced global CBF (Rogers et al., 1983; Rogers et al., 1985; Mathew, & Wilson, 1991). Other studies found evidence that current smoking increases the CBF (Paulson & Vigdis, 2020; Elbejjani et al., 2019). This needs to be considered when interpreting group comparisons with NS. However, a bias would be mainly expected in form of weaker effects in smokers (Durazzo et al., 2015), meaning that the presented results would rather underestimate higher effects than postulating false positive effects.

Future studies are needed to further elaborate on these points. Longitudinal designs are clearly necessary to disentangle whether the described alterations precede the onset of a TUD (making subjects even more vulnerable to start smoking) or if the alterations are caused by smoking and worsen with a chronic progression of TUD. Longitudinal designs would further allow to track the progression of brain changes and test for the interaction of the processes described in this thesis.

Furthermore, investigating a group of more and heavier dependent smokers who are not motivated to quit could help to clarify some of the observed effects. It remains to be elaborated if the observed results can be transferred to these groups of smokers. Future studies should additionally specify the effects of reward, threat and cognitive control processing, for example by the use of different categories of drug-related negative cues (e.g., pictures used on cigarette packets vs. novel cues) and positive cues (e.g., pictures showing individualized alternative rewards of each subject).

As experiment II represents a preliminary investigation of the modulating impact of actual smoking behavior on commonly observed brain structures in PD patients, future studies are encouraged to investigate this interaction in more depth, including elaborate measures on

smoking behavior. Furthermore, it remains to be investigated if smoking has a modulating impact in patients suffering from other mental disorders which could also be related to the aversion related brain network and to chronic smoking behavior (e.g., depressive disorders).

From a network perspective, upcoming studies investigating different processes of reward, threat and cognitive control, could use resting state data or tractography to test for alterations of the described networks. They may thereby be able to identify neural pathways that allow refining the presented model and provide targets for possible future interventions to promote smoking cessation.

Finally, it seems highly important to link the observed brain alterations to smoking cessation therapy success. Therefore, longitudinal smoking cessation studies are needed to investigate a predictive value of these aberrations in TUD cessation success. Furthermore, it would be of interest to investigate whether activations in the identified brain regions predict behavioral measures, such as the ability to resist craving or if the described alterations are changing after successful cessation. Therefore, longitudinal study designs in combination with multimodal data sets and advanced statistical methods (e.g., machine learning classification or clustering approaches) are desirable as they allow identifying individuals who are more or less likely to quit smoking successfully.

5.4 Conclusion

In conclusion, results gained by the experiments of this thesis support hypotheses on neural alterations in TUD subjects and suggest an integrative reward, threat/ punishment and cognitive control processing model of TUD. They can enlarge our understanding of brain alterations related to TUD and to translate implications for prevention strategies and smoking cessation interventions.

From a theoretical perspective, results gained by this thesis provide empirical evidence for hypotheses derived from previous studies and theoretical models of addiction. Results confirm alterations in brain networks related to reward, threat/ punishment and cognitive control processes in TUD subjects und thus suggest an integrative model. However, further research is clearly need to replicate these findings in a large and independent sample.

From a clinical mechanism-based perspective, brain alterations related to TUD and joined into an integrative model can be used to inform smoking prevention and cessation interventions. Alterations in the brain reward network in TUD subjects may suggest to apply strategies to

enhance the value and processing of alternative rewards (e.g., using CBT-techniques). A confrontation with long-term consequences of chronic smoking behavior may be more appropriate for light smokers, since heavier TUD subjects could present a desensitization of the aversion related brain network. Cognitive down-regulation of craving alterations in TUD subjects might be targeted using NF techniques (e.g., real-time fMRI NF) to enhance prefrontal control activation. Implicit cognitive control over reward processes are applied by TUD subjects when confronted with long-term consequences of consumption. These might be used as a novel part of smoking cessation therapy, where unknown aversive drug-related cues could be paired with individualized appetitive drug-related cues in a conditioning paradigm. These implications remain to be investigated using clinical designs in TUD subjects.

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SUPPLEMENTS

A Research articles

Experiment I

Kunas, S. L., Stuke, H., Heinz, A., Ströhle, A., & Bermpohl, F. (2021). Evidence for a "hi-jacked" brain reward system but no desensitized threat system in quitting motivated smokers: An fMRI study. *Addiction*, 2021;1-12.

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Experiment II

Kunas, S. L., Hilbert, K., Yang, Y., Richter, J., Hamm, A., Wittmann, A., ... & Lueken, U. (2020). The modulating impact of cigarette smoking on brain structure in panic disorder: a voxel-based morphometry study. *Social cognitive and affective neuroscience*, *15*(8), 849-859.

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Experiment III

Kunas, S. L., Stuke, H., Plank, I.S., Laing, E.M., Bermpohl, F., & Ströhle, A. (submitted). Neurofunctional alterations of cognitive down-regulation of craving in quitting motivated smokers

Experiment IV

Kunas, S.L., Bermpohl, F., Plank, I.S., Ströhle, A., & Stuke, H. (2021). Aversive drug cues reduce cigarette craving and increase prefrontal cortex activation during processing of cigarette cues in quitting motivated smokers. *Addiction Biology*, 2021;e13091.

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RESEARCH REPORT

ADDICTION SSA

Evidence for a hijacked brain reward system but no desensitized threat system in quitting-motivated smokers: An fMRI study

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Abstract

Background and aims: Several aspects of how quitting-motivated tobacco use disorder (TUD) subjects and never-smokers differ in terms of reward and threat processing remain unresolved. We aimed to examine aberrant reward and threat processes in TUD and the association with smoking characteristics.

Design: A between- and within-subjects functional magnetic resonance imaging (fMRI) experiment with a 2 (groups) × 4 (stimulus type) factorial design. The experimental paradigm had four conditions: pictures of (1) cigarettes served as drug-related-positive cues, (2) food as alternative reward cues, (3) long-term consequences of smoking as drug-related-negative cues and (4) neutral pictures as control.

Setting/participants: Adult participants ($n = 38$ TUD subjects and $n = 42$ never-smokers) were recruited in Berlin, Germany.

Measurements: As contrasts of primary interest, the interactions of group × stimulus-type were assessed. Significance threshold correction for multiple testing was carried out with the family-wise error method. Correlation analyses were used to test the association with smoking characteristics.

Findings: The 2 × 2 interaction of smoking status and stimulus type revealed activations in the brain reward system to drug-related-positive cues in TUD subjects (between-subjects effect: P-values ≤ 0.036). As a response to drug-related-negative cues, TUD subjects showed no reduced activation of the aversive brain network. Within the TUD group, a significant negative association was found between response of the aversive brain system to drug-related-negative cues (within-subjects effect: P-values ≤ 0.021) and the number of cigarettes smoked per day (right insula $r = -0.386$, $P = 0.024$; left insula $r = -0.351$, $P = 0.042$; right ACC $r = -0.359$, $P = 0.037$).

Conclusions: Moderate smokers with tobacco use disorder appear to have altered brain reward processing of drug-related-positive (but not negative) cues compared with never smokers.

KEYWORDS
Cue-reactivity, fMRI, quitting motivation, reward processing, threat processing, tobacco use disorder

INTRODUCTION

Throughout the 20th century, tobacco smoking contributed to the death of approximately 100 million people [1]. It is associated with health consequences such as lung cancer or pulmonary disease [2], which result in premature death in approximately 50% of smokers [3]. This makes smoking the primary cause of preventable deaths [4]. Only approximately 7% of dependent smokers attempting to quit remain abstinent after 12 months [5]. In order to develop new and improve already existing strategies to aid abstinence in quitting-motivated smokers, it is of great importance to understand the mechanisms which underlie tobacco use disorder (TUD) and can provide promising targets for successful smoking cessation interventions.

One previously investigated mechanism involved in the maintenance of TUD is a disruption of reward processing [6,7]. According to incentive–sensitization theory [8,9], addiction and craving develop as a consequence of neuroadaptations induced by repeated consumption of drugs. It is proposed that the mesocorticolimbic brain system, which is involved in the assignment of incentive salience to rewarding stimuli, gradually becomes sensitized to drug-related stimuli and desensitized to non-drug-related alternative rewards [6–9]. Brain structures involved in the cortico–striatal–limbic reward pathway include the amygdala, ventral tegmental area (VTA), hippocampus, ventral pallidum, nucleus accumbens (NAc), medial thalamus and orbitofrontal/medial pre-frontal cortex (mPFC) [10].

The construct of hypersensitivity to drug-associated rewards is supported by several functional magnetic resonance imaging (fMRI) investigations that found heightened activity in mesocorticolimbic areas (e.g. ventral striatum, NAc) in smokers following presentation of drug-related cues compared to healthy controls or neutral cues (e.g. [11–15]). Furthermore, previous studies could demonstrate a reduced activation in smokers as a response to non-drug alternative rewards compared to healthy controls or neutral cues (e.g. [16–21]); for a meta-analysis, see Lin *et al.* [6]. However, many questions still remain unanswered. Non-quitting-motivated smokers were investigated in most studies (e.g. [22,23]), making it more difficult to derive suggestions for smoking cessation programs. In addition, former studies typically compared drug-related or alternative reward cues directly to neutral cues or compared smokers with non-smokers, while not investigating the function of smoking status and reward processing together (e.g. [24,25]). Finally, mainly non-individualized monetary cues were used as alternative rewards (e.g. [26–28]), thus limiting the external validity of studies. Therefore, several aspects of how quitting-motivated TUD subjects and never-smokers differ in terms of reward processing still remain unresolved. Moreover, it is not clear whether such changes are related to the severity of addiction or other characteristics of TUD subjects.

In addition to a potentially 'hijacked' brain reward system, TUD is marked by persistent drug use despite experience or knowledge of its negative consequences. According to Campbell [29], this decreased sensitivity to the negative aspects of consumption is not only a key factor in the maintenance of addiction in general, but one of its defining characteristics. From a theoretical perspective, addiction is marked by a decreased sensitivity to the negative aspects of consumption [30]. Hayes & Northoff [31] identified a core aversion-related brain network associated with the processing of threat stimuli, encompassing cortical and subcortical areas [e.g. amygdala, anterior cingulate cortex (ACC), hippocampus, thalamus, insula, DMPFC, secondary motor cortex]. From a neurofunctional perspective, it can be assumed that this network is also involved in the processing of aversive aspects of drug use and may be altered in subjects suffering from substance use disorders.

To date, only few studies have attempted to elucidate a disruption in the processing of aversive aspects of smoking addiction in regular smokers [32–37]. Dinh-Williams and colleagues [33] showed that non-quitting-motivated chronic smokers display greater activations in regions of the visual association cortex and extended visual system as well as in pre-frontal and limbic brain structures in response to aversive smoking-related images compared to neutral cues. However, they did not include a control group of non-smokers. Therefore, it remains unclear whether quitting-motivated TUD subjects present an aberrant processing of drug-related-negative cues which could constitute an important mechanism underlying the maintenance of TUD.

Summarizing the above, a 'hijacked' reward system and a desensitized aversive system may represent two mechanisms of smoking preservation which are, to date, not sufficiently understood.

To address these issues, we examined quitting-motivated TUD subjects and applied a novel extended cue-reactivity paradigm. The primary aim of this study was to investigate aberrant reward and threat processes in TUD subjects and the association with behavioral smoking characteristics; therefore, we hypothesized that:

1. increased activations elicited by drug-related-positive cues in mesocorticolimbic brain structures in quitting-motivated TUD subjects compared to never-smokers as well as decreased functional activation elicited by alternative rewards;
2. stronger activations in a network characteristic for threat processing in response to drug-related-negative cues (e.g. lung cancer) in never-smokers compared to quitting-motivated TUD subjects; and
3. that heavier and more dependent TUD subjects would show greater activations in mesocorticolimbic brain areas during altered reward processing and a reduced response to drug-related-negative cues in areas related to threat processing.

Additionally, for sensitivity analysis, we investigated general reward and threat processing among both groups, and for the sake of completeness and to replicate findings of previous investigations we examined the effects of the different stimulus types separated for both groups.

MATERIALS AND METHODS

Participants

The present study was conducted within the framework of the German Collaborative Research Center (TRR 265: 'Losing and regaining control over drug intake'), funded by the German research foundation (DFG). In total, 82 participants (39 TUD subjects and 43 never-smokers) underwent fMRI scanning. Due to technical issues, 38 TUD subjects (55.26% female) and 42 never-smokers (73.81% female) were included in the present analysis (for a consort flow-chart see Supporting information, Fig. S1). Participants were recruited in Berlin using advertising and flyers. Inclusion criteria for TUD subjects were (a) current DSM-5-TR diagnosis of TUD verified by a structured clinical interview for DSM-5-TR [38]; and (b) aged between 18 and 65 years. Exclusion criteria were (a) comorbid DSM-5-TR mental disorder within the last 12 months; (b) life-time history of any substance-use disorder other than TUD and bipolar or psychotic disorders; (c) current suicidal intent; (d) concurrent psychopharmacological or psychotherapeutic/psychiatric treatment; (e) history of brain injury; and (f) pregnancy. Participants were classified as never-smokers if they had smoked fewer than 10 cigarettes during their life-time. The never-smoker group was free of current or past medical, neurological or mental illness. Healthy controls as well as TUD subjects received financial compensation (€50) for their participation in the study. After the examination all TUD subjects took part in a free, 6-week smoking cessation intervention, as all of them were quitting-motivated. Furthermore, half the participants were randomized to an additional sport intervention. The study was approved by the local ethics committee and all subjects gave written, informed consent prior to participating in the study.

As the primary research question and analysis plan of this study were not pre-registered on a publicly available platform, the results should be considered exploratory.

Clinical assessments

During the first session, all participants completed the multiple-choice vocabulary test (MWT; range = 0–37) [39] to assess their global level of intelligence, the trait part of the State–Trait Anxiety-Inventory (STAI-T; range = 20–80) [40] and the short version of the General Depression Scale [anxiety and depression scale (ADS-K); range = 0–45] [41]. The Fagerström Test for Nicotine Dependence (FTND; range = 0–10) [42] was used to assess severity of nicotine dependence. Furthermore, information regarding frequency of alcohol use was acquired (drinking days/week). For more details regarding the tests see also Supporting information, Text S1.

Extended cue-reactivity task

We established a novel extended cue-reactivity task, which was performed during fMRI. We asked participants to abstain from smoking and eating for 3 hours. This duration of abstinence was chosen to ensure a sufficient level of craving for cigarettes, but avoid severe withdrawal in the moderately dependent TUD group at the time of the fMRI scanning. The task was used to study drug-related-positive, drug-related-negative and alternative reward cue-reactivity at the psychological and neural level. The experimental paradigm consisted of four conditions: established photographs displaying cigarette items were used as drug-related-positive cues, pictures of healthy, low-fat, attractive food were used as alternative reward cues, pictures showing long-term consequences of smoking (e.g. bronchial carcinoma) were used as drug-related-negative cues and pictures displaying neutrally valenced items were presented during neutral control conditions. Before the fMRI session, participants rated a set of 144 drug-related-positive, alternative reward and drug-related-negative pictures each. For drug-related-positive and alternative reward pictures, the question 'how strong is your desire to consume this now?' was used. For drug-related-negative cues, the question 'how deterrent do you experience this picture?' was asked, using an eight-point Likert-scale from 'not at all' to 'very much'. For the experiment, the 50% most rewarding/threatening stimuli were automatically selected in order to maximize effects (for an example run and more details regarding the task see Fig. 1, and for more details regarding the selected cues see Supporting information, Text S2).

Statistical analysis of behavioral data

To examine differences in drug and food craving ratings (as well as in threat ratings) before and within the task the Scheirer–Ray–Hare test was used, as specific assumptions for an analysis of variance were violated (see Supporting information, Text S2). To specify the direction of the effects, we used Mann–Whitney *U*-tests. For analysis of the differences in craving ratings between the two groups at the end of each run, we also used the Mann–Whitney *U*-test. A paired *t*-test was conducted to quantify the impact of drug-related-negative cues on subjective desire for cigarettes; therefore, we calculated the difference between craving ratings at the end of each run when preceded by drug-related-negative cues in comparison to the other categories for the TUD group. Furthermore, the difference between alternative reward and drug-related-positive cues in TUD subjects was examined using a paired *t*-test (see also Supporting information, Text S2).

fMRI data acquisition and pre-processing

The study was conducted with a 3-Tesla Siemens Magnetom Prisma scanner. Functional images were acquired using T2-weighted

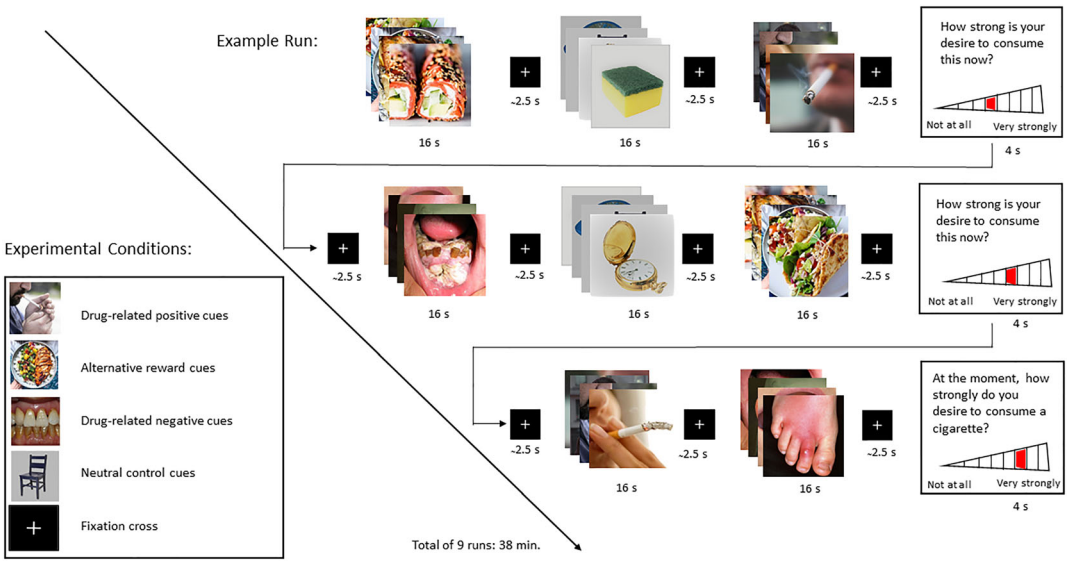

FIGURE 1 Four pictures of one category were presented per block. Each block lasted 16 seconds and ended with the presentation of a fixation cross [intertrial interval (ITI)], jittered around 2.5 seconds. In each run, two blocks of each of the four categories were presented. Subjects were instructed to attend to all stimuli and to rate their current desire to consume some of these items (cigarette or food) twice per run, by pressing one of eight buttons covering an eight-point scale ranging from 'not at all' to 'very strongly'. At the end of each run, participants were additionally asked to rate how strongly they desire to smoke a cigarette, using the same rating scale as described above. In total, the task consisted of nine runs, which altogether lasted 38 minutes [Color figure can be viewed at wileyonlinelibrary.com]

gradient-echo echoplanar imaging (TR 869 ms, TE 38 ms, voxel size 2.4 × 2.4 × 2.4 mm) and anatomical images were acquired using a T1-weighted MPRAGE sequence (voxel size 1 × 1 × 1 mm) using a 64-channel head coil. To minimize movement artifacts, participants' heads were positioned on a pillow and fixed using foam pads surrounding the head. Image pre-processing was performed using statistical parametric mapping (SPM12; http://www.fil.ion.ucl.ac.uk/spm/software/spm12) and MATLAB R2020a (Mathworks, Sherborn, MA, USA)-based scripts and comprised slice timing with reference to the middle slice, SPM12 standard re-alignment and unwarping including correction for field deformations based on a previously acquired field map, co-registration, normalization to Montreal Neurological Institute (MNI) stereotactic space using unified segmentation based on the SPM tissue probability map for six tissue classes and spatial smoothing with 8-mm full-width at half-maximum isotropic Gaussian kernel. Following pre-processing, all nine runs were visually inspected, for each subject separately, in order to perform a visual quality control.

fMRI data analysis pathway

First-level analysis was carried out as described in Supporting information, Text S3. For second-level analysis, group effects were assessed by a 2 × 4 analysis of covariance (ANCOVA) using a full factorial model in SPM12, encompassing the factors 'group' (TUD subjects and never-smokers) and 'stimulus-type' (neutral, alternative reward, drug-related-positive and drug-related-negative). The continuous variables of the STAI-T and ADS-K were included as covariates of no interest because of significant group differences in these measures (see below). In accordance with our hypotheses, we tested the group × stimulus interactions (drug-related-positive versus alternative reward cues and drug-related-negative versus neutral cues). For sensitivity analyses, we investigated the effect of reward processing across both groups and stimulus types (drug-related-positive and alternative reward > neutral) as well as the effect of threat responsivity across both groups (threat > neutral). For the sake of completeness and to replicate findings of previous investigations, we report the effects of drug-related-positive, alternative reward and drug-related-negative cues contrasted to neutral cues within each of the two groups separately. *F*-contrasts were computed followed by post-hoc *t*-contrasts to specify the direction of the effects.

Region of interest (ROI) and whole brain analysis

As small subcortical brain regions (e.g. VTA) are difficult to investigate using a whole brain approach, an anatomical ROI analysis of a priori-defined subcortical brain areas was conducted. Based on the literature of brain regions involved in reward processing [10,43], the reward system was defined to include NAc, amygdalae, hippocampi, thalamus, pallidum and mid-brain (including VTA) for hypothesis 1. Based on the model proposed by Hayes & Northof [31], the core aversive system was defined to include amygdalae, hippocampi, insulae, ACC and thalamus for hypothesis 2. The a priori-defined anatomical regions of interest were built combining definitions from the Automated Anatomical Labeling Atlas [44], implemented in the Wake Forest

University PickAtlas [45]. The bilateral ROIs were investigated using one single mask. Small volume correction on this single mask was applied using a family-wise error (fwe)-corrected threshold of $P_{fwe} < 0.05$ with a minimum cluster size of $k = 10$ contiguous voxels. ROI analyses were followed by whole brain analyses. To correct for multiple comparisons on a whole brain level, group-level results were thresholded at $P < 0.05$ fwe-corrected.

Correlation analysis

To evaluate the relationship of altered reward processing and threat responsivity with dimensional measures of nicotine addiction in TUD subjects only, Pearson's correlations were calculated between extracted beta-values of significantly activated brain regions identified in the ROI analysis for the two contrasts of interest [TUD subjects: (drug > alt) and (threat > neutral)] with the FTND (as a dimensional measure of nicotine dependence), cigarettes smoked per day (implying that heavy smokers consume a higher number of cigarettes per day) and pack-years (calculated as the product of smoking amount and time). Moreover, the difference between craving ratings at the end of each run when preceded by other categories in comparison to drug-related-negative cues (mean craving rating after other cues minus mean craving rating after drug-related-negative cues) were correlated with the FTND, cigarettes smoked per day and pack-years. The toolbox marsbar (http://marsbar.sourceforge.net) was used in SPM12 to extract the beta-weights using a sphere of 5 mm around the peak voxel of significant ROIs (see Tables 2 and 3 for MNI coordinates). Age served as covariate in the partial correlation analysis of pack-years (see also Supporting information, Text S2 for more details).

RESULTS

Sample characteristics

Demographic data and smoking characteristics are shown in Table 1. TUD subjects were moderately nicotine-dependent, as evidenced by FTND and average cigarettes smoked per day. STAI-T and ADS-K scores were, although subclinical in both groups, significantly higher in TUD subjects, which is in line with results from previous investigations [46].

Subjective craving ratings

Within-task craving ratings showed the expected main effect of group ($H_{(1/156)} = 33.115$, $P < 0.001$, $\eta^2 = 0.179$), stimulus ($H_{(1/156)} = 128.579$, $P < 0.001$, $\eta^2 = 0.458$) and group × stimulus interaction ($H_{(1/156)} = 27.851$, $P < 0.001$; $\eta^2 = 0.155$); see also Table 1 and Supporting information, Fig. S3. In the group of TUD subjects, final craving ratings were significantly lower when drug-related-negative cues compared to other cues preceded the rating ($t_{(36)} = -6.09$, $P < 0.001$, $d = 1.348$). Within the task, TUD subjects rated their craving for food significantly higher compared to cigarettes ($t_{(35)} = -5.453$, $P < 0.001$, $d = 1.263$), and at the end of each run they rated a medium desire to smoke a cigarette now (for ratings conducted before the fMRI session see Supporting information, Fig. S2).

fMRI results

Altered reward processing

The 2 × 2 interaction of smoking status and stimulus type [TUD subjects (drug > alt) > never-smokers (drug > alt)] revealed stronger activation in the bilateral hippocampi and thalamus as well as in the left mid-brain (including VTA) in TUD subjects regarding drug-related-positive cues in the ROI analysis (Table 2, Figure. 2). On a whole brain level, the OFC was significantly activated.

Altered threat responsivity

The 2 × 2 interaction of smoking status and stimulus type [never-smokers (threat > neutral) > TUD subjects (threat > neutral)] reached no significant results, neither on a ROI nor on a whole brain level (Table 2).

Sensitivity analysis

The processing of reward in general (drug-related-positive and alternative reward) against neutral cues elicited brain activation in the bilateral thalamus, hippocampi, mid-brain (including VTA) and pallidum in the ROI analysis (Supporting information, Table S1) among both groups. On a whole brain level, frontal, parietal, temporal occipital as well as subcortical brain areas (ACC) were activated (Supporting information, Text S4). The effect of threat responsivity reached significant activation in the bilateral insulae, hippocampi, thalamus and in the right ACC in the ROI analysis (Supporting information, Table S1) among both groups. On a whole brain level, frontal, parietal, temporal and occipital brain regions were activated (Supporting information, Text S4 and Supporting information, Table S1).

Investigating the two groups separately regarding threat responsivity, TUD subjects showed significant activation in structures belonging to the aversive brain system (bilateral insulae, right ACC and hippocampus). On a whole brain level, TUD subjects showed significantly activated brain regions in the lingual and occipital gyrus, temporal gyrus, inferior frontal gyrus, superior and inferior parietal gyrus and in the right insula and ACC (Table 3 and Fig. 3). Conversely, never-smokers showed no significant brain activation in the ROI analysis. On a whole brain level, significantly activated brain regions in the occipital and parietal cortex could be observed (Table 3).

TABLE 1 Socio-demographic and psychometric characteristics of the smoker and never-smoker sample.

Sample characteristic	TUD subjects $n = 38$	Never-smokers $n = 42$	Statistic	P
Age (mean, SD)	35.18 (10.57)	32.36 (10.97)	$t_{(78)} = -1.17$	0.245
Female gender (*n*, %)	21 (55.26)	31 (73.81)	$\chi^2_{(1)} = 3.016$	0.090
Right-handedness (*n*, %)	38(100)	39(92.86)	$\chi^2_{(1)} = 2.747$	0.100
Level of education				
A-level[a] (*n*, %)	30 (78.95)	35 (83.33)	$\chi^2_{(1)} = 0.252$	0.616
Monthly income in € (*n*, %)				
< 1000	7 (18.42)	16 (38.10)	$\chi^2_{(4)} = 6.401$	0.171
1000–2000	12 (31.58)	12 (28.57)		
2000–3500	16 (42.11)	11 (26.19)		
3500–4500	2 (5.26)	–		
> 4500	1 (2.63)	1 (2.38)		
MWT (mean, SD)	28.32 (4.67)	29.38 (3.26)	$t_{(78)} = 1.192$	0.237
Craving ratings (median, IQR)				
Alternative reward	5.66 (1.60)	5.36 (2.95)	$U_{(78)} = 0.130$	0.896
Cigarette cues	3.56 (2.22)	1.00 (0.28)	$U_{(78)} = 7.387$	< 0.001**
Final craving rating	5.00 (3.50)	1.00 (0.00)	$U_{(80)} = 7.557$	< 0.001**
Drinking days (per week) (mean, SD)	1.89 (1.13)	1.26 (0.87)	$t_{(78)} = -2.64$	0.012*
STAI-T (mean, SD)	38.90 (7.42)	31.09 (6.33)	$t_{(78)} = -5.02$	< 0.001**
ADS-K (mean, SD)	7.97 (5.05)	4.12 (2.87)	$t_{(78)} = -4.09$	< 0.001**
FTND (mean, SD)	4.03 (2.27)			
Pack-years (mean, SD)	10.75 (9.55)			
Cigarettes/day (mean, SD)	14.40 (6.05)			

Abreviations: ADS-K = general depression scale; FTND = Fagerström Test for Nicotine Dependence. Missing values: monthly income: 1; FTND: 2; cigarettes/day: 2; craving ratings for alternative reward and cigarette cues 2; missing values were treated with listwise deletion. For the Mann–Whitney *U*-test we report the standardized test statistic; IQR = interquartile range; MWT = Mehrfachwahl–Wortschatz test (identification test); SD = standard deviation; STAI-T = trait part of the State–Trait Anxiety Inventory.
*$P < 0.05$; **$P < 0.001$.
[a]Abitur.

Regarding the effects of drug-related-positive cues and alternative rewards, separated for the two groups, please refer to Supporting information, Text S4 and Supporting information, Tables S3 and S4.

Correlation analysis

No significant correlations between activated brain regions and dimensional measures of smoking behavior could be obtained for altered reward processing in TUD subjects (Supporting information, Table S2).

Regarding threat processing, correlation analyses revealed significant negative associations between the number of cigarettes smoked per day and extracted beta weights of the left ($r = -0.351$; $P = 0.042$) and right insula cortex ($r = -0.386$; $P = 0.024$) and ACC ($r = -0.359$; $P = 0.037$) in TUD subjects, showing that brain activations of heavy smokers were less influenced by aversive drug cues. The difference between craving ratings when preceded by other cues minus drug-related-negative cues was significantly and negatively correlated with the number of cigarettes smoked per day ($r = -0.319$; $P = 0.040$) and FTND scores ($r = -0.412$; $P = 0.017$; see also Supporting information, Table S5). Heavy and more dependent smokers exhibited a lower difference between craving ratings, implying a lower impact of drug-related-negative cues on craving. No significant correlations between pack-years and functional activation of brain regions/differences in craving ratings could be observed.

DISCUSSION

In the present study we found evidence for a 'hijacked' brain reward system in TUD subjects, as they presented an increased functional activation of mesocorticolimbic brain areas elicited by drug-related-positive versus alternative reward cues when compared to never-smokers. We did not observe a reduced activation of the so-called aversive brain network during the processing of drug-related-negative cues in TUD subjects compared to never-smokers. However, within the TUD group, limbic brain structures belonging to the core aversive

TABLE 2 Locations of significantly activated brain regions during processing of cigarette cues compared to alternative rewards in TUD subjects versus never-smokers (a); results of the interaction contrast of drug-related-negative cues versus neutral cues in TUD subjects versus never-smokers (b).

Contrast/region	Side	Voxels	x	y	z	*F* or *t*	*P* < 0.05 fwe-corrected
a. Altered brain reward processing							
F-contrast							
Interaction TUD versus NS (drug versus alt)							
Region of interest analysis							
Hippocampus	L	37	−20	−18	−16	16.93	0.020
Mid-brain (incl. VTA)	L	34	−2	−18	−8	16.01	0.022
Thalamus	L	17	−4	−16	−2	15.10	0.035
Hippocampus	R	12	24	−38	−2	13.52	0.045
Thalamus	R	10	8	−14	−2	12.53	0.043
Whole brain analysis							
Orbitofrontal cortex	R	18	0	42	−8	25.00	0.016
Post-hoc *t*-contrast							
TUD (drug > alt) > NS (drug > alt)							
Region of interest analysis							
Hippocampus	L	91	−20	−18	−16	4.02	0.018
Mid-brain (incl. VTA)	L	42	−2	−18	−8	4.00	0.027
Thalamus	L	139	−4	−16	−2	3.92	0.036
Hippocampus	R	39	24	−38	−2	3.94	0.033
Thalamus	R	12	8	−14	−2	3.54	0.016
Whole brain analysis							
Orbitofrontal cortex	R	69	2	42	−8	5.00	0.006
a. Altered threat responsivity							
F-contrast							
Interaction TUD versus NS (threat versus neutral)							
ROI analysis				No differential activation			
Whole brain analysis				No differential activation			

Note: L: left; R: right; voxels: number of voxels per cluster; x, y, z: MNI coordinates; TUD: tobacco use disorder subjects, NS: never-smokers; drug: drug-related-positive cues; alt: alternative rewards; threat: drug-related-negative cues; fwe = family-wise error; VTA = ventral tegmental area.
P < 0.05 fwe-corrected: for region of interest (ROI) analyses a family-wise error-corrected threshold of $P_{fwe} < 0.05$ with k > 10 voxels on a peak level was used. For whole-brain analyses a threshold of $P_{fwe} < 0.05$ was applied.

network were activated during the presentation of drug-related-negative cues, and this activation was negatively correlated with the number of cigarettes smoked per day.

An important component of the mesocorticolimbic brain system is dopaminergic projections from the VTA and related brain stem areas to subcortical (e.g. NAc, thalamus, hippocampus) and pre-frontal brain regions (e.g. OFC), as these pathways appear to be critical in drug-induced reward processing [47–50]. According to the incentive–sensitization theory [8], it can be assumed that brain areas belonging to the reward pathway of TUD subjects gradually became sensitized to tobacco cues and desensitized to alternative reward cues as, in our case, food cues. The main functional activation effects of drug-related-positive cues in TUD subjects are in accordance with results of previous studies [16,19,21]. However, to the best of our knowledge, previous studies did not directly investigate the interaction effect of smoking status and (food) reward processing. For the first time we observed alterations in processing of drug cues versus alternative reward cues related to smoking status and can therefore draw the conclusion of a 'hijacked' brain reward system in TUD. Contrary to our expectations, functional activation of the mesocorticolimbic reward system was not related to the number of cigarettes smoked per day, FTND or pack-years. Further studies need to assess alterations of reward processing over time during the development and maintenance of TUD. Results for subjective craving ratings were inconsistent with the fMRI findings, as TUD subjects rated their craving for food cues significantly higher than for drug-related-positive cues within the task. This phenomenon can be possibly explained by the abstinence motivation of TUD subjects included in

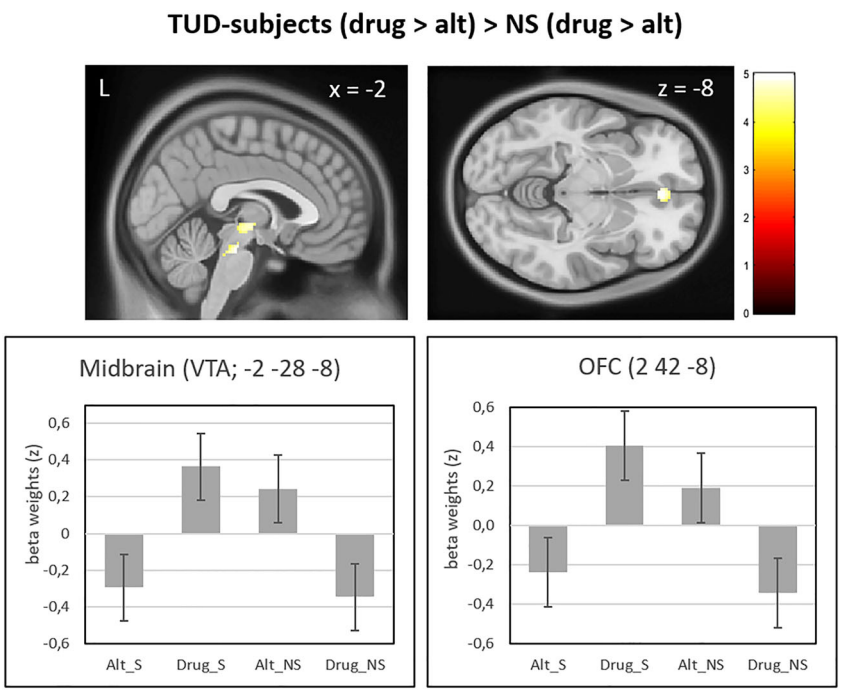

FIGURE 2 Neural correlates of altered reward processing for drug-related-positive cues in tobacco use disorder (TUD) subjects compared to alternative rewards and never-smokers identified in the region of interest (ROI) (VTA) and whole brain analyses (OFC). VTA = ventral tegmental area; OFC = orbitofrontal cortex; drug = drug-related-positive cues; alt = alternative reward. Significance threshold is $P < 0.05$ family-wise error (fwe)-corrected. Bars represent the estimated, standardized beta values of the corresponding brain region. Error bars represent the standard error of the mean [Color figure can be viewed at wileyonlinelibrary.com]

our study. It may be that the motivation to quit impacts upon the craving ratings given during the task. However, craving ratings are often not predictive of smoking behaviors, which may have a strong habitual component [51].

The second mechanism under examination was functional activation elicited by drug-related-negative cues in a brain network associated with threat processing. We could not confirm our hypothesis of altered processing of drug-related-negative stimuli in TUD subjects compared to never-smokers, pointing to the fact that there may be no general desensitization of the aversive system in TUD. However, investigating the TUD group separately revealed activation of threat-related brain regions in response to drug-related-negative stimuli, consistent with findings of previous studies [32–36], which was not observed in healthy controls. These findings suggest that when quitting-motivated TUD subjects are exposed to the negative value of smoking these cues can, to some degree, be processed as unpleasant and engage structures associated with negative emotions [52,53], even though no significant group difference with never-smokers was found. This result is complemented by our behavioral finding that prior presentation of drug-related-negative cues reduced subjective cigarette craving.

Importantly, the observed activation of the aversive brain network is driven by light smokers, as we observed a negative correlation of medium effect size between brain activation with the number of cigarettes smoked per day. This result suggests that, relative to light smokers, heavy smokers present a desensitization of the aversive brain network; i.e. they are no longer responding strongly to the negative aspects of smoking. Interestingly, this finding was, again, paralleled by behavioral analyses of craving ratings preceded by drug related-negative cues versus other cues. Here, a negative association of medium effect size between the difference in final craving ratings and smoking behavior suggests that heavy and more dependent smokers were less influenced in their craving when drug-related-negative cues were presented beforehand. An alternative explanation for this association could be that TUD subjects who are less sensitive to drug-related-negative cues tend to consume more cigarettes per day. Thus, as our study is limited by its cross-sectional design, longitudinal studies are needed to assess the role of threat processing as a potential marker of vulnerability for smoking onset and maintenance.

When defining the ROIs for the present analysis we chose, based on the literature, to assign some regions to both the brain reward and aversive system (e.g. amygdala and hippocampus). Both reward- and threat-related stimuli are highly emotional, a fact that might be reflected in the common activation of brain regions [31]. In addition, some areas code for multiple, even apparently opponent processes (e.g. aversion and reward). There are numerous cell types with various response characteristics (e.g. throughout the amygdala) which may respond to the presence of rewarding, aversive or both types of stimuli [31]. Conversely, some areas previously also found during processing of drug-related-positive cues (e.g. insula and ACC) were assigned to the aversive system ROI, but not included in the ROI of the reward system. This approach was chosen because previous research linked these brain regions not primarily to reward processes, but rather control, conflict and interoceptive processes during the processing of drug-related-positive cues [53,54].

Pharmacotherapy, such as nicotine substitution and bupropion, have been proved to be effective mainly in reducing withdrawal symptoms experienced during cessation [44]. Additionally, non-pharmacological treatment is important to address the full spectrum of neurobiological mechanisms that underlie TUD. In this context, our results are clinically relevant as they offer important starting-points for interventions. It can be suggested that smoking cessation

TABLE 3 Significantly activated brain regions contrasting drug-related-negative cues against neutral cues in TUD subjects (a) and never-smokers (b) separately.

Contrast/region	Side	Voxels	x	y	z	t	$P < 0.05$ fwe-corrected
(a) Effect of threat responsivity in TUD subjects							
Post-hoc *t*-contrast							
TUD (threat > neutral)							
ROI analysis							
Insula	R	331	40	8	−12	4.87	< 0.001
ACC	R	542	0	14	26	4.77	0.002
Hippocampus	R	46	26	−40	−2	4.49	0.005
Insula	L	276	−42	8	−10	4.47	0.006
Whole brain analysis							
Lingual gyrus	R	746	16	−82	−8	8.70	< 0.001
Lingual gyrus	L	528	−24	−76	−6	7.61	< 0.001
Middle occipital gyrus	L	170	−28	−78	20	6.27	< 0.001
Middle occipital gyrus	R	116	32	−74	22	5.78	< 0.001
Middle temporal gyrus	R	164	50	−66	8	5.35	< 0.001
Inferior frontal gyrus	R	61	46	6	24	5.33	< 0.001
Middle temporal gyrus	L	102	−48	−80	10	5.27	< 0.001
Superior parietal gyrus	L	100	−26	−50	56	5.26	< 0.001
Inferior parietal gyrus	R	41	28	−46	48	5.23	0.012
Supramarginal gyrus	R	20	58	−22	36	4.91	0.014
Insula	R	15	40	8	−12	4.87	0.017
ACC	R	15	0	14	26	4.82	0.021
(b) Effect of threat responsivity in NS							
Post-hoc *t*-contrast							
NS (threat > neutral)							
Whole brain analysis							
Lingual gyrus	R	825	16	−82	−6	8.99	< 0.001
Lingual gyrus	L	435	−13	−88	−6	6.53	< 0.001
Middle occipital gyrus	L	129	−48	−78	22	6.23	< 0.001
Middle occipital gyrus	R	54	30	−76	22	5.16	0.004
Inferior parietal gyrus	L	26	−28	−52	52	4.83	0.016

Note: L: left; R: right; voxels: number of voxels per cluster; x, y, z: MNI coordinates; TUD: tobacco use disorder subjects, NS: never-smokers; threat: drug-related-negative cues; fwe = family-wise error; ACC = anterior cingulate cortex.
$P < 0.05$ fwe-corrected: for region of interest (ROI) analyses a family-wise error-corrected threshold of $P_{fwe} < 0.05$ with k > 10 voxels on a peak level was used. For whole-brain analyses a threshold of $P_{fwe} < 0.05$ was applied.

treatment should address strategies to enhance the meaning and processing of alternative rewards as, for instance, intended by psycho-education and enjoyment training, in all stages of TUD. Cognitive–behavioral therapy (CBT) approaches could include an individualized training session to identify and activate alternative rewards in the treatment of quitting-motivated TUD subjects. Conversely, the confrontation with long-term consequences of chronic smoking behavior seems to be more efficient for light smokers. Additionally, our results can be used to inform alternative and novel intervention strategies targeting the brain, such as repetitive transcranial magnetic stimulation, deep brain stimulation and real-time fMRI neurofeedback. Such approaches represent potentially useful and clinically meaningful treatment modalities for TUD [55,56], but further research is needed to detect involved brain regions and conditions. Our results can suggest new target regions for such interventions (e.g. OFC and hippocampus) as well as applying new strategies (e.g. enhancing alternative reward processing).

Future studies should investigate the role of aversive processing to inform health advertisement campaigns as well as the use of long-term negative consequences in smoking cessation therapy. Therefore,

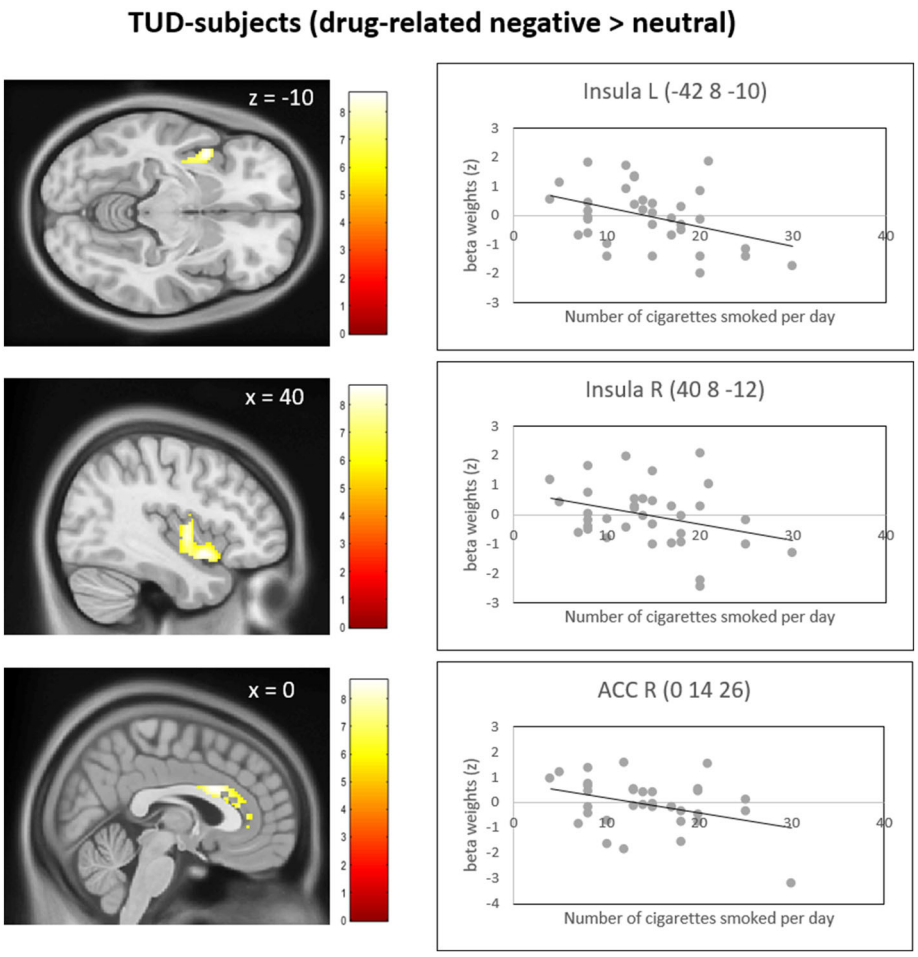

FIGURE 3 Neural correlates of altered threat processing compared to neutral cues in tobacco use disorder (TUD) subjects only (left side). Scatterplots show the negative relationship between extracted beta weights and the number of cigarettes smoked per day (right side). R = right; L = left; Significance threshold is $P < 0.05$ family-wise error (fwe)-corrected. Dots represent the estimated, Z-standardized beta values of the corresponding brain region [Color figure can be viewed at wileyonlinelibrary.com]

it may be important to specify the effects of threat processing; for example, by including a group of heavy and light smokers who are not motivated to quit and by the use of different categories of drug-related-negative cues (e.g. pictures used on cigarette packets versus unknown pictures). Furthermore, it would be of interest to investigate whether activations in the identified brain regions predict behavioral measures, such as the ability to resist craving or successful smoking cessation.

Strengths and limitations

Complementing previous investigations on smoking cue-reactivity, we recruited a well-defined sample of TUD subjects who were dependent according to DSM-5-TR criteria and motivated to quit. Participants in our study were free of psychotropic medication and did not suffer from any mental disorder that could influence the processing of the applied cue categories. The groups were well matched for gender, age, handedness, education and income. Cues presented during the task were selected by the participants beforehand to match their individual preferences. Of special note, results are corrected for differences in subclinical trait anxiety and current depression scores, which could represent a potential bias in neurofunctional processes. To the best of our knowledge, this is the first study investigating altered reward and threat processing within one paradigm using individualized pictures. This offers the opportunity to examine interaction effects between TUD subjects and never-smokers to gain a clearer understanding of two promising mechanisms of smoking initiation and preservation which can be used to modify and improve treatment and prevention strategies.

However, several limitations must also be considered. As our study has a cross-sectional design, we cannot infer causal interactions between smoking behavior and neurofunctional findings. It may be possible that TUD subjects already present aberrant reward processes before starting to smoke; this could even present a risk factor for smoking initiation which has to be elaborated in longitudinal designs. Furthermore, it could be possible that heavy smokers were more used to pictures showing drug-related-negative consequences, as they are more confronted with pictures used for health campaigns on cigarette packets. To avoid this potential confound, we explicitly used pictures which are not used by health campaigns and which are not used on cigarette packets. While individualized cues are a strength of the study this requires additional consideration, as any differences in the selected cues between the groups could create a bias. However, we found no systematic preference for a specific food/threat category in one of the two groups and the groups did not differ in their ratings for single cues. Thus, we believe that the risk of bias is somewhat low and does not account for the findings. The inclusion of a younger age

group (e.g. 18 years) may represent a potential bias, as smoking may not yet have been established and the brain is still in its maturation at this age. However, there were only three participants between the ages of 20 and 25 years included in our analysis, assuming that this level of bias is rather low. In addition, TUD subjects were only moderately nicotine-dependent, and stronger dependency could lead to other results which have to be elaborated in future studies. Additionally, it is important to keep in mind that the use of food cues as alternative rewards may not perform identically to other alternative reward categories (e.g. money), as previous studies have shown that nicotine can act as an appetite suppressant [57] or increased appetite can represent a withdrawal symptom [58].

Summarized, our results suggest that altered reward processing is found in moderately dependent TUD subjects and may hence be addressed at all stages of cessation intervention, while the confrontation with long-term consequences might be more promising in light smokers. From a clinical point of view, already existing intervention strategies (e.g. CBT approaches) can be enhanced and new treatment modalities (e.g. real-time fMRI neurofeedback) can be informed by our results. However, these practical consequences still have to be verified in clinical studies.

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DECLARATION OF INTERESTS

None.

AUTHOR CONTRIBUTIONS

Stefanie L. Kunas: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; software; visualization; writing-original draft; writing-review & editing. **Heiner Stuke:** Conceptualization; data curation; formal analysis; funding acquisition; methodology; software; supervision; validation. **Andreas Heinz:** Funding acquisition; project administration; resources; supervision; validation; visualization. **Andreas Ströhle:** Conceptualization; funding acquisition; project administration; resources; supervision. **Felix Bermpohl:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; software; supervision; validation; visualization.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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Supplementary Materials

Evidence for a hijacked brain reward system but no desensitized threat system in quitting motivated smokers: An fMRI study

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Content

Supplementary Texts

Supplementary Tables

Supplementary Figures

Supplementary Figure S1: consort flow-chart

Supplementary Figure S2: subjective ratings conducted before the fMRI session

Supplementary Figure S3: craving ratings within the enhanced cue reactivity task

Supplementary Texts

Supplementary Text 1: Details on clinic assessments

Multiple-choice vocabulary test (MWT): The MWT is objective and can be administered several times without learning effects. It takes about 5 min and is completed like a self-evaluation scale. It comprises 37 items and consists of lines, each comprising five words. One is an authentic word from the dictionary, while four are fictitious. The subject is asked to find the "correct" one and to underline it. The familiarity of the words differs widely. Each word correctly recognized gives a point which is added to the total score (range 0-37) [1].

State-Trait-Anxiety-Inventory, Trait part (STAI-T): The STAI is a frequently used measure of anxiety. The STAI-T was designed to measure a stable propensity to experience anxiety and tendencies to perceive stressful situations as threatening. The trait scale consists of 20 statements that require individuals to rate how they generally feel on a four-point scale (1-4). Test-retest reliabilities for the trait scale are high, ranging from 0.73-0.86. Concurrent validity with other anxiety questionnaires ranges from 0.73-0.85) [2].

General Depression Scale, short version (ADS-K): The short version of the General Depression Scale is a frequently used self-report to measure depressive mood. The short version consists of 15 items which require individuals to rate how frequently typical depressive symptoms occurred within the last week on a four-point scale (0-3). Test-retest reliabilities are high (0.87) and concurrent validity with other depressions scales ranges from 0.64-0.88. A cut-off value for clinically relevant depressive disorders was defined as ≥ 17 [3].

Fagerstroem Test for Nicotine Dependence (FTND): The FTND is a widely used standard instrument for assessing the dimensional level of physical addiction to nicotine. It contains six

items that evaluate the quantity of cigarette consumption, the compulsion to use and dependence. Yes/ No items are scored from 0 to 1 and multiple-choice items are scored from 0-3 yielding a total score of 0-10. Low physical dependency is defined by 0-2, medium dependency by 3-4, high dependency by 5-6 and very high physical dependency by 7-10 [4].

Supplementary Text 2: Statistical analysis of behavioral data and subjective craving ratings

Assumption checks

To investigate the main and interaction effects of drug-related positive, alternative reward and drug-related negative craving and threatening ratings (before and within the fMRI task), we checked the outcome variable regarding normal distribution using the Shapiro-Wilk test ($p < 0.001$) and equal variances, using the Levene test ($p < 0.001$). Both tests showed a significant result, indicating that the data is not normally distributed and has unequal variances. Therefore, we performed the non-parametric Scheirer-Ray-Hare test as alternative for a univariate analysis of variance (ANOVA). Concerning the t-tests we also tested for normal distribution and equal variances, when assumptions were violated, we conducted the Mann-Whitney-U test. The following assumptions were tested before conducting the Person correlation analysis: normality (Shapiro-Wilk test), equal variances (Barlett's test), linearity (scatterplots) and presence of outliers (boxplots). Outliers were defined as a deviation of more than two standard deviations from the mean, no outliers could be detected None of the assumptions were violated.

Subjective craving ratings within the groups for specific cue categories

Concerning the alternative reward (food) condition, it is important to mention, that only pictures of heathy, low-calorie and low-fat food were included (e.g., salad or rice with vegetables). We selected different categories of healthy food: sweet (e.g., fruits with yoghurt) and savory. Within the savory condition, pictures were balanced between meat and vegetarian/ vegan dishes. We investigated the ratings for each single picture, separately for the two groups. There was no single picture, where both groups differed considerably in their rating (all difference scores < 1), which means, that they were very similar in their preferences. We further compared the pictures with the 50% highest desirability ratings of both groups (which were automatically selected for the task), and found that these included all conditions in both groups (sweet, savory with and without meat). The same procedure was performed for drug-related negative cues. Here again, we could show that both groups rated

each single picture quite similar (all difference scores < 0.9). Five pictures were rated as rather low threatening in both groups (mean rating = 4.63) and were not automatically selected for any participant. These five pictures showed relatively mild long-term consequences, like a man who is treated with oxygen. The 50% most threatening pictures included all categories (e.g. external visible
consequences like smokers' leg, and different kinds of carcinomas) equally in both groups.

Supplementary Text 3: Functional imaging data analysis

On a single subject level, brain activation differences related to presentation of the different stimuli were analyzed using a general linear model (GLM) in SPM12. The blood oxygen level dependent response was modeled by a canonical hemodynamic response function for each of six conditions: neutral cues, alternative reward cues, drug-related negative cues, drug-related positive cues, button-presses and ratings, resulting in 4 regressors of interest and two noise regressors (button-press and rating). Model parameter estimates and the resulting t-statistic images were submitted to group level analysis.

Supplementary Text 4: Sensitivity analysis

F-contrasts were computed for the main effects of group and stimulus type. To specify the direction of the observed main effects we report (in accordance with the aim of our study), the post-hoc conducted t-tests.

General reward processing across both groups
In the whole brain analysis, a significant cluster of 66321 voxels was identified. For reasons of simplicity, significantly activated brain areas are summarized: frontal areas (e.g. superior frontal gyrus, middle frontal gyrus, inferior frontal gyrus, precentral gyrus, supplementary motor area), parietal areas (e.g. precuneus, angular gyrus, supramarginal gyrus, inferior parietal gyrus, superior parietal lobule), temporal areas (e.g. insula, middle temporal gyrus, superior temporal gyrus, fusiform gyrus, postcentral gyrus), occipital areas (lingual gyrus, calcarine sulcus, cuneus, middle occipital gyrus, superior occipital gyrus), parts of the cerebellum and subcortical brain areas (e.g. hippocampus, ACC, thalamus, parahippocampal

gyrus, pallidum). Regarding the results of the region of interest (ROI) analysis, please refer to Table S1.

General threat responsivity across both groups

In the whole brain analysis, the right lingual gyrus (16, -82, 6), the left middle occipital gyrus (-28, -78,22), the right (28, -46, 48) and left (-26, -50, 52) superior parietal gyrus, the right (46, 4, 26) and left (-40, -6, 42) precentral gyrus , the right (60, -18, 30) and left (-58, -20, 32) supramarginal gyrus, the right (38, -4, 8) and left (-40, -6, 2) insula cortex, the right (44, -46, -18) and left (-42, -48, -18) fusiform gyrus and the right supplementary motor area (8, 10, 56) were significantly activated. Significantly activated ROIs are presented in Table S1.

Effects of drug-related positive and alternative reward cues separated for the two groups

Investigating the effects of drug-related positive cues and alternative reward cues within the two groups separately in the predefined ROIs, TUD subjects presented a significant activation in the mesocorticolimbic brain reward system (e.g. bilateral thalamus, hippocampi, midbrain and pallidum) to drug-related positive cues (nico > neutral). On a whole brain level, prefrontal regions (e.g. inferior frontal gyrus, OFC, ACC, superior frontal gyrus) and temporal regions (middle temporal gyrus) as well as parts of the cerebellum were significantly activated (see Table S2). Contrasting alternative rewards to neutral cues (alternative reward > neutral), TUD subjects showed a significant activation in the left hippocampus in the ROI analysis and in the left lingual gyrus and superior frontal gyrus in the whole brain analysis. Never-smokers presented activated brain clusters in the left pallidum and the right midbrain in the ROI analysis for drug-related positive cues contrasted to neutral cues (nico > neutral). On a whole brain level, significant activations could be observed in frontal (inferior frontal gyrus, precentral gyrus), temporal (middle temporal) and occipital (lingual gyrus) brain regions. As response to alternative rewards, never-smokers exhibited a significant activation in the left thalamus and bilateral hippocampi in the ROI analysis (alternative reward > neutral). On a whole brain level, prefrontal (e.g. OFC, superior frontal gyrus, precuneus), subcortical (thalamus) and occipital (lingual gyrus, middle occipital gyrus) brain regions could be obtained in never-smokers (see Table S3).

Supplementary Tables

Supplementary Table S1: Significantly activated brain regions during general reward and threat processing across both groups

Table S1. Significantly activated brain regions during general reward (A) and threat (B) processing across both groups in the ROI analysis.

Contrast/ Region	Side	Voxels	x	y	z	*t*	p_{fwe} corrected
(A) Effect of general reward processing							
Post-hoc t-contrast							
Reward > Neutral (drug & alt > neutral)							
Region of interest analysis							
Thalamus	R	841	18	-32	0	8.39	< 0.001
Thalamus	L	714	-6	-16	-2	7.57	< 0.001
Thalamus	L		-2	-28	-4	7.00	< 0.001
Hippocampus	L	131	-22	-38	0	7.89	< 0.001
Hippocampus	L		-20	-38	-2	7.04	< 0.001
Hippocampus	R	130	24	-38	-2	7.79	< 0.001
Midbrain	L	1169	-8	-18	-6	7.85	< 0.001
Midbrain	R		6	-28	-24	7.83	< 0.001
Pallidum	R	320	20	2	2	5.27	< 0.001
Pallidum	L	103	-20	-8	2	5.00	< 0.001
(B) Effect of general threat processing							
Post-hoc t-contrast							
Threat > Neutral							
Region of interest analysis							
Insula	L	546	-40	-6	2	5.53	< 0.001
Insula	R	445	38	-4	8	5.88	< 0.001
ACC	R	441	4	12	26	4.48	< 0.001
Hippocampus	R	67	26	-40	-2	5.56	< 0.001
Thalamus	L	44	-4	-22	-2	4.59	0.004
Thalamus	R	20	4	-14	-2	4.37	0.011
Hippocampus	L	11	-22	-40	0	4.00	0.040

L: left; R: right; voxels: number of voxels per cluster; x, y, z: MNI coordinates; nico: drug-related positive cues; alt: alternative rewards neutral: neutral cues

Supplementary Table S2: Correlations between significantly activated brain regions and smoking behavior during altered reward processing.

Table S2. Correlations between significant activated brain regions and measurements of smoking behavior. Brain areas were identified in the ROI analysis during the processing of drug-related positive cues compared to alternative rewards in TUD subjects [TUD(drug > alt)]. Beta weights were extracted using the toolbox marsbar and a sphere around the significant peak voxel of 5 mm.

	Hippocampus L	Hippocampus R	Thalamus L	Thalamus R	Midbrain L
Cigarettes per day	0.050	0.027	0.004	0.026	-0.072
	(0.775)	(0.875)	(0.983)	(0.882)	(0.680)
FTND	-0.136	-0.040	-0.059	-0.086	-0.132
	(0.437)	(0.818)	(0.737)	(0.622)	(0.451)
Pack years	0.012	-0.011	-0.011	0.075	-0.056
	(0.944)	(0.947)	(0.946)	(0.660)	(0.742)

Note: R = right, L = left; FTND = Fagerstrom Test for nicotine dependence; Missing values: cigarettes per day = 2; Missing values were treated with listwise deletion.

Supplementary Table S3: Significantly activated brain regions contrasting drug-related positive and alternative reward cues against neutral cues in the TUD group only

Table S3. Significantly activated brain regions contrasting cigarette cues (part A) and alternative reward cues (part B) against neutral cues in the TUD group only.

Contrast/ Region	Side	Voxels	x	y	z	*t*	p_{fwe} corrected
(A) Effect of drug in TUD subjects							
Post-hoc t-contrast							
TUD (drug > neutral)							
Region of interest analysis							
Thalamus	R	835	18	-32	3	6.11	< 0.001
Thalamus	L	86	-6	-12	18	4.25	< 0.001
Hippocampus	R	319	20	-36	0	6.88	< 0.001
Hippocampus	L	302	-22	-40	0	5.96	< 0.001
Midbrain (incl. VTA)	R	400	2	-34	-24	5.63	< 0.001
Pallidum	R	22	16	2	2	3.87	0.044
Whole brain analysis							
Middle temporal gyrus (incl. Precuneus)	R	18176	48	-64	10	18.61	< 0.001
Middle temporal gyrus (incl. inferior parietal gyrus and angular gyrus)	L	4433	-46	-74	8	16.44	< 0.001
Fusiform gyrus	L	518	-40	-50	-16	11.05	< 0.001
Inferior frontal gyrus	R	3063	42	18	26	7.89	< 0.001
Cerebellum	L	325	-8	-54	-42	7.07	< 0.001
Middle temporal gyrus	L	719	-50	-14	-16	7.12	< 0.001
Precentral gyrus (incl. Middle frontal gyrus & insula)	L	2617	-30	26	-4	6.99	< 0.001
Orbitofrontal gyrus (incl. rectus)	R	328	4	46	-18	6.39	< 0.001
Supplementary motor area	R	651	4	16	46	5.96	< 0.001
Thalamus	R	388	2	-32	-24	5.75	< 0.001
Superior frontal gyrus	R	52	20	34	40	5.38	< 0.001
ACC	R	70	6	48	26	5.14	0.004
Cerebellum	L	40	-30	-60	-30	4.92	0.011
(B) Effect of alt in TUD subjects							
Post-hoc t-contrast							
TUD (alt > neutral)							
Region of interest analysis							
Hippocampus	L	58	-20	-28	-2	4.50	0.004
Whole brain analysis							
Lingual gyrus (incl. Middle occipital gyrus)	L	3857	-14	-92	-4	11.37	< 0.001
Superior frontal gyrus	L	46	-20	-8	54	5.08	0.006

L: left; R: right; voxels: number of voxels per cluster; x, y, z: MNI coordinates; S: TUD subjects, NS: non-smoker; nico: drug-related positive cues; alt: alternative rewards; neutral: neutral cues

Supplementary Table S4: Significantly activated brain regions contrasting drug-related positive and alternative reward cues against neutral cues in the never-smoker group only

Table S4. Significantly activated brain regions contrasting cigarette cues (part A) and alternative reward cues (part B) against neutral cues in the never-smoker group only.

Contrast/ Region	Side	Voxels	x	y	z	*t*	p_{fwe} corrected
(A) Effect of drug in NS							
Post-hoc t-contrast							
NS (drug > neutral)							
Region of interest analysis							
Midbrain	R	92	6	-28	-4	4.53	0.005
Pallidum	L	129	-26	20	-4	4.43	0.008
Whole brain analysis							
Middle temporal gyrus (incl. occipital gyrus)	L	1790	-48	-74	10	16.03	< 0.001
Fusiform gyrus	R	292	42	-46	-20	11.83	< 0.001
Precuneus	R	3998	22	-72	-4	7.95	< 0.001
Lingual gyrus (incl. Cerebellum)	L	427	-10	-82	-6	6.89	< 0.001
Precentral gyrus	R	964	36	10	28	6.66	< 0.001
Inferior parietal gyrus	L	724	-30	-54	46	6.62	< 0.001
Inferior frontal gyrus	L	372	-36	16	26	6.15	< 0.001
Precentral gyrus	L	301	-34	0	54	5.90	< 0.001
(B) Effect of alt in NS							
Post-hoc t-contrast							
NS (alt > neutral)							
Region of interest analysis							
Thalamus	L	333	-2	-22	-4	4.89	<0.001
Hippocampus	R	77	24	-30	-4	4.41	0.006
Hippocampus	L	60	-22	-30	-4	4.38	0.007
Whole brain analysis							
Lingual gyrus	R	3669	16	-92	2	12.52	< 0.001
Orbitofrontal gyrus	L	41	-24	32	-12	6.03	< 0.001
Middle occipital gyrus	L	52	-28	-80	22	5.36	< 0.001
Superior frontal gyrus	L	107	-24	-4	52	5.24	0.006
Precentral gyrus	R	72	32	-6	54	5.05	< 0.001
Thalamus	L	73	-2	-22	-4	4.89	< 0.001

L: left; R: right; voxels: number of voxels per cluster; x, y, z: MNI coordinates; S: TUD subjects, NS: non-smoker; nico: drug-related positive cues; alt: alternative rewards; neutral: neutral cues

Supplementary Table S5: Correlations between brain regions and smoking behavior in TUD subjects during the processing of drug-related negative cues

Table S5. Correlations between significant activated brain regions- identified in the ROI analyses – and differences in craving ratings with smoking behavior in TUD subjects during the processing of drug-related negative cues vs. neutral cues [TUD (threat > neutral)]. Beta weights were extracted using the toolbox marsbar and a sphere around the significant peak voxel of 5 mm.

	Difference in craving ratings	Insula R	Insula L	Hippocampus	ACC
Cigarettes per day	**-0.319***	**-0.386***	**-0.351***	-0.321	**-0.359***
	(0.040)	(0.024)	(0.042)	(0.06)	(0.037)
FTND	**-0.412***	-0.100	-0.092	-0.102	-0.162
	(0.017)	(0.610)	(0.606)	(0.564)	(0.359)
Pack years	-0.211	-0.048	-0.135	0.083	-0.017
	(0.218)	(0.779)	(0.426)	(0.627)	(0.918)

Note: R = right, L = left; FTND = Fagerstrom Test for nicotine dependence; Missing values: cigarettes per day = 2; Missing values were treated with listwise deletion.
*$p < 0.05$

Supplementary Figures

Supplementary Figure S1: consort flow-chart

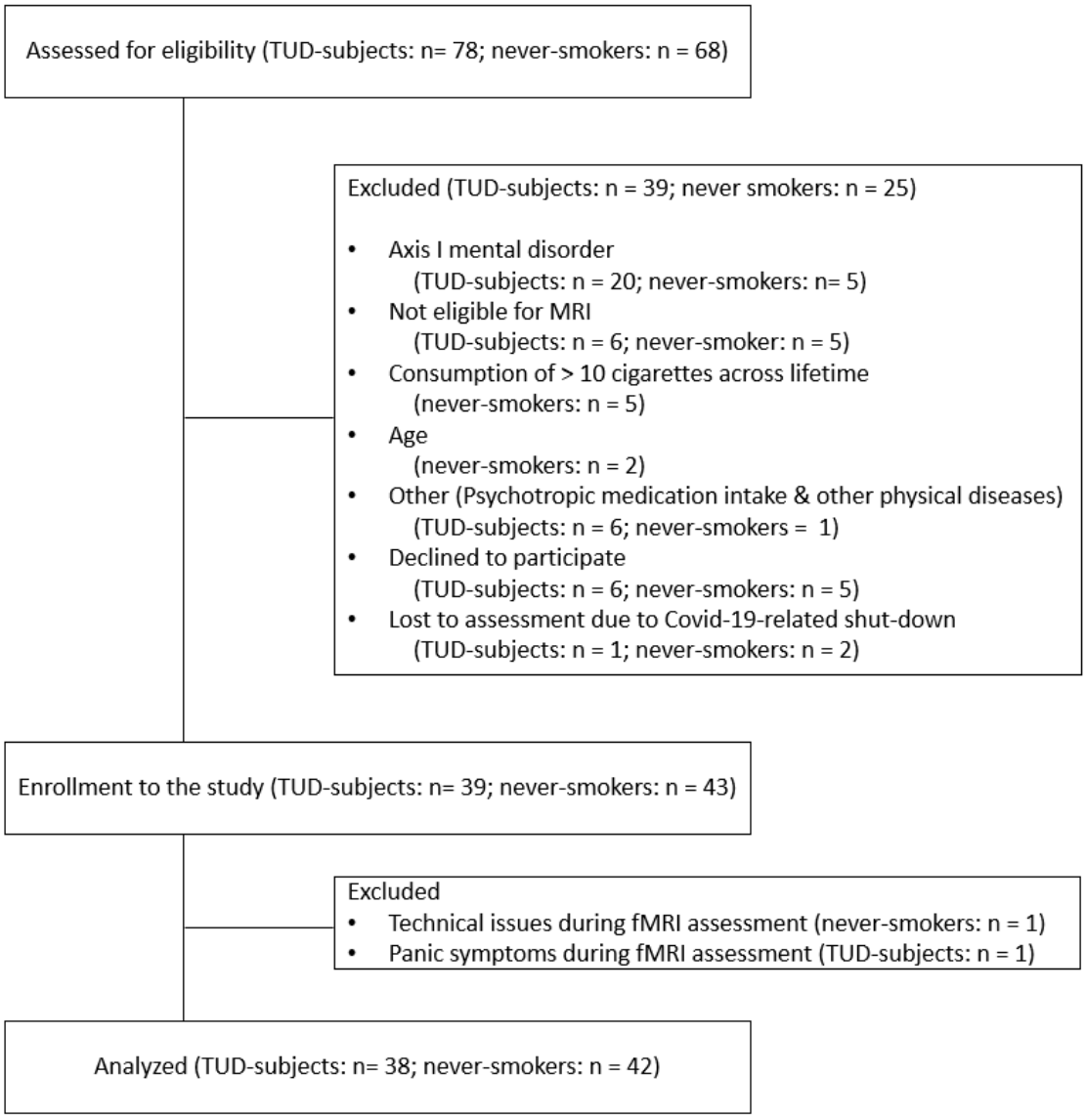

Figure S1: Consort flow-chart of included participants.

Supplementary Figure S2: subjective ratings conducted before the fMRI session

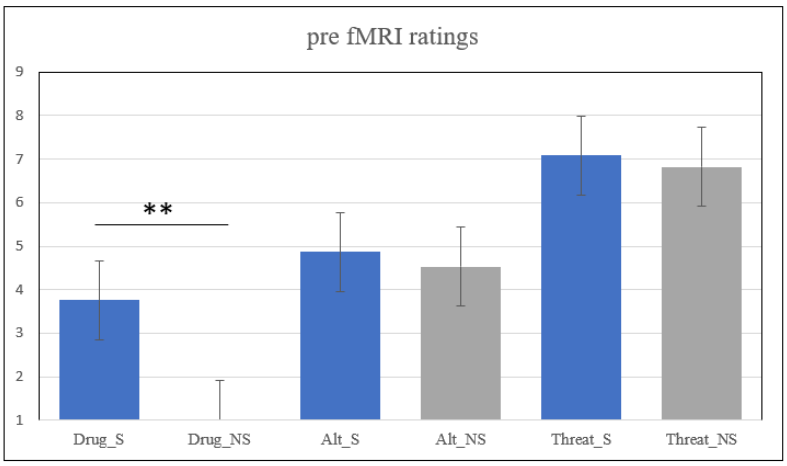

Figure S2: subjective ratings of drug-related positive (drug), alternative reward (alt) and drug-related negative (threat) cues before the fMRI measurement, which were presented in the scanner. S = TUD subjects; NS = never-smoker. Before the fMRI session, participants rated a set of 140 pictures of each category. For the experiment, the 50% most rewarding / threatening stimuli were automatically selected. We found a significant main effect of group ($H_{(1/234)} = 31.786$; $p < 0.001$, $\eta^2 = 0.127$) and stimulus type ($H_{(2/234)} = 194.014$; $p < 0.001$, $\eta^2 = 0.639$) as well as a significant group-by-stimulus interaction ($H_{(2/234)} = 15.517$; $p < 0.001$, $\eta^2 = 0.124$). Post-hoc conducted Mann-Whitney-U tests indicate a significant difference between the two groups regarding drug-related positive cues ($U(80) = 6.874$, $p < 0.001$, $\eta^2 = 0.283$). No significant difference between alternative rewards ($U(80) = 0.987$; $p = 0.323$, $\eta^2 = 0.027$) and drug-related negative cues ($U(80) = 0.689$; $p = 0.491$, $\eta^2 = 0.087$) could be observed.

Supplementary Figure S3: craving ratings within the enhanced cue reactivity task

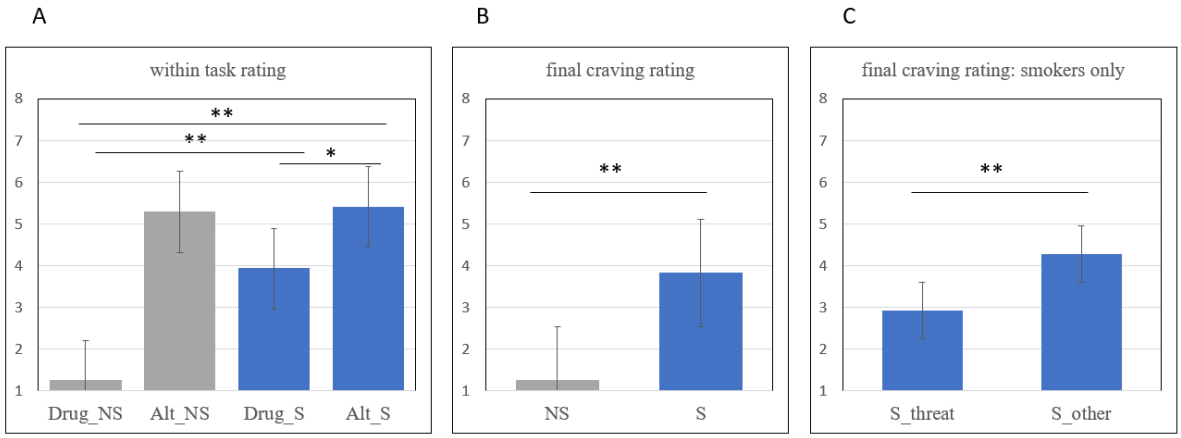

Figure S3: subjective craving ratings within the enhanced cue-reactivity task. S = TUD subjects; NS = never-smoker. **A)** Ratings separated for the two groups, Nico = rating following drug-related positive cues; Alt = ratings following alternative reward cues; **B)** final craving ratings were presented at the end of each run and refer to craving for cigarettes at the moment, separated for the two groups; **C)** final craving ratings in the group of TUD-subjects only, separated for ratings preceded by drug-related negative cues (threat) vs. alternative reward, drug-related positive and neutral cues (other).

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The modulating impact of cigarette smoking on brain structure in panic disorder: a voxel-based morphometry study

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Abstract

Cigarette smoking increases the likelihood of developing anxiety disorders, among them panic disorder (PD). While brain structures altered by smoking partly overlap with morphological changes identified in PD, the modulating impact of smoking as a potential confounder on structural alterations in PD has not yet been addressed. In total, 143 PD patients (71 smokers) and 178 healthy controls (62 smokers) participated in a multicenter magnetic resonance imaging (MRI) study. T1-weighted images were used to examine brain structural alterations using voxel-based morphometry in a priori defined regions of the defensive system network. PD was associated with gray matter volume reductions in the amygdala and hippocampus. This difference was driven by non-smokers and absent in smoking subjects. Bilateral amygdala volumes were reduced with increasing health burden (neither PD nor smoking > either PD or smoking > both PD and smoking). As smoking can narrow or diminish commonly observed structural abnormalities in PD, the effect of smoking should be

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considered in MRI studies focusing on patients with pathological forms of fear and anxiety. Future studies are needed to determine if smoking may increase the risk for subsequent psychopathology via brain functional or structural alterations.

Key words: smoking; gray matter volume; panic disorder; amygdala; hippocampus

Introduction

Smoking behavior is overrepresented in patients with mental disorders in general (Lasser *et al.*, 2000; Cook *et al.*, 2014) and in patients with anxiety disorders particularly (Johnson *et al.*, 2000). Among anxiety disorders, panic disorder (PD) is linked to cigarette smoking in many epidemiological investigations using cross-sectional designs (Goodwin and Hamilton, 2002; Lawrence *et al.*, 2010). Prospective epidemiological studies support smoking to increase the likelihood of developing panic attacks and PD (Breslau and Klein, 1999; Johnson *et al.*, 2000; Isensee *et al.*, 2003; Breslau *et al.*, 2004). Furthermore, PD patients who smoke report significantly more intense anxiety symptoms and greater severity of panic symptoms than those who do not smoke (Zvolensky *et al.*, 2005). In addition, they show increased problems to stop smoking compared to healthy smokers (Piper *et al.*, 2010). However, while the link between smoking and PD is well established epidemiologically, its neurobiological basis remains mostly unclear.

Persistent smoking has been related to a number of structural brain changes following nicotine consumption, as demonstrated by previous cross-sectional studies (Brody *et al.*, 2004; Gallinat *et al.*, 2006; Zhang *et al.*, 2011; Liao *et al.*, 2012; Pan *et al.*, 2013; Fritz *et al.*, 2014). Voxel-based morphometry (VBM) analyses found reduced gray matter volumes (GMV) in the anterior cingulate cortex (ACC; Brody *et al.*, 2004; Yu *et al.*, 2011; Liao *et al.*, 2012), the dorsolateral prefrontal cortex (Brody *et al.*, 2004; Gallinat *et al.*, 2006; Liao *et al.*, 2012; Fritz *et al.*, 2014), the orbitofrontal cortex (OFC; Kühn *et al.*, 2010; Morales *et al.*, 2012; Fritz *et al.*, 2014), the fusiform gyrus (Gallinat *et al.*, 2006), the cerebellum (Brody *et al.*, 2004; Kühn *et al.*, 2012) and in the left thalamus (Liao *et al.*, 2012; Hanlon *et al.*, 2016). Associations between cigarette smoking and brain volumes were also identified for striatal nuclei, with smaller nucleus accumbens volumes (Das *et al.*, 2012) and greater putamen volumes (Das *et al.*, 2012; Franklin *et al.*, 2014) in otherwise healthy smokers. Contradictory evidence is available for amygdala volume, where Durazzo *et al.* (2017) reported smaller GMV, whereas Shen *et al.* (2017) did not find any differences in healthy smokers *vs* non-smokers.

Neural system models for PD emphasize altered functionality of a network conferring defensive reactivity, which encompasses the insula, ACC, thalamus, hippocampus, amygdala and regions of the brain stem (midbrain, periaqueductal gray; Dresler *et al.*, 2013). Structural alterations have been reported for limbic structures (amygdala, hippocampus), cortical areas (ACC), the brain stem (midbrain, pons), basal ganglia (caudate, putamen) and the thalamus (Massana *et al.*, 2003; Uchida *et al.*, 2003; Asami *et al.*, 2009; Hayano *et al.*, 2009; Del Casale *et al.*, 2013; Dresler *et al.*, 2013). Reduced volumes of cortico-limbic structures were associated with PD symptoms and maintenance (Dresler *et al.*, 2013). It appears that brain structural abnormalities in smokers substantially overlap with those associated with PD pathophysiology in terms of fronto-limbic circuits (e.g. ACC, amygdala). As smoking behavior is overrepresented among PD patients, it may represent a potential confounder. Thus, differences between PD patients and healthy controls (HC) may have been over- or underestimated in previous investigations as a result of smoking. The same may be true for studies comparing PD patients with other patient groups with lower smoking prevalence.

Only a handful of neuroimaging studies have focused on understanding the neural mechanisms of smoking and comorbid mental illness, and the majority of these studies have concentrated on comorbid schizophrenia (Tregellas *et al.*, 2007; Schneider *et al.*, 2014; Jørgensen *et al.*, 2015). No investigation has previously examined the effect of smoking on brain morphology in patients with PD. For those areas, overlapping in both conditions, it is unclear whether smoking enhances or obscures the effect of PD.

To address this issue, we here intended to further clarify the modulating impact of smoking on brain morphological correlates in PD patients free of psychopharmacotherapy. First, we aimed to confirm smoking effects on brain morphology in healthy smokers and non-smokers. Second, based on the above-cited VBM studies, we hypothesized GMV reductions in fronto-limbic circuitry, frequently observed as a feature of PD pathophysiology, may be partly driven by differential rates in smoking behaviors. Third, we examined a possible additive effect of smoking on brain morphology in PD patients.

Materials and methods

Participants

The study was part of the German national research network PANIC-NET (second funding period). Magnetic resonance imaging (MRI) measurements were conducted in five German centers (Marburg, Berlin, Dresden, Greifswald and Muenster), which are participating centers for the national research initiative PANIC-NET (funded by the German Federal Ministry of Education and Research, BMBF). These centers have a long-standing tradition of collaborative multicenter functional magnetic resonance imaging (fMRI) studies (e.g. Kircher *et al.*, 2013). The current analysis encompasses a *post-hoc* research question supplementing the main study outcomes. In total, 157 PD patients and 187 HC subjects underwent MRI scanning. Inclusion criteria for patients were as follows: (i) a current DSM-IV-TR (Diagnostic and Statistical Manual of Mental Disorders 4th Edition) primary diagnosis of PD (American Psychiatric Association, 2000) evidenced by the Composite International Diagnostic Interview (CAPI-WHO-CIDI; DIAX-CIDI version) and validated by clinical experts; (ii) a score $\geq$3 on the Clinical Global Impressions Scale and (iii) an age of 18–65 years. Exclusion criteria were as follows: (i) comorbid DSM-IV-TR psychotic or bipolar I disorder; (ii) current alcohol dependence/current abuse or dependence on benzodiazepine and other psychoactive substances; (iii) current suicidal intent; (iv) borderline personality disorder; (v) concurrent ongoing psychopharmacological treatment for PD or another mental disorder and (vi) antidepressant or anxiolytic pharmacotherapy. The HC group was free of current or past medical, neurological or mental illness as evidenced by a clinical interview. Additional MRI-related exclusion criteria such as ferromagnetic metal implants applied to both groups. Smoking status was assessed on a categorical level (yes/no) by

Table 1. Sociodemographic characteristics of the smoker and non-smoker sample and clinical characteristics of the PD patients sample, only

	Smokers, mean +/− s.d. or no. (%) n = 133				Non-smokers, mean +/− s.d. or no. (%) n = 188			
Sociodemographic characteristics								
	PD (n = 71) 53%	HC (n = 62) 47%	Statistic *t* or χ^2	*P*	PD (n = 72) 38%	HC (n = 116) 62%	Statistic *t* or χ^2	*P*
Age [mean (s.d.)]	34.13 (10.7)	31.23 (9.5)	1.643	0.103	33.18 (11.3)	31.85 (10.8)	0.804	0.423
Female gender [n (%)]	45 (63)	34 (55)	1.001	0.317	44 (61)	67 (58)	0.206	0.650
Years of education [n (%)]								
8	5 (7)	0	10.413	0.005	4 (6)	1 (1)	16.427	<0.001
10	23 (32)	10 (16)	26 (36)	17 (15)				
12–13	43 (61)	52 (84)	42 (58)	97 (84)				
Right-handedness [n (%)]	69 (97)	62 (100)	0.904	0.342	69 (96)	112 (97)	0.850	0.654
Clinical characteristics								
	PD patients, mean +/− s.d. or no. (%) n = 143				HC subjects, mean +/− s.d. or no. (%) n = 178			
	Smokers (n = 71) 50%	Non-smokers (n = 72) 50%	Statistic *t* or χ^2	*P*	Smokers (n = 62) 35%	Non-smokers (n = 116) 65%	Statistic *t* or χ^2	*P*
SIGH-A	20.4 (7.6)	18.6 (8.8)	1.276	0.204	2.3 (2.3)	1.8 (2.1)	1.170	0.244
ASI	32.8 (11.5)	30.0 (11.5)	1.449	0.150	10.31 (6.3)	10.0 (6.7)	0.305	0.761
BSI	62.4 (32.9)	51.6 (34.0)	1.900	0.060	6.9 (7.1)	8.0 (9.2)	−0.735	0.453
BDI	14.8 (8.5)	12.3 (8.3)	1.760	0.081	2.2 (2.9)	2.0 (2.6)	0.374	0.709
MI total	2.2 (0.9)	2.1 (0.7)	0.851	0.396				
MI AAC	1.9 (0.9)	1.8 (0.7)	0.653	0.515				
MI AAL	2.5 (0.9)	2.4 (0.9)	0.782	0.436				
CGI	4.5 (1.2)	4.2 (0.9)	1.530	0.128				
PAS	23.2 (8.5)	21.7 (9.4)	0.994	0.322				
Comorbid DEP	32 (45.10)	19 (26.39)	5.437	0.020				
Comorbid diagnoses	1.54 (1.34)	1.14 (1.43)	1.70	0.10				
Nicotine dependence[a]	20 (28.17)	6 (8.33)						

Notes: PD, panic disorder; HC, healthy controls; CGI, Clinical Global Impression Scale; SIGH-A, Hamilton Anxiety Scale; PAS, Panic and Agoraphobia Scale; ASI, Anxiety Sensitivity Index; BDI-II, Beck Depression Inventory; BSI, Brief Symptom Inventory; MI total, Mobility Inventory total score; MI AAC, Mobility Inventory Avoidance Accompanied; MI AAL, Mobility Inventory Avoidance alone; DEP, depressive disorders.
Missing values in HC subjects: SIGH-A: 15 S, 33 NS; ASI: 11 S, 6 NS; BSI: 11 S, 7 NS.
[a]The diagnosis of nicotine dependence is based on the 12-month prevalence assessed by the CIDI interview, the six subjects in the non-smoker group can be characterized as ex-smoker.

self-report and compared with 12-month DSM-IV-TR diagnosis of nicotine dependence in the PD group, assessed in the clinical interview. After exclusion of participants with missing data regarding smoking status and quality control, MRI data from 143 PD patients [72 non-smokers (PD/NS) and 71 smokers (PD/S)] and 178 HC participants [116 non-smokers (HC/NS) and 62 smokers (HC/S)] were included in the present analysis (see Figure S1 in the supplement). Sociodemographic and clinical characteristics of the final, 'quality controlled' sample are shown in Table 1. The study was approved by the ethics committees of all participating universities. All subjects gave written, informed consent before participating in the study.

MRI acquisition

MRI data were acquired using 3T scanners. The following scanners were used: a 3T Philips Achieva scanner (Philips Medical Systems, Best, The Netherlands) in Münster; a 3T Siemens Trio scanner (Siemens AG, Erlangen, Germany) in Dresden and Marburg; a 3T Siemens Verio scanner (Siemens AG, Erlangen, Germany) in Greifswald; a 3T General Electric Healthcare scanner (General Electric Healthcare, Milwaukee, WI) and a 3T Siemens Trio scanner in Berlin. MP-RAGE T1-weighted images were acquired with the following parameters: voxel size = 1 × 1 × 1 mm^3; repetition time (TR) = 1900 ms; inversion time (TI) = 900 ms; field of view (FOV) = 256 × 256 mm^2; slices per slab = 176; thickness = 1 mm; flip angle = 9, echo time (TE) = 2.26 ms.

Preprocessing and statistical analyses

We used SPM12 (www.fil.ion.ucl.ac.uk) and the CAT12 toolbox (http://www.neuro.uni-jena.de/cat/) implemented in MATLAB R2016a (MathWorks, Sherborn, MA) to pre-process and analyze the neuroimaging data. Brain scans were segmented in gray matter, white matter and cerebrospinal fluid and subsequently normalized to the Montreal Neurological Institute reference brain in CAT12. The voxel size was re-sampled to 1.5 × 1.5 × 1.5 mm^3 during this step. The resulting images were quality controlled via visual inspection and CAT12-based outlier checks (homogeneity analysis). All scans that were scored as having low quality in one of the assessments were rejected, which lead to the exclusion of $n = 16$ subjects. Scans from the remaining subjects were smoothed with an 8 mm full-width at half-maximum Gaussian kernel. To confirm pervious findings, we investigated the effect of smoking in HC subjects on a whole-brain level 'as well as in specific regions pertaining to the pathophysiology of smoking and PD' (HC/S < HC/NS). Subsequently, we examined our a priori defined hypothesis if actual smoking status confounds differences in specific

fronto-limbic circuitry and on a whole-brain level between HC subjects and PD patients by conducting t-tests for the smoker and non-smoker groups separately [PD < HC (non-smokers only); PD < HC (smokers only)]. In addition, we examined in an explorative manner the main and interaction effects of smoking and diagnosis [(PD < HC (whole sample); S < NS (whole sample); S < NS (PD only); PD < HC (non-smokers < smokers)]. Furthermore, an explorative analysis on white matter volume differences in pre-defined regions of interest (ROIs) was performed using the above-mentioned contrasts. Age, gender, education, total intracranial volume, study center and Beck Depression Inventory (BDI-II; Beck *et al.*, 1996) scores were included as covariates of no interest. For all analyses, an implicit mask with an absolute threshold of 0.15 was applied, allowing us to include only those voxel showing an increased probability to contain the analyzed tissue type. An anatomical ROI of the a priori defined brain areas (amygdala, ACC, hippocampus, insula, thalamus, OFC) was calculated combining the definitions from the Automated Anatomical Labeling Atlas (Tzourio-Mazoyer *et al.*, 2002) as implemented in the Wake Forest University PickAtlas (Maldjian *et al.*, 2003) in SPM12 in one mask. Small volume correction on this ROI masks was applied using a cluster-forming threshold of $P < 0.001$ on the voxel level and a clusterwise familywise error-corrected threshold of $P_{fwe} < 0.05$ with a minimum cluster size of $k = 10$ contiguous voxels. For the exploratory whole-brain analysis, as recommended in cluster-extent-based thresholding in fMRI analysis (Woo *et al.*, 2014), a cluster-extent threshold was applied to correct for multiple comparisons using $P < 0.001$ as significance threshold on the voxel level and $k = 200$ contiguous voxels on the cluster level. In addition, we performed Pearson's correlation analyses between significant reduced GMV in PD patients (PD < HC) identified in our ROI analysis and clinical scores (Hamilton Anxiety Scale and Panic and Agoraphobia Scale). To further examine an additive effect of smoking and PD, we conducted a linear regression model in SPM using three groups with increasing health burden (group 1: neither PD nor smoking; group 2: either PD or smoking; group 3: both PD and smoking) as independent variable. We examined a positive and negative linear effect on the previous identified brain regions of the group analysis only.

Results

Sample characteristics

Smokers were significantly more frequent in PD patients than in HC subjects [χ^2 (1) = 7.176, $P = 0.007$]. Demographic data and clinical characteristics are shown in Table 1, where differences within the smoker and non-smoker groups (PD/S *vs* HC/S and PD/NS *vs* HC/NS) are reported. Age, gender and handedness were matched between the two diagnostic groups (PD *vs* HC) and did not significantly differ. However, there was a difference regarding the years of education, with HC subjects showing more years of education than PD patients irrespective of their smoking status. For that, education was added as covariate of no interest in the models. Regarding the clinical characteristics of the PD patient sample only (PD/S *vs* PD/NS), PD/S showed trend wise higher Brief Symptom Inventory and BDI-II scores, reflecting the previously reported association of smoking with symptom severity (Zvolensky *et al.*, 2005). Furthermore, significantly more PD/S suffered from a comorbid depressive disorder (Table 1), for that, we included the BDI-II scores as covariate of no interest in the models. Based on the 12-month prevalence, assessed by the clinical interview, 20 PD/S patients were characterized as nicotine dependent according to DSM-IV criteria and six PD/NS patients presented nicotine dependence within the past 12 months and can be characterized as ex-smoker.

VBM: ROI analysis

Compared to non-smokers, we identified significant regional GMV reductions in smokers in the right insula cortex and ACC in HC subjects (Table 2). Investigating the modulating impact of smoking on PD pathophysiology, only in non-smokers, PD patients showed significantly reduced regional GMV in the bilateral amygdalae and hippocampi compared to non-smoking HC subjects. In the smoker group, no main effect of diagnosis could be shown (Figure 1 and Table 2).

Irrespective of smoking status, a GMV reduction in the right amygdala was identified in the PD group compared to HC subjects in our explorative conducted ROI analysis (Figure 1 and Table 2). Furthermore, we found significant smaller regional GMV in smokers compared to non-smokers regardless of diagnosis in the left orbitofrontal gyrus, the right insular cortex and the left ACC. Regarding PD patients, a significant GMV reduction could be obtained in the left thalamus in smokers compared to non-smokers. No significant effects could be obtained in the exploratory conducted interaction analysis (Table 2).

However, we found a significant negative correlation between Structured Interview Guide for the Hamilton Anxiety Scale (SIGH-A) scores and the right amygdala volume ($r = -0.173$, $P = 0.040$) only in the group of non-smokers [PD < HC (non-smokers only)]. When smokers of both groups were included in the analysis, the correlation was not significant ($r = -0.098$, $P = 0.107$). No other significant correlations could be obtained between the right amygdala and the other clinical scores (Figure 1 and Table 3).

Analyses of white matter volume revealed no significant alterations in smokers compared to non-smokers and in PD patients compared to HC subjects in the a priori defined ROIs.

VBM: explorative whole-brain analysis

In the HC group, significant regional GMV reductions were found in smokers compared to non-smokers in the right fusiform gyrus, the right precentral gyrus, the left precentral gyrus and the right supplementary motor area (Table 4).

Related to HC/NS subjects, PD/NS patients showed significantly reduced regional GMV in the left and right parahippocampal area and the right cerebellum. No significant differences in GMV could be obtained for PD/NS > HC/NS. In the smoker group only, no main effect of diagnosis could be shown (Table 4).

The exploratory conducted main effect of diagnosis, irrespective of smoking status, showed a significant reduced GMV in PD patients in the left middle temporal gyrus (Table 4). Irrespective of diagnosis, we found significant smaller regional GMV in smokers compared to non-smokers in the right fusiform gyrus, the right and left precentral gyrus, the left orbitofrontal gyrus inclusive ACC and in the left middle frontal gyrus (Table 4). No additional structural alterations were identified in PD/S patients compared to PD/NS patients on a whole-brain level. The interaction analysis of smoking and diagnosis revealed no significant results.

Regression analyses

We performed a regression analysis to examine a potential additive effect of health burden on the previous identified brain

Table 2. Locations of significant gray matter volume differences identified in the ROI analysis in PD and HC subjects as a function of smoking status on peak level with MNI coordinates of local maxima

Contrast/region	Side	Cluster size in voxel	x	y	z	t-value	P FWE corrected
S < NS (healthy controls only)							
Insula	R	60	42	0	8	3.95	0.002
ACC	R	16	14	47	12	3.05	0.034
S > NS (healthy controls only)			No differential effect				
PD < HC (non-smokers only)							
Amygdala	L	92	−18	0	−22	3.71	0.003
Amygdala	R	101	24	0	−22	3.69	0.003
Hippocampus	L	10	−18	−4	−22	3.34	0.038
Hippocampus	R	30	−2	−22	3.70	0.013	
PD > HC (non-smokers only)			No differential effect				
PD < HC (smokers only)			No differential effect				
PD > HC (smokers only)			No differential effect				
PD < HC (whole sample)							
Amygdala	R	25	24	0	−22	3.36	0.022
PD > HC (whole sample)			No differential effect				
S < NS (whole sample)							
Orbitofrontal gyrus	L	134	−6	42	−12	3.84	0.001
Insula	R	27	−2	4	3.37	0.049	
ACC	L	20	−6	45	−4	3.28	0.050
S > NS (whole sample)			No differential effect				
S < NS (PD only)							
Thalamus	L	50	−4	−8	14	3.54	0.001
S > NS (PD only)			No differential effect				
Interaction of smoking and diagnosis			No differential effect				

Notes: PD, panic disorder; HC, healthy control; ROI, region of interest; NS, non-smoker; S, smoker; L, left; R, right; x, y, z, MNI coordinates; MNI, Montreal Neurological Institute; ACC, anterior cingulate cortex; FWE, familywise error.
$P < 0.05$ FWE corrected with a minimum cluster size of 10 voxel.

Table 3. Pearson's correlation analyses between significant reduced GMV in PD patients compared to HC subjects identified in the ROI analysis with clinical scores

	SIGH-A (*p*)	PAS (*p*)
Amygdala R (non-smoker)	−0.173 (0.040)*	−0.076 (0.295)
Amygdala R (whole group)	−0.098 (0.107)	−0.076 (0.295)

Notes: GMV, gray matter volumes; PD, panic disorder; HC, healthy control; ROI, region of interest; SIGH-A, Hamilton Anxiety Scale; PAS, Panic and Agoraphobia Scale; R, right.
Missing values in HC subjects: SIGH-A: 48; PAS: 31.

regions. We found a linear decrease in the left and right amygdala with increasing health burden in the ROI analysis (Figure 2 and Table 5 as well as Figure S2 in the supplement).

Results did not change when excluding the six ex-smokers from the group of PD/NS.

Discussion

As supported by the present findings, smoking behavior is highly prevalent in PD patients. Structural abnormalities characterizing the neurobiology of smoking do, to some extent, overlap with brain circuits involved in the pathophysiology of PD. Despite this high co-occurrence, the modulating impact of smoking on regional gray matter abnormalities in PD patients was not explicitly targeted before. Present findings partly confirm pervious identified morphological differences between healthy smokers and non-smokers and indicate that GMV reductions in the amygdala and hippocampus commonly associated with PD pathophysiology are mainly driven by non-smokers. These effects appear to diminish in smokers, which can be attributed to already reduced GMV in healthy smoking subjects. Furthermore, bilateral amygdala volumes show a linear decrease with increasing health burden, possibly indicating additive effects of smoking and PD.

Regarding PD patients, GMV reductions in the bilateral amygdalae and hippocampi were more pronounced in the non-smoker group only than in the combined sample. Furthermore, comparing the smoker group of PD patients and HC subjects yielded comparable GMV in the above-mentioned brain structures. Results of the correlation analysis support this finding. We found a non-significant relationship between amygdala volume and SIGH-A scores, when smokers of both groups were included in the analysis and a significant negative relation in non-smokers only. GMV reductions in the amygdala and hippocampus can be related to the pathophysiology of PD, evidenced by previous research (Del Casale *et al.*, 2013; Pannekoek *et al.*, 2013). These findings on a morphological level can be matched by the presence of neurochemical alterations. PD patients demonstrate lower N-acetylaspartate in the hippocampus (Trzesniak *et al.*, 2010), and reduced binding properties for the serotonin 5-HT_{1A} receptor are evident in the amygdala and hippocampus in PD patients (Nash *et al.*, 2008). Considering the results of the whole-brain approach, the parahippocampal gyrus, a brain region linked to the pathophysiology of PD in several previous studies (Del Casale *et al.*, 2013; Dresler *et al.*, 2013), was significant only when smokers of both groups were excluded from the analysis. Findings indicate that current smoking has indeed a modulating impact on brain morphological correlates in PD patients potentially blurring group differences associated with psychopathology. We thus conclude that current smoking behavior in PD patients and HC subjects can narrow or diminish commonly observed structural

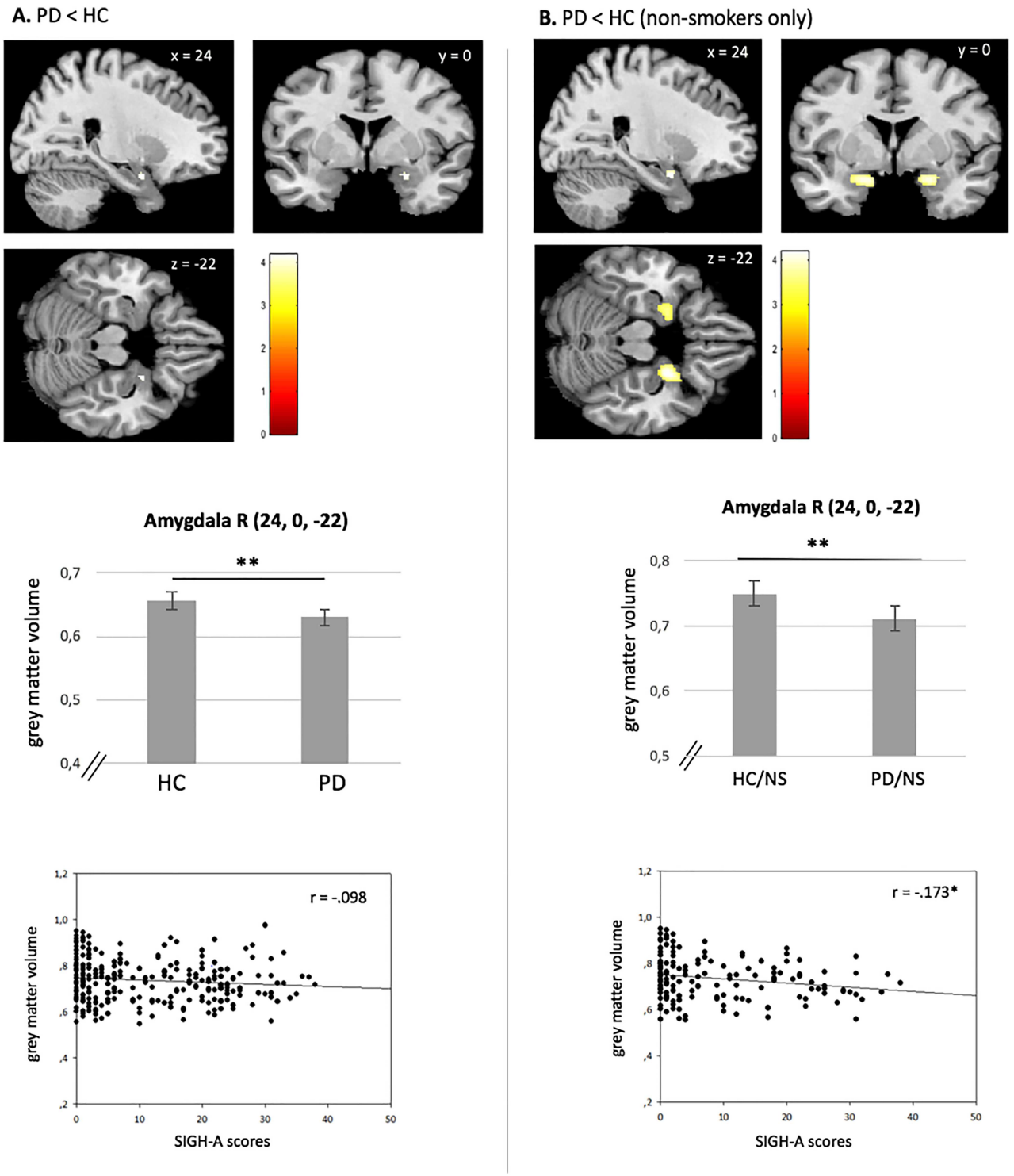

Fig. 1. (A): Main effect of PD on GMV across the whole sample of PD patients and HC subjects. PD patients show reduced volumes compared to HC participants in the right amygdala. 'No significant correlation between GMV and SIGH-A scores'. (B): Significant differences between HC/NS and PD/NS in the right amygdala. Reduced GMVs are negatively correlated with SIGH-A scores. Bars represent the estimated gray matter volumes of the corresponding brain region; error bars represent SEM. R = right. $^{*}P < 0.05$; $^{**}P < 0.001$.

abnormalities in PD patients. If this confounder is not considered by matching or as a covariate, which was the case in previous examinations (i.e. Massana *et al.*, 2003; Asami *et al.*, 2009, 2018; Hayano *et al.*, 2009; Uchida *et al.*, 2003), findings on structural abnormalities in PD patients pertaining to key regions involved (e.g. amygdala and hippocampus) may be biased. As such, it can be speculated if previous studies including both smoking and non-smoking subjects may even have underestimated the true effect of volume reduction in the amygdala and hippocampus.

Table 4. Locations of significant gray matter volume differences identified in the whole-brain analysis in PD and HC as a function of smoking status on peak level with MNI coordinates of local maxima

Contrast/region	Side	Cluster size in voxel	x	y	z	t-value	P uncorrected
S < NS (healthy controls only)							
Fusiform gyrus	R	847	28	−42	−18	4.33	<0.001
Precentral gyrus	L	761	−24	−21	69	4.20	<0.001
Precentral gyrus	R	521	46	−15	56	4.17	<0.001
Supplementary motor area	R	222	2	−2	48	3.69	<0.001
S > NS (healthy controls only)			No differential effect				
PD < HC (non-smokers only)							
Parahippocampal area	R	552	0	−22	3.76	0.001	
Parahippocampal area	L	210	−18	0	−22	3.76	<0.001
Cerebellum	R	409	32	−75	−50	3.57	<0.001
PD > HC (non-smokers only)			No differential effect				
PD < HC (smokers only)			No differential effect				
PD > HC (smokers only)			No differential effect				
PD < HC							
Middle temporal gyrus	L	267	−40	6	−27	3.97	<0.001
PD > HC			No differential effect				
S < NS (whole sample)							
Fusiform gyrus	R	1463	28	−45	−16	5.20	<0.001
Precentral gyrus	L	337	−36	−16	60	3.71	<0.001
Precentral gyrus	R	325	38	−20	42	3.99	<0.001
Orbitofrontal gyrus inclusive ACC	L	208	−6	42	−12	3.84	<0.001
Middle frontal gyrus	L	219	−21	21	48	3.72	<0.001
S > NS (whole sample)			No differential effect				
S < NS (PD only)			No differential effect				
S > NS (PD only)			No differential effect				
Interaction of smoking and diagnosis			No differential effect				

Notes: PD, panic disorder; HC, healthy control; NS, non-smoker; S, smoker; L, left; R, right; x, y, z, MNI coordinates; MNI, Montreal Neurological Institute; ACC, anterior cingulate cortex.
$P < 0.001$ uncorrected with a minimum cluster size of 200 voxels.

Across the whole sample, PD patients exhibited reduced GMV in a subcortical brain structure compared to HC participants. Studies with different functional and structural imaging modalities have consistently reported abnormalities of the amygdala in PD patients, consistent with animal work on fear conditioning and prominent neuroanatomical models of PD (Gorman *et al.*, 2000; Pannekoek *et al.*, 2013). Reduced amygdala volumes in PD patients may represent the structural basis of the functional abnormalities that have been reported in the neuroimaging literature (Sakai *et al.*, 2005; Pillay *et al.*, 2006; Nash *et al.*, 2008; Chechko *et al.*, 2009; Tuescher *et al.*, 2011).

To further examine if smoking results in an additive effect on the previously identified brain structures, we conducted a regression model. Considering three groups with increasing health burden, a linear negative effect of decreasing GMV with increasing burden was observed for bilateral amygdalae volumes, suggesting that the combined impact of health burden (smoking and PD) may be reflected in the structure of the amygdalae. Previous research linked structural alterations of this brain region to both conditions (Dresler *et al.*, 2013; Durazzo *et al.*, 2017). However, alternatively it needs to be considered that the observed GMV reductions in the amygdalae may have existed even before subjects began smoking and developing panic symptoms and PD. Reduced GMV in the amygdalae could therefore also represent a potentially predisposing factor to smoking behavior or PD, or a shared factor for the development of both conditions.

Considering the HC group, GMV reductions in smokers with respect to *the* 'insula, ACC' and fusiform gyrus are consistent with results from previous VBM investigations (Brody *et al.*, 2004; Gallinat *et al.*, 2006; Yu *et al.*, 2011; Liao *et al.*, 2012; Stoeckel *et al.*, 2016). When evaluating the whole sample, smoker showed significant GMV reductions in 'prefrontal cortex' regions identified in various studies before, like in the OFC and middle frontal gyrus (Brody *et al.*, 2004; Morales *et al.*, 2012; Fritz *et al.*, 2014), which have been related to the neurobiology of substance addiction, including smoking (Goldstein and Volkow, 2002).

Cigarette smoke components, including nicotine and free radicals, facilitate negative effects on various neurotransmitter systems, neurobiology, the respiratory system and normal neurodevelopmental processes (Niedermaier *et al.*, 1993; Zvolensky *et al.*, 2003; Dwyer *et al.*, 2008; Moylan *et al.*, 2013). Evidence into the pathogenesis of PD supports a role of these developments (Moylan *et al.*, 2013). The importance of specific neurotransmitter systems has been extensively demonstrated in anxiety disorders, with current first-line pharmacological therapies interacting predominantly with the serotonergic, noradrenergic, cannabinoid, cholinergic and dopaminergic systems (Moylan *et al.*, 2013). However, some of these agents are also effective in enhancing smoking cessation (Jorenby *et al.*, 1999), suggesting a plausible biological interaction between these systems and nicotine dependence. Furthermore, free radicals, another highly concentrated component of cigarette smoke, were linked to a relative deficit in both, tryptophan and serotonin, which could be related to increased anxiety symptoms (Bell *et al.*, 2005; Kulz

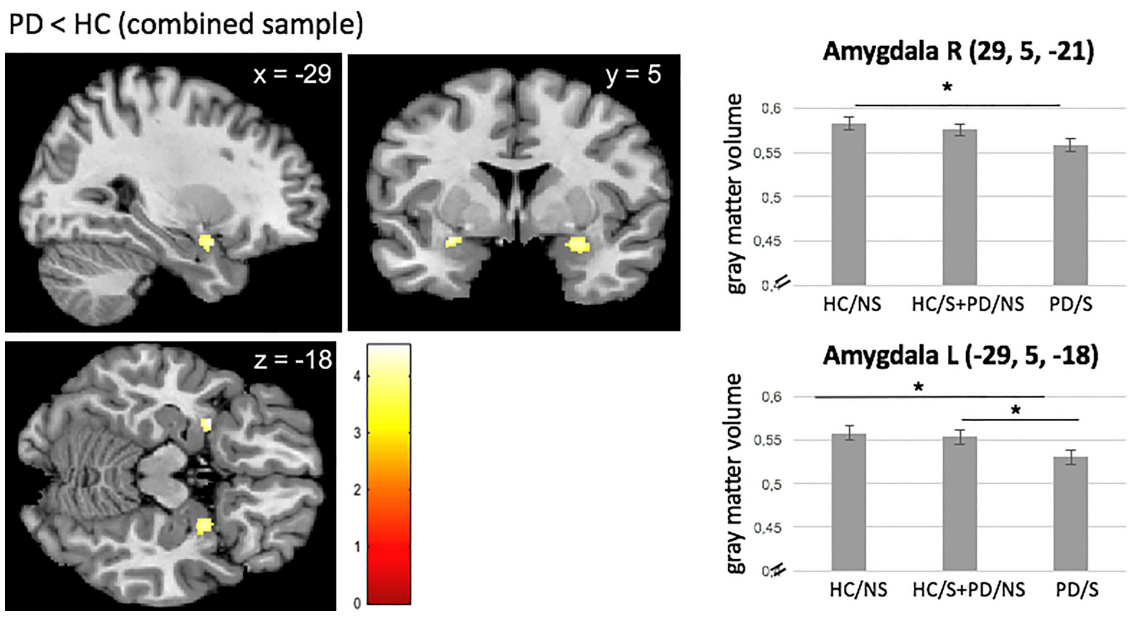

Fig. 2. Linear negative association between health burden and bilateral amygdalae GMV. Bars represent the estimated gray matter volumes of the corresponding brain region; error bars represent SEM. R = right, L = left. *$P < 0.05$.

Table 5. Regression analysis of the whole sample, separated in three groups with increasing burden of health (group 1: neither PD nor smoking; group 2: either PD or smoking; group 3: both PD and smoking)

	Side	Cluster size in voxel	x	y	z	*t*-value	*P* FWE corrected
Linear negative effect							
Amygdala	L	22	−29	5	−18	3.80	0.017
Amygdala	R	57	29	5	−21	4.4	0.010
Linear positive effect			No differential effect				

Notes: PD, panic disorder; L, left; R, right; x, y, z, MNI coordinates; MNI, Montreal Neurological Institute; FWE, familywise error.
Cluster represents region of interest analysis with a cluster-based threshold of $P < 0.05$ FWE corrected and with a minimum cluster size of 10 voxel.

et al., 2007). Numerous population-based studies demonstrated smoking as being prospectively associated with increased rates of anxiety disorders and PD (Breslau and Klein, 1999; Johnson *et al.*, 2000). The effects caused by cigarette smoking may at least partially underpin the biological mechanisms through which smoking might contribute to the development of PD. Hence, it can be speculated that smoking may act as a brain structural vulnerability factor. Like on neurotransmitter systems, smoking cigarettes impacts on specific brain structures also involved in the pathogenesis of PD and thereby possibly predisposing smokers for the development of anxiety symptoms and PD.

Conversely, nicotine can exert an anxiolytic effect as well through rapid desensitization of nicotine acetylcholine receptors in the brain (Gentry and Lukas, 2002; Picciotto *et al.*, 2008) and thereby reduce anxiety symptoms, known as the theory of self-medication or self-treatment. Evidence supports that nicotine exposure does produce a subjective calming effect, although this is coupled with an increase in objective measures of physiological arousal (Perkins, 1995), which could contribute to the maintenance or an increased frequency of smoking in PD patients.

However, as our study is limited by its cross-sectional design, longitudinal studies are clearly needed to assess the role of smoking as a potential marker of vulnerability for PD on a brain structural level.

Although our study benefits from a large sample size, we have to consider some limitations. The study was not primarily designed to examine the effect of smoking, so we had only limited data available on smoking behavior; though a more detailed assessment of smoking severity, history and dependence could shed more light on the observed differences. Furthermore, smoking status was based exclusively on self-report and was not verified by parameters like CO levels or plasma cotinine. As all patients were medication-free, the interaction of cigarette smoking and selective serotonin re-uptake inhibitor (SSRI) treatment could not be investigated. Smokers differ from non-smokers in pharmacokinetics (Zevin and Benowitz, 1999; Kroon, 2007), thus it is plausible to assume an additional interaction with SSRI treatment on symptomatology and possibly brain structure and function. As SSRIs are a first-line treatment, studying the interaction effects with smoking in PD patients would be of relevance from an ecological validity perspective. Significantly more PD/S patients suffered from a comorbid depressive disorder, which seems plausible, considering the link between smoking and symptom severity (Covey *et al.*, 1998; Zvolensky *et al.*, 2005), although our effects remained stable after controlling for depressive symptom scores (BDI-II). Because of the cross-sectional design of our study, we cannot exclude the possibility of any pre-existing structural differences between the analyzed smokers and non-smokers as well as between HC participants

and PD patients. As our study represents a preliminary investigation of the modulating impact of actual smoking behavior on commonly established brain structures in PD patients, future studies are encouraged to investigate this interaction in more depth, including elaborate measures on smoking behavior.

We conclude that current cigarette smoking impacts on neural pathways associated with PD pathophysiology in smokers, differences in GMV reductions in the amygdala and hippocampus diminished. In line, we could demonstrate an additive effect of smoking and PD on amygdalae volumes, a brain structure involved in both, the pathophysiology of PD and nicotine dependence. From a methodological perspective, current smoking status should be considered as an important covariate in future neuroimaging studies focusing on PD. From a mechanism-based perspective, the frequently observed co-occurrence of smoking and PD may be reflected by partly overlapping neurostructural correlates. Longitudinal studies are needed to assess whether smoking (which onset precedes the one of PD) may confer its risk properties also via the neural systems level. Early preventive approaches on smoking cessation particularly in PD vulnerable individuals may specifically ameliorate the adverse effects of smoking also on a neural systems level.

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Conflict of interest

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Supplementary data

Supplementary data are available at *SCAN* online.

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Supplementary Material

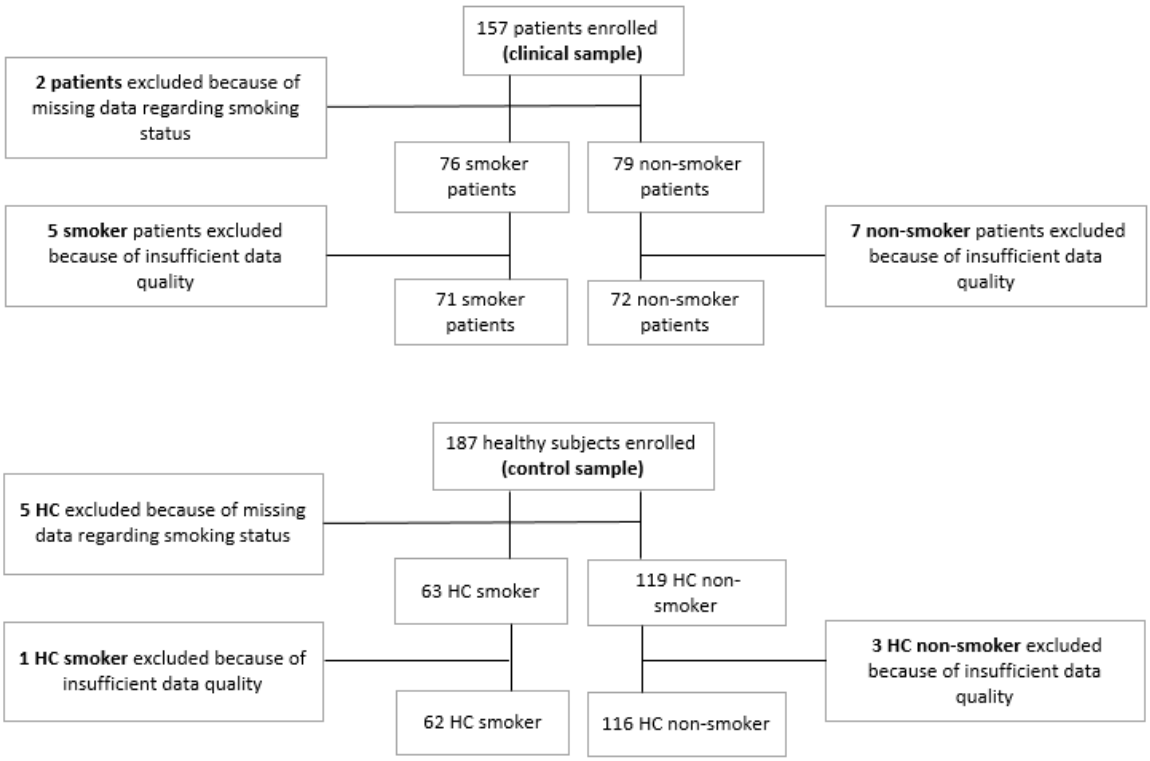

Figure S1: Flowchart of the sample, separated for PD patients and HC subjects.

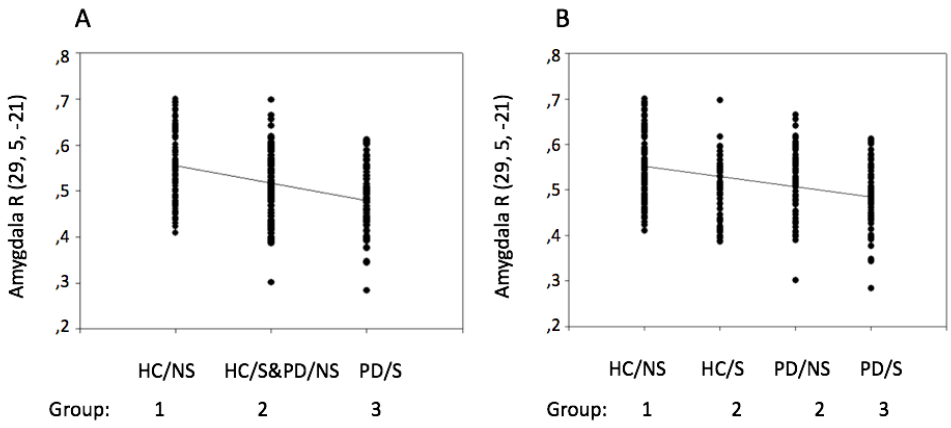

Figure S2: Scatterplot of right amygdala volumes (extracted from a sphere with 5mm diameter around the peak identified in the regression analysis). Plot A pictures the three groups of the regression analysis and plot B separates group 2 in HC/S & PD/NS.

Submitted version of Experiment III

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Neurofunctional alterations of cognitive down-regulation of craving in quitting motivated smokers

Abstract

Cognitive down-regulation of craving involves a neural network within the prefrontal cortex. Tobacco use disorder (TUD) and trait impulsivity has been associated with prefrontal cortex impairments and down-regulation deficits. However, general deficits in down-regulation of craving (regarding non-drug-related cues) compared to never-smokers (NS), differential alterations between drug-related and non-drug-related cues, as well as its links to subject characteristics (smoking severity, trait impulsivity) have so far sparsely been investigated in TUD. In this study, 78 subjects (37 TUD & 42 NS) underwent fMRI scanning while performing a down-regulation of craving task. Two reward cue-types were presented (drug cues and alternative rewards). Subjects applied down-regulation of craving during a LATER condition and up-regulated their craving during a NOW condition. Subjective craving ratings were assessed after each trial. To evaluate down-regulation of craving, we investigated the LATER vs. NOW condition. TUD subjects showed no differences in down-regulation on a behavioral level, neither compared to NS nor between the two reward cue-types. On a neurofunctional level, we found evidence for differences between the two groups in the alternative reward condition in form of a stronger BOLD response in the middle temporal gyrus in TUD. No evidence for differences between the two reward cue-types was found within TUD subjects. During down-regulation across both reward cue-types, we identified significant negative associations between activation of control areas and smoking severity. Results indicate that smokers present general alterations in down-regulation of craving on a neurofunctional level, compared to NS, but no differences between drug-related and alternative reward cues.

1. Introduction

Different behavioral and neurofunctional processes are proposed to be altered in case of a substance use disorder in general and tobacco use disorder (TUD) specifically, thereby influencing its progression and maintenance. Such processes address for example reward learning (Robinson and Berridge 1998), punishment processing (Kunas et al., 2021) or cognitive control over automatically triggered impulses (Baler & Volkow, 2006; McClure &

Bickel, 2014). Automatic impulses are defined as strong desires or urges to consume a specific substance (craving) and lead to an activation in mesolimbic brain regions including the ventral striatum, amygdala, caudate, pallidum, thalamus, and ventral tegmental area (VTA; Koob & Moal, 2001; Boswell & Kober, 2016).

One of the proposed mechanisms of TUD is a deficit in down-regulation of craving processes (McClure & Bickel, 2014): The lack of control over drug use may be regarded as an imbalance between implicit (craving related) and explicit (control related) systems, in which explicit processes aimed at self-regulation are unable to down-regulate urge-related processes (McClure & Bickel, 2014). The prefrontal cortex (PFC) has been implicated in cognitive down-regulation of craving, besides its role in decision making, inhibitory control and affect regulation (Royall et al., 2002; Boswell & Kober, 2016; Demos McDermott et al., 2019; Giuliani et al., 2013; Giuliani & Pfeifer, 2015; Siep et al., 2012). Cognitive neuroscience research suggests that successful cognitive down-regulation is dependent on top-down control from the prefrontal cortex over subcortical regions involved in reward and emotion (Heatherton & Wagner, 2011).

In TUD, neuroimaging studies have demonstrated abnormal PFC functions (Sutherland et al., 2012). Such alterations in the PFC could possibly build the neurobiological basis of down-regulation impairments in TUD. However, only few neuroimaging studies are available specifically investigating down-regulation of craving processes within smokers. These studies found an increased activation in the prefrontal cortex and related areas (angular gyrus, supplementary motor area SMA, middle temporal gyrus MTG and anterior cingulate gyrus ACC) and a decreased activation in craving-related mesolimbic regions during down-regulation of cigarette craving compared to passive viewing (Brody et al., 2007; Hartwell et al., 2011; Kober et al., 2010; Zhao et al., 2012). As far as we know, no study has yet compared down-regulation effects between smokers and non-smokers. This might be explained by the difficulty to investigate down-regulation of craving processes for cigarettes in non-smokers. However, this strain could be solved by using an alternative reward condition (e.g., food) that can induce craving in both groups. As studies comparing down-regulation of (alternative reward) craving between smokers and non-smokers are lacking, general (e.g., both drug-related and non-drug-related) down-regulation deficits in smokers remain unknown.

It is also unclear whether the described changes in the down-regulation of coveted stimuli are specific for drug-related cues or are also present in non-drug-related alternative reward cues in

smokers. This question suggests itself, given that smokers recruit similar brain circuits during regulation for different appetitive stimuli or emotional states, like for instance for cigarettes, food and negative affect (Tabibnia et al., 2014). However, to date only one study directly investigated cognitive down-regulation of craving for drug-related cues compared to alternative reward cues and found a stronger dorsomedial PFC activation during reappraisal of craving for alternative reward compared to cigarette cues (Kober et al., 2010).

Furthermore, smoking and personality characteristics were found to be related to craving, relapse and prefrontal cortex activation (Copersi et al., 2017; Dalley et al., 2011; Azizian et al., 2008). The severity of abuse was associated with impairments in frontostriatal circuits and general cognitive processes in smokers (Azizian et al., 2008; Yuan et al., 2016). Trait impulsivity could be related to higher levels of craving for cigarettes (Doran et al., 2004), as well as to aberrant activity in the prefrontal cortex (Bloom et al., 2014). However, so far it was not investigated if smoking severity and impulsivity are related to down-regulation of craving deficits in smokers.

To sum up, it is unclear, whether processes of down-regulation of craving differ between smokers and non-smokers, whether they differ between drug related cues and alternative rewarding cues in smokers, and whether they depend on smoking severity and impulsivity. The aim of this study was therefore to examine cognitive down-regulation of craving processes in TUD subjects via cognitive reappraisal. Reappraisal in this case refers to changing one's interpretation of a situation to alter craving. To classify behavioral and neural correlates of cognitive down-regulation processes in TUD subjects as a function of smoking status (compared to NS) and reward cue-type (drug cues vs. alternative rewards), we investigated the following hypotheses:

1. On a behavioral level, it was hypothesized that TUD subjects show lower reductions in craving ratings after down-regulation of craving for alternative rewards, compared to NS (between-subjects) which would reflect a general (i.e., both drug-related and non-drug-related) deficit in cognitive down-regulation of craving in TUD (1.1). Moreover, it was expected that TUD subjects present higher reductions in craving ratings for alternative rewards compared to drug-related cues (within-subjects), which would reflect an additional drug-specific deficit in cognitive down-regulation of craving in TUD (1.2).

2. On a neurofunctional level, we expected decreased activations in the (prefrontal) control network as well as a decreased deactivation of mesolimbic brain regions in TUD subjects compared to NS during down-regulation of craving for alternative rewards (between-subjects, 2.1), reflecting a general deficit in down-regulation in TUD. Within TUD subjects, stronger (prefrontal) activations and mesolimbic deactivations were expected during down-regulation for alternative rewards, compared to drug-related cues (within-subjects, 2.2), indicating an additional drug-specific impairment.
3. Furthermore, an association between behavioral and neurofunctional correlates of down-regulation of craving with smoking severity and trait impulsivity was hypothesized (within-subjects).

2. Materials and Methods

2.1 Participants

The present study was part of the German Collaborative Research Center (TRR 265: Losing and regaining control over drug intake), funded by the German research foundation (DFG), representing one of the sub-projects of the consortium. For the cross-sectional part of the project, 82 participants (39 TUD subjects and 43 NS) underwent fMRI scanning. Due to technical issues with the scanner during data acquisition, 37 TUD subjects (56.80 % female) and 41 NS (73.17 % female) were included in the present analysis. Subjects were recruited through (online) advertising and flyers in the Berlin metropolitan area. Inclusion criteria for TUD subjects comprised a current DSM-5-TR diagnosis of TUD evidenced by a structured clinical interview for DSM-5-TR (First et al., 2016) and an age range between 18 - 65 years. Exclusion criteria were a comorbid DSM-5-TR mental disorder within the last 12 months; a lifetime history of any substance-use disorder other than TUD and any bipolar or psychotic disorders; a current suicidal intent; concurrent psychotherapeutic/ psychiatric or psychopharmacological treatment; a history of brain injury and pregnancy. NS were defined with less than 10 cigarettes smoked during lifetime. Furthermore, the NS group was free of any current or past medical, neurological or mental illness. All participants received financial compensation (50€) for their participation. Additionally, all TUD subjects were offered a free, 6-week smoking cessation intervention after the measurements, and half of them were randomized into an additional sport intervention (lasting 12 weeks). TUD subjects were informed about the study regularities before inclusion and were thus highly quitting

motivated. The study was approved by the local ethics committee and all subjects gave written, informed consent before participating in the study.

2.2 Clinical assessments

During a first session, all participants completed the multiple-choice vocabulary test (MWT; range 0-37; Lehrl et al., 1995) to measure their global level of intelligence, the trait part of the State-Trait-Anxiety-Inventory (STAI-T; range 20-80; Spielberger et al., 2012) and the short version of the General Depression Scale (ADS-K; range 0-45; Hautzinger et al., 2012). Furthermore, information regarding frequency of alcohol use was acquired (drinking days/week). The Fagerstroem Test for Nicotine Dependence (FTND; range 0-10; Heatherton et al., 1991) served to assess the severity of nicotine dependence in the TUD group and the Germen version of the Barratt Impulsiveness Scale, short version (BIS-15; range: 15-60; Meule et al., 2011) to measure self-reported impulsivity within the three established subscales (attention, motor impulsiveness and non-planning). To quantify the motivation of abstinence and therapy expectancies, TUD subjects performed the goal attainment scaling (Kiresuk & Sherman, 1968) and answered five questions concerning their motivation and therapy expectancies (for details see Supplementary Text 1).

2.3 Cognitive down-regulation of craving task

Abstinence from eating and smoking for at least three hours before the fMRI session was instructed to ensure a sufficient level of craving for both cigarettes and food but to avoid severe withdrawal in the scanner in a group of moderate smokers. Before starting the regulation of craving task, participants performed another two tasks in the scanner (lasting about 40 minutes) which are not subject of the present study. The experimental paradigm of the task consisted of five conditions: drug-related cues preceded by the instruction NOW and LATER, food cues preceded by the instruction NOW and LATER and a neutral control condition (glass of water) preceded by the instruction NOW (similar to previous tasks studying regulation of craving, e.g., Kober et al., 2010). NOW cues instructed participants to consider the positive feelings of now consuming the depicted item without thinking of any negative consequences. LATER cues instructed participants to down-regulate their craving for the depicted item by using reappraisal techniques (thinking of the negative long-term consequences of smoking e.g., bronchial carcinoma). A neutral control category was included only for the NOW condition (glass of water). Before the fMRI session, participants received an extensive description of the task and the possibility to practice the LATER condition, furthermore, participants rated a set of 50 food pictures with two questions each in sequence:

"how strong is your desire to consume this now?" and afterwards "how deterrent do you expect this in the long-term?", using an eight-point Likert scale from "not at all" to "very much". For the experiment, the 50% most rewarding and concurrently most detrimental stimuli were automatically selected in order to maximize effects (for more details see Figure 1).

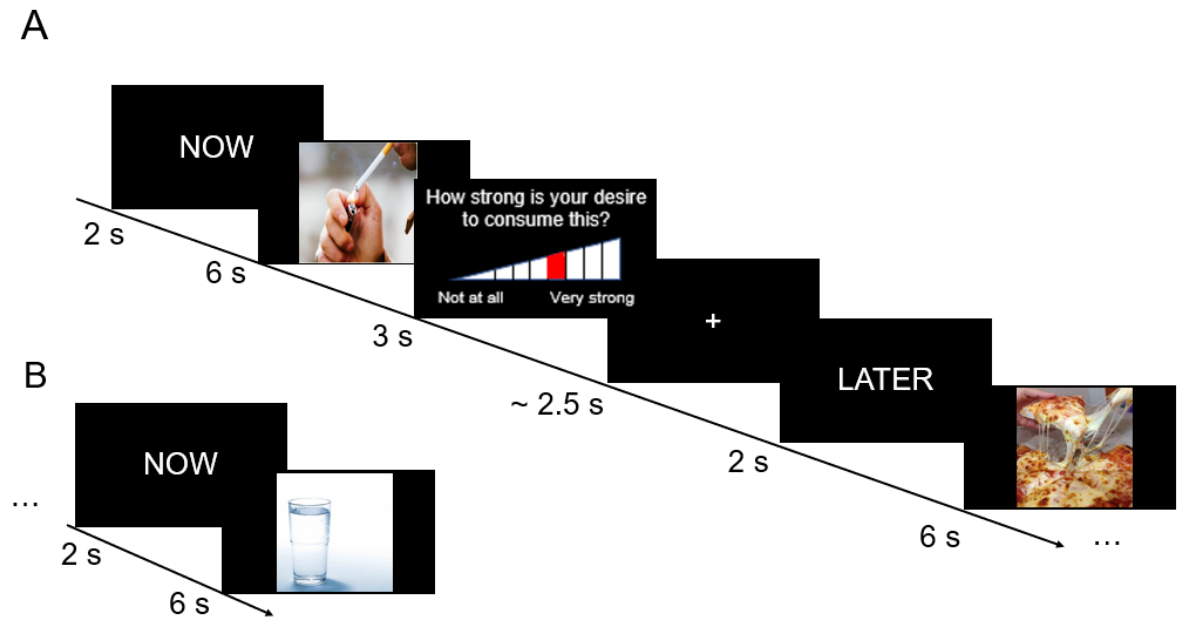

Figure 1: A) Example of a full Cigarette NOW trial followed by Food LATER, cues and instructions were counterbalanced across subjects. B) Example of the Neutral NOW condition, with similar cues (a glass of water) across all neutral trials. The cue categories (drug, alternative reward and neutral cues) were presented for 6 seconds each, followed by a rating (presented for 3 seconds) where participants indicated how much they want to consume the depicted item now using an eight-point Likert scale ranging from "not at all" to "very much". One-hundred trials (20 NOW drug, 20 NOW food, 20 LATER drug, 20 LATER food and 20 NOW neutral) were presented with an ITI between the conditions jittered around 2,5 seconds. The total task duration was appr. 25 minutes and consisted of 5 runs. After the first run participants had the possibility to ask questions concerning the instructions of the task.

2.4 Statistical analysis of behavioral rating data

For between-subject analysis we focused on the alternative reward condition, as craving ratings for cigarettes were very low in the NS group, as expected (M=1.115, SD = 0.497 for NOW and M = 1.298, SD = 0.199 for LATER). To examine differences between TUD subjects and NS in down-regulation of craving for alternative reward based on behavioral ratings, a two-way repeated measure analysis of variance (ANOVA) with the factors smoking status (TUD subjects & NS) and reappraisal (LATER and NOW) was used (hypothesis 1.1).

For within-group analysis of TUD subjects, differences in down-regulation for the different reward cue-types (drug & alternative reward) was assessed using a two-way repeated measures ANOVA (hypothesis 1.2). Please refer to Supplementary Text 2 for assumptions concerning the tests.

2.5 fMRI data acquisition and pre-processing

Functional images were acquired using a Siemens simultaneous multi-slice T2*-weighted gradient-echoplanar imaging (EPI) sequence (TR = 869 ms, TE = 38 ms, 60 slices, slice thickness = 2.4 mm, voxel size 2.4 × 2.4 × 2.4 mm). Field map images were obtained using a Siemens dual gradient-echo sequence (TR = 698 ms, TE1 = 5.19 ms, TE2 = 7.65 ms, 64 slices). High-resolution anatomical images were acquired using a T1-weighted MPRAGE sequence (TR = 2000 ms, TE= 2.01 ms, TI = 880ms, 208 sagittal slices, voxel size 1 × 1 × 1 mm). For more details please refer to Supplementary Text 3. Image preprocessing was performed using statistical parametric mapping (SPM12; http://www.fil.ion.ucl.ac.uk/spm/software/spm12) and MATLAB R2020a (Mathworks, Sherborn, Massachusetts) based scripts and comprised slice timing with reference to the middle slice, SPM12 standard realignment and unwarping including correction for field deformations based on a previously acquired field map, co-registration, normalization to MNI stereotactic space using unified segmentation based on the SPM tissue probability map for six tissue classes, and spatial smoothing with 8 mm full-width at half-maximum isotropic Gaussian kernel. Following preprocessing, all five runs were visually inspected, for each subject separately, in order to perform a visual quality control.

2.6 fMRI data analysis pathway

For first level analysis, brain activation differences related to the presentation of the different stimuli were analyzed using a general linear model (GLM) in SPM12 (for more details see Supplementary Text 3). For second level analysis, group effects were assessed with a 2x2x2 analysis of covariance (ANCOVA) using a full factorial model in SPM12, encompassing the factors “smoking status” (TUD subjects & NS), “reward cue-type” (drug & alternative rewards) and “reappraisal” (NOW & LATER). The neutral control condition was excluded of the analysis as we were not interested in cue-reactivity effects. The continuous variables of the STAI-T and ADS-K were included as covariates of no interest in the between-subject analyses, as groups differed significantly in these measures (see below). As a first analysis, to validate the paradigm and to show that our task elicited the proposed activations in the (prefrontal) control and mesolimbic brain network, we investigated the effect of “reappraisal”

(LATER vs. NOW), pooled across both conditions, reward cue-types (drug and alternative reward) and smoking status (TUD subjects & NS). Furthermore, we calculated the effect of "reappraisal" within the NS group and the TUD group, separately. To test our *a priori* formulated hypotheses, we then investigated the smoking status-by-reappraisal interaction (2.1) for alternative rewards ("altLATER > altNOW" for "NS > TUD subjects" & "altLATER > altNOW" for "TUD > NS subjects"). Within TUD subjects, the reward cue-type-by-reappraisal interaction [(altlater > altnow) > (druglater > drugnow) & (altnow > altlater) > (drugnow > druglater)] was investigated (hypothesis 2.2).

2.7 Region of interest analysis

To identify neural correlates of cognitive down-regulation of craving we searched for increased activity in the (prefrontal) control network and reduced activation in caving-related mesolimbic brain regions during LATER compared to NOW trials (Heatherton & Wagner, 2011). An anatomical region of interest (ROI) analysis of these *a priori* defined brain areas was conducted. Based on the literature (Kober et al., 2010 & Brandl et al., 2020), we included the dlPFC, vlPFC, SMA, MTG and angular gyrus in the ROI analysis concerning the (prefrontal) control network. The mesolimbic reward network included: the amygdala, ventral striatum, pallidum, caudate, thalamus, and VTA (Koob & Moal, 2001). Anatomical ROIs were build combining the definitions from the Automated Anatomical Labeling Atlas (Maldjian et al., 2003). To further specify subcortical and prefrontal cortex regions (vlPFC, dlPFC and ventral striatum), we used the corresponding Brodmann areas. The bilateral ROIs were investigated using one single mask for the control network and one single mask for the reward network. Small volume correction on this single mask was applied using a family wise error corrected threshold of $p_{fwe} < 0.05$ with a minimum cluster size of $k = 10$ contiguous voxels.

2.8 Correlation analysis

To evaluate the relationship between behavioral and neural correlates of cognitive down-regulation and smoking severity and trait impulsivity, we extracted beta weights of significantly activated ROIs for the within-subjects contrasts in TUDs: LATER > NOW & NOW > LATER (pooled for both reward cue-types). The toolbox marsbar was used to extract the values using a sphere of 5 mm around the peak voxels of the significant clusters of the ROIs (for MNI coordinates see Table 2). Furthermore, the difference between craving ratings (NOW minus LATER) was calculated as behavioral correlate of down-regulation, where higher values implicate a stronger craving reduction. Pearson and, when assumptions were violated, Spearman correlations were calculated between behavioral and neural correlates of

reappraisal with smoking severity (pack years & FTND) and with trait impulsivity (BIS-15). We corrected for multiple testing using the false discovery rate (FDR) to control for false positive results (see also Supplementary Text 2).

3. Results

3.1 Sample characteristics

Demographic characteristics and smoking data are shown in Table 1. TUD subjects showed a moderate nicotine dependence, according to FTND scores, pack years and average cigarettes smoked per day. STAI-T and ADS-K scores were significantly higher, though subclinical, in the TUD group, which is consistent with previous literature (Morrell & Cohen, 2006). Included subjects were highly abstinence motivated, evidenced by the questions regarding therapy expectancies (TE).

Table 1: Sociodemographic and psychometric characteristics of the smoker and never-smoker sample.

Sample characteristic	**TUD subjects** N = 37	**Never-smokers** N = 41	**Statistic**	***p***
Age [M(SD)]	35.60 (10.40)	32.27 (11.01)	$t(76) = -1.363$	0.177
Female gender [n(%)]	21 (56.80)	30 (73.17)	$x^2(1) = 2.315$	0.128
Right-handedness [n(%)]	37(100)	39(95.12)	$x^2(1) = 2.741$	0.100
Level of education				
A-Level[a] [n(%)]	30 (81.08)	34 (82.93)	$x^2(1) = 0.045$	0.832
Monthly income in € [n(%)]	7 (18.91)	16 (39.02)	$x^2(4) = 6.902$	0.141
< 1.000	11 (29.73)	12 (29.27)		
1.000 – 2.000	16 (43.24)	10 (24.39)		
2.000- 3.500	2 (5.41)	-		
3.500 – 4.500	1 (2.70)	1 (2.43)		
> 4.500				
BMI [M(SD)]	24.63(4.89)	23.23(3.53)	t(75) = 1.433	0.157
MWT [M(SD)]	28.60 (4.40)	29.41 (3.29)	$t(76) = 0.938$	0.351
Drinking days/week [M(SD]	1.89 (1.13)	1.26 (0.87)	$t(76) = -2.721$	0.008*
STAI-T [M(SD]	38.90 (7.42)	31.09 (6.33)	$t(76) = -5.042$	< 0.001**
ADS-K [M(SD)]	8.06 (5.253)	4.200 (2.95)	$t(76) = -3.994$	< 0.001**
BIS (total) [M(SD)]	32.63 (7.134)			
BIS (non-planning)	11.44(3.627)			
BIS (motor)	11.31(2.717)			
BIS (attentional)	9.88 (2.791)			
TE [M (SD)]	40.92 (6.901)			
FTND [M(SD)]	4.00 (2.30)			
Pack Years [M(SD)]	10.91 (9.63)			
Cigarettes/day [M(SD)]	14.29 (6.11)			

Note. M = mean; SD = standard deviation; BMI = Body Mass Index; MWT = Mehrfachwahl – Wortschatz – Test. Identification Test. STAI-T = trait part of the State-Trait-Anxiety-Inventory; ADS-K = General Depression Scale; BIS-11: Baratt Impulsiveness Scale, short version; TE = therapy expectancies; FTND = Fagerstroem Test for Nicotine Dependence. Missing values: BMI: 1 (NS); Monthly income: 1; TE = 1; FTND: 3; Cigarettes/day: 2; BIS-11: 5. Missing values were treated with listwise deletion.

[a] Abitur

*p < 0.05

** p < 0.001

3.2 Behavioral craving ratings

Between-subject analyses of craving ratings for alternative rewards reached a significant main effect for LATER vs. NOW ($F(146,1) = 26.31$; $p < 0.001$, $\eta^2 = 0.149$), indicating lower craving ratings after the LATER compared to the NOW condition, as expected. We found no significant main effect of smoking status ($F(146,1) = 3.668$, $p = 0.057$, $\eta^2 = 0.021$), indicating that TUD subjects showed no differences in craving ratings compared to NS. No significant

interaction effect of smoking status-by-reappraisal ($F(146,1) = 0.353$, $p = 0.553$, $\eta^2 = 0.002$) could be obtained, contrary to hypothesis 1.1.

Within TUD subjects, we found a significant main effect for LATER vs. NOW ($F(34,1) = 60.74$, $p < .001$, $\eta^2 = 0.640$), indicating again lower craving ratings after the LATER compared to the NOW condition. No significant main effect for reward cue-type could be obtained ($F(34,1) = 3.527$, $p = .069$, $\eta^2 = 0.090$), indicating that TUD subjects showed no differences in craving ratings between alternative rewards and drug cues. Contrary to our hypothesis 1.2, we found no significant interaction between reward cue-type and reappraisal ($F(34,1) = 2.70$, $p = .110$, $\eta^2 = .070$).

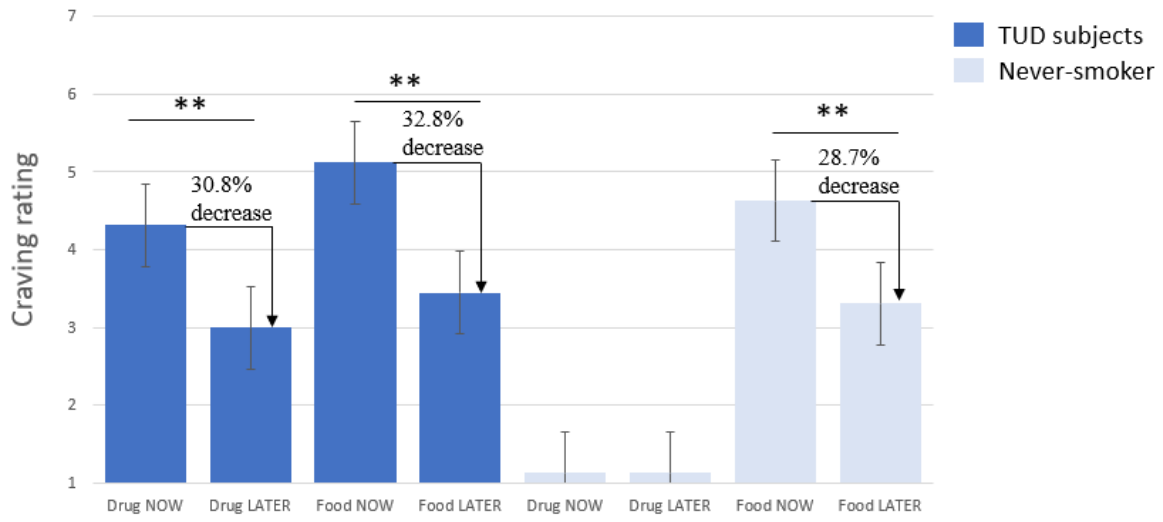

Figure 2: means of behavioral craving ratings separated by smoking status and reward cue-type. Error-bars present the standard error of the mean.
*p <0.05
**p < 0.001

3.3 fMRI results

3.3.1 Validation of the cognitive control of craving task

When data were pooled across both reward cue-types (drug & alternative reward) and smoking status (TUD subjects & NS), we found prefrontal cortex activation and mesolimbic top-down regulation during down-regulation of craving (LATER vs NOW), indicating that the

experimental paradigm worked. A full description of the results of this analysis can be found in Supplementary Text 4 and Table S1.

We found no significant effects (LATER vs NOW) in the separate analysis of the NS group only, neither in the control ROI nor in the mesolimbic ROI. The effect of down-regulation was absent for all analyses, the condition with both reward cue types collapsed as well as for both reward cue types separately (drug & alternative reward; see also Table S1, B).

Within TUD subjects, we observed an effect in the LATER > NOW contrast, when both reward cue-types were pooled, in the MTG, SMA, angular gyrus, dlPFC and vlPFC in the ROI analysis (see Table 2 & Figure 3B). In the NOW > LATER contrast the caudate, putamen and thalamus were significantly activated in the ROI analysis (Table 2). For a separate investigation of both reward cue-types within TUD subjects see Table S1, C.

Table 2: Significant results in the LATER vs. NOW condition in TUD subjects across both reward cue-types.

Contrast/ Region	Side	Voxels	x	y	z	*t*	BA	*p <0.05* fwe corrected
LATER > NOW								
Control ROI								
MTG	L	53	-52	-20	-20	5.35	21	< 0.001
SMA	L	62	-40	10	52	5.31	6	< 0.001
Angular gyrus	L	81	-46	-58	28	4.86	39	< 0.001
dlPFC	L	56	-8	48	44	4.89	8	< 0.001
vlPFC	L	33	-50	26	-4	4.31	47	< 0.001
NOW > LATER								
Mesolimbic ROI								
Caudate	L	10	-8	19	-6	4.13	/	0.018
Putamen	L	87	-32	-14	-2	3.98	/	0.025
Thalamus	L	96	-12	-22	6	3.54	/	0.043

L: left; R: right; voxels: number of voxels per cluster; x, y, z: MNI coordinates; BA = Brodmann-area; MTG = middle temporal gyrus; SMA = supplementary motor area; dlPFC = dorsolateral prefrontal cortex; vlPFC = ventrolateral prefrontal cortex; ROI = region of interest

$p < 0.05$ fwe corrected: for ROI analyses a family-wise-error corrected threshold of $pfwe < 0.05$ with k > 10 voxels on a peak level was used.

3.3.2 Confirmatory analysis

Concerning hypothesis 2.1 (differences between TUD subjects and NS in the alternative reward condition), the interaction effect of smoking status-by-reappraisal showed no greater brain activations in NS. We exploratively searched for stronger activations in TUD subjects and found (contrary to our assumption) that TUD subjects showed a stronger activation in the

left MTG (-52, -20, -20; $t = 3.580$; $p = 0.004$) compared to NS in the ROI analysis (Figure 3A). No effects could be obtained in the mesolimbic ROI analysis.
Concerning hypothesis 2.2 (within-subject differences between reward cue-types), no significant interaction effects of reappraisal-by-reward cue-type could be obtained in both directions in TUD subjects.

Concerning hypothesis 3 (associations of behavioral and neurofunctional correlates with measures of smoking severity and trait impulsivity), behavioral correlates of down-regulation (LATER minus NOW craving ratings) showed no associations with smoking severity and trait impulsivity (Table 3). Significant negative correlations were identified between pack years and BOLD responses (LATER > NOW, pooled across reward cue-types) in the left vlPFC and SMA as well as between FTND scores and BOLD responses in the left vlPFC (Table 3 & Figure 3B). For trait impulsivity, no associations were observed with the total BIS-15 score. In an explorative analysis of the sub-scales of the BIS-15, we observed a negative association between the non-planning subscale and BOLD responses (LATER > NOW, pooled across both reward cue-types) in the left vlPFC (see Table S3). No other correlations reached significance after controlling for multiple testing using the FDR method.

Table 3: Correlations of smoking characteristics/ trait impulsivity with significant BOLD activations (LATER vs NOW, pooled across reward cue-types) and craving ratings in TUD subjects. Beta weights were extracted using the toolbox marsbar and a sphere around the significant peak voxel of 5 mm. P-values are based on false discovery rate correction for multiple testing.

	FTND	Pack years	BIS-15
LATER > NOW			
dlPFC	-0.350* (0.176)	-0.245 (0.143)	-0.133 (0.927)
vlPFC	**-0.554*** (0.036)	**-0.524*** (0.043)	-0.392 (0.130)
MTG	-0.456 (0.060)	-0.396(0.123)	0.088 (0.858)
SMA	-0.428 (0.090)	**-0.471*** (0.043)	-0.012 (0.724)
Angualr gyurs	-0.174 (0.702)	-0.313 (0.126)	0.044 (0.927)
NOW > LATER			
Caudate (L)	-0.059 (0.927)	0.141 (0.413)	-0.254 (0.506)
Thalamus (L)	-0.396 (0.123)	-0.408 (0.012)	-0.188 (0.690)
Putamen (L)	-0.266 (0.409)	-0.141 (0.407)	-0.135 (0.723)
NOW – LATER (craving rating)	-0.006 (0.724)	0.309 (0.151)	-0.318 (0.724)

Note: R = right, L = left; dlPFC = dorsolateral prefrontal cortex: vlPFC = ventrolateral prefrontal cortex, MTG = middle temporal gyrus; SMA = supplementary motor area, FTND = Fagerstrom Test for nicotine dependence; Missing values: BIS-15 = 5, FTND = 3, Subjective craving ratings = 2. Missing values were treated with listwise deletion.
craving ratings represent the difference between the NOW and LATER condition (NOW minus LATER)
*$p < 0.05$
**$p < 0.001$

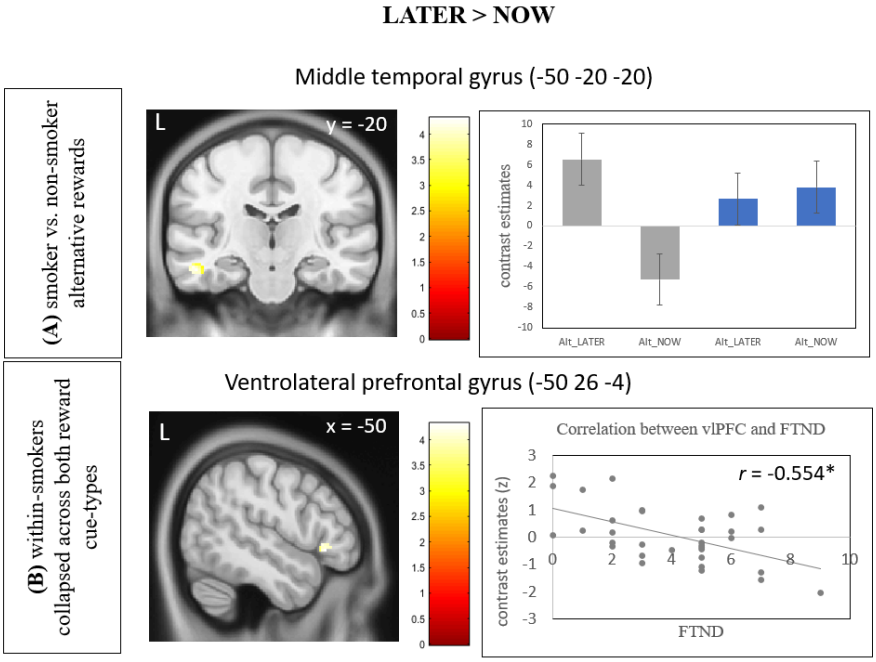

Figure 3: **(A)** Stronger BOLD responses in the left MTG in TUD subjects compared to NS in the alternative reward condition (hypothesis 2.1). **(B)** Stronger activations in the (prefrontal) control network (vlPFC) during down-regulation of craving (LATER > NOW, pooled across both reward cue-types) in TUD subjects (left side) and significant correlations between FTND scores and vlPFC activation during down-regulation of craving (LATER > NOW) (right side). Contrast estimates were transformed to z values for the correlation analysis. L = left.

4. Discussion

The present study extends research on cognitive down-regulation of craving in quitting motivated TUD subjects and offers starting points to better understand general and specific alterations in this group. Both TUD subjects and NS showed a reduction of craving ratings after cognitive down-regulation (NOW minus LATER) on a behavioral level. No difference in behavioral craving reduction could be observed between TUD subjects and NS in the alternative reward condition, nor within TUD subjects between the different reward cue-types. On a neurofunctional level we found alterations in down-regulation of craving (LATER > NOW) in form of a stronger BOLD response in the middle temporal gyrus in TUD subjects compared to NS in an alternative reward (food) condition. No differences were found for cognitive down-regulation between different reward cue-types within TUD subjects. Significant negative associations between BOLD responses in the vlPFC and SMA (LATER >

NOW, pooled across both reward cue-types) with smoking severity (pack years, FTND) implicate a role of these characteristics in down-regulation of craving, at least on a neurofunctional level.

4.1 Cognitive down-regulation of craving ratings

As expected, craving ratings were significantly reduced in LATER versus NOW trials independent of reward cue-type and smoking status. This result is consistent with previous research testing intentional down-regulation strategies to target craving for food (Wolz et al., 2020), cigarettes (e.g., Kober et al., 2010; Zhao et al., 2012) or emotions in general (Morawetz et al., 2017). The present study adds to these studies in that it provides a direct comparison of craving regulation between NS und TUD. Compared to NS, TUD subjects showed no significant differences in craving regulation for food on a behavioral level. This result is compatible with findings by Wu et al. (2015) in emotion regulation and suggests that TUD subjects may not exhibit general deficits in the cognitive down-regulation of craving for rewards. Within the TUD group, the reduction of perceived levels of craving was similar for alternative rewards and drug-related cues, thus suggesting that TUD subjects do not present a specific form of cognitive down-regulation deficit exclusively related to drug-related cues. In summary, our behavioral findings suggest that highly quitting motivated TUD subjects do exhibit neither a general nor a specific deficit in cognitive down-regulation of craving.

4.2 Neurofunctional correlates of cognitive down-regulation of craving

Contrary to our hypothesis 2.1, TUD subjects showed a stronger MTG activation compared to NS in the alternative reward condition during cognitive down-regulation of craving. The MTG is in the present study considered as part of the control network and may in particular play a role for the reenacting of an emotional scene (together with SMA and angular gyrus) which potentially represents the execution of regulation (Johnson-Frey et al., 2005). While decreased activations in control areas (representing regulation deficits) were hypothesized in TUD, the observed stronger activation in the MTG might be interpreted as a compensatory process, reflecting higher down-regulation efforts in TUD subjects compared to NS. On a behavioral level both groups showed similar craving reductions for alternative rewards, thus different explanations may be plausible for a compensatory process in TUD subjects on a neurofunctional level. First, it might be speculated that TUD subjects need to compensatorily down-regulate stronger mesolimbic cue-reactivity, elicited during the NOW condition, to reach similar craving ratings. Previous studies found stronger mesolimbic bottom-up processes, characterizing smoking behavior (Nestor et al., 2011). Second, it may be possible that a stronger

MTG activation represents a compensation of a dysfunction in other cognitive control areas (e.g., vlPFC) to reach similar outcomes compared to NS on a behavioral level. Considering these two possible explanations, it is important to note that we did not observe any differences in mesolimbic cue-reactivity for alternative rewards between TUD subjects and NS. This speaks in favor of the second explanation (increased MTG activation in smokers as a compensation of possible dysfunctions in other cognitive control areas), which would point to a general smoking-associated neurofunctional alteration during down-regulation of craving.

In contrast to our hypothesis 2.2 that functional activation in (prefrontal) control areas would be greater during down-regulation of craving for alternative rewards (food) versus drug cues, we did not find any significant differences in TUD subjects. Interestingly, contrary to our findings, Kober and colleagues (2010) found that the dorsomedial PFC was more strongly activated during regulation of craving for food compared to cigarettes. An explanation for the discrepancy between findings may be that TUD subjects were less severely dependent in the present study compared to Kober et al. (2010). Due to the on average moderate nicotine dependency, it is possible that down-regulation of craving for both stimulus types was similarly challenging for TUD subjects and that therefore no differential activation was apparent in the interaction. Our results thus suggest no cognitive down-regulation impairments specific to smoking cues in light to moderate TUD subjects.

4.3 Associations between cognitive down-regulation of craving and smoking characteristics

Concerning hypothesis 3, we found significant relations between smoking severity and BOLD responses in control areas (vlPFC and SMA) during cognitive down-regulation of craving, with stronger activation in less severe smokers. Two explanations of this finding are possible: First, stronger down-regulation efforts (as reflected in stronger activation of control areas) may be present in more light smokers which may decline when individuals develop more severe smoking behavior. Second, it is also well possible that some individuals exhibit reduced down-regulation efforts (as reflected in lower activation in control areas) form the very beginning and consequently develop more severe smoking behavior. Our cross-sectional findings do now allow conclusions on which explanation might be correct.

A negative association of the non-planning subscale of trait impulsivity with vlPFC activation during down-regulation of craving seems to be in accordance with previous findings in other SUDs (Goldstein & Volkow, 2012). The non-planning subscale of the BIS-15 represents a measure of hasty decision-making, or a lack of decision making, related to planning for the future (Spinella, 2007). Thus, it seems plausible that TUD subjects with higher scores in non-planning impulsiveness show reduced activation in the vlPFC (representing a control area),

during down-regulation processes which refer to the negative long-term consequences of smoking.

4.4 Limitations

The rather homogenous group of TUD subjects with high levels of income, education and without any mental or physical disorders may be limited in its external validity. Furthermore, BIS-15 total scores showed no deviation from means of standard control groups (Spinella, 2007). This indicates that TUD subjects in our sample were not highly impulsive, as would have been expected because of their addictive behavior. Therefore, investigating a group of more impulsive, more dependent smokers who are not quitting motivated and present a lower socioeconomic status may lead to different results.

4.5 Conclusion

Based on our results it can be proposed that TUD subjects show general alterations in cognitive down-regulation of craving on a neurofunctional level compared to NS (between-subjects) but no drug-specific alterations compared to other alternative rewards (within-subjects). Smoking severity represents a potential influencing factor on cognitive down-regulation of craving effort, while trait impulsivity showed only weak associations. A stronger MTG activation in TUD subjects compared to NS during down-regulation of craving may be interpreted as compensatory activation to adjust for deficits in other parts of the control network (e.g., vlPFC). In parts of prefrontal control areas (e.g., vlPFC, SMA) a hypoactivation during down-regulation of craving might correspond to a possible deficit in down-regulation processes within TUD subjects, which might be present especially in heavy smokers.

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Supplementary Materials

Neurofunctional alterations of cognitive down-regulation of craving in quitting motivated smokers

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Content

Supplementary Texts

Supplementary Text 1: Details on clinic assessments

Therapy expectancies: Five questions were asked regarding therapy expectancies and motivation for therapy, which can be translated as following:

1. How logical does the therapy appears to you, as it was described to you?
2. How successful does the therapy appears to you?
3. Would you recommend the therapy to a friend of you?
4. How strong is your motivation to take part in the program?
5. How successful do you think will the therapy be in your case?

The questions were answered using a 10-point Likert-scale ranging from 1 (not at all) to 10 (very much). The question regarding therapy motivation (4) reached a mean of 9.61 with a standard deviation of 0.83 implicating a very high therapy motivation of the participating TUD subjects.

Supplementary Text 2: Statistical analysis of behavioral data and correlation analysis

To examine differences in subjective down-regulation of craving ratings between the two groups (for the alternative reward condition only), we checked the outcome variable regarding normal distribution using the Shapiro-Wilk test and equal variances, using the Levene test. Both tests showed a non-significant result, indicating that the data is normally distributed and has equal variances. Both tests showed non-significant results for the outcome variable for the within subject repeated measures ANOVA as well. Concerning the t-test we also tested for normal distribution and equal variances, all assumptions were met. The following assumptions were tested before conducting the Person correlation analysis: normality (Shapiro-Wilk test), equal variances (Barlett's test), linearity (scatterplots) and presence of outliers (boxplots). Outliers were defined as a deviation of more than two standard deviations from the mean, no outliers could be detected. As pack years were not normally distributed, according to the Shapiro-Wilk test ($p = 0.001$) Spearman correlations were conducted instead of Pearson correlations.

Supplementary Text 3: fMRI data acquisition and 1st level analysis

Specific parameters of the T2*-weighted gradient-echo planar imaging sequence: (TR = 869 ms, TE = 38 ms, 60 slices, slice thickness = 2.4 mm, voxel size 2.4 × 2.4 × 2.4 mm, no inter-slice gap, field of view (FoV) = 210 mm, matrix size 88 x 88, acquisition orientation T > C, interleaved slice order, acceleration factor slice = 6, flip angle = 58°, bandwidth = 1832 Hz/Px, prescan normalize, weak raw data filter, fat sat).
Specific parameters of the field map images: (TR = 698 ms, TE1 = 5.19 ms, TE2 = 7.65 ms, 64 slices, slice thickness = 2.4 mm, no slice gap, voxel size 2.4 × 2.4 × 2.4 mm, field of view (FoV) = 210 mm, matrix size 88 x 88, acquisition orientation T > C, interleaved slice order, flip angle = 54°, bandwidth = 279 Hz/Px).
Specific parameters of the T1-weighted MPRAGE sequence: (TR = 2000 ms, TE= 2.01 ms, TI = 880ms, FoV = 256 mm, 208 sagittal slices, voxel size 1 × 1 × 1 mm, flip angle = 8°, GRAPPA factor 2 (PE), 24 ref. lines, prescan normalize, 23.1% slice oversampling, bandwidth = 240 Hz/Px).
To minimize movement artifacts, participants' heads were positioned on a pillow and fixated using foam pads surrounding the head.

1st level analysis
The blood oxygen level dependent response was modeled by a canonical hemodynamic response function for each of eight conditions: drug NOW and LATER, food NOW and LATER, neutral NOW, instruction, button-presses and ratings, resulting in 4 regressors of interest and 4 noise regressors (instruction, neutral NOW, button-press and rating). Model parameter estimates and the resulting t-statistic images were submitted to group level analysis.

Supplementary Text 4: Explorative analysis

Pooled across both groups and reward cue-types, the ROI analysis reached significance in brain regions of the prefrontal control network (left SMA, angular gyrus, vlPFC, dlPFC, MTG) in the reappraisal condition (LATER > NOW). For the opposite contrast, representing deactivations during reappraisal (NOW > LATER), significant activations in the mesolimbic brain reward system (bilateral amygdala, left putamen, striatum, thalamus and caudate) could be shown (Table S1, B) in the ROI analysis.

Supplementary Tables: S1: Validation of the down-regulation of craving paradigm

Table S1. Locations of significantly activated brain regions for the effect of down-regulation, across and separated for the two groups and reward cue types.

Contrast/ Region	Side	Voxels	x	y	z	*t*	*p <0.05* fwe corrected
A) Effect of down-regulation across reward cue types and groups							
Later > Now							
Region of interest analysis							
SMA	L	62	-40	10	52	5.46	0.001
Angular gyrus	L	81	-44	-56	28	5.07	0.027
vlPFC (BA 47)	L	113	-50	26	-4	5.07	0.036
dlPFC	L	56	-8	50	42	4.95	0.033
Middle temporal gyrus	L	34	-52	-20	-20	4.21	0.016
Now > Later							
Region of interest analysis							
Thalamus	L	159	-12	-22	6	3.97	0.009
Caudate	L	28	-6	20	-6	4.30	0.012
Amygdala	L	40	-28	-6	-12	4.11	0.019
Amygdala	R	27	28	-2	16	3.89	0.041
Putamen	L	37	-32	8	-8	3.82	0.049
Striatum	L	10	-6	16	-6	3.36	0.035
B) Effect of down-regulation within the NS group							
NS (LATER > NOW)						No differential effect	
NS (NOW > LATER)						No differential effect	
NS (AltLater > altNOW)						No differential effect	
NS (AltNOW > AltLATER)						No differential effect	
NS (DrugLATER > drugNOW)						No differential effect	
NS (DrugNOW > drugLATER)						No differential effect	
C) Effect of down-regulation within TUD subjects							
S (AltLATER > AltNOW)							
Region of interest analysis							
SMA	L	62	-40	10	52	5.46	0.001
Angular gyrus	L	81	-44	-56	28	5.07	0.027
vlPFC	L	113	-50	26	-4	5.07	0.036
dlPFC	L	56	-8	50	42	4.95	0.033
MTG	L	34	-52	-20	-20	4.21	0.016
S (AltNOW > AltLATER)							
Region of interest analysis							
Thalamus	L	159	-12	-22	6	3.97	0.009
Caudate	L	28	-6	20	-6	4.30	0.012
Amygdala	L	40	-28	-6	-12	4.11	0.019
Amygdala	R	27	28	-2	16	3.89	0.041
Putamen	L	37	-32	8	-8	3.82	0.049
Striatum	L	10	-6	16	-6	3.36	0.035
S (DrugLATER > DrugNOW)							
Region of interest analysis							
dlPFC	L	21	-8	50	44	3.58	0.045
MTG	L	29	-50	-18	-20	3.78	0.001
S (DrugNOW > DrugLATER)							
Region of interest analysis						No differential effect	

L: left; R: right; voxels: number of voxels per cluster; x, y, z: MNI coordinates; MTG = middle temporal gyrus; SMA = supplementary motor area; dlPFC = dorsolateral prefrontal cortex; vlPFC = ventrolateral prefrontal cortex; ROI = region of interest; LATER > NOW means that both reward cue-types were collapsed

$p < 0.05$ fwe corrected: for ROI analyses a family-wise-error corrected threshold of $pfwe < 0.05$ with k > 10 voxels on a peak level was used

S2: Explorative correlation analysis

Table S2: Correlations between behavioral craving ratings and significant activated brain regions - in the ROI analyses – with sub-scales of the BIS-15 in TUD subjects. Beta weights were extracted using the toolbox marsbar and a sphere around the significant peak voxel of 5 mm. All correlations were corrected for multiple testing using the false-discovery-rate.

	BIS-NP	BIS-MI	BIS-AI
LATER > NOW			
dlPFC	-0.216	-0.068	-0.027
	(0.723)	(0.574)	(0.858)
vlPFC	**-0.284***	-0.102	-0.070
	(0.043)	(0.691)	(0.724)
MTG	-0.094	0.240	0.112
	(0.373)	(0.858)	(0.817)
SMA	-0.109	0.089	0.009
	(0.374)	(0.927)	(0.927)
Angualr gyurs	0.017	0.050	0.043
	(0.927)	(0.928)	(0.927)
NOW > LATER			
Caudate (L)	-0.033	-0.487	-0.218
	(0.972)	(0.059)	(0.724)
Thalamus (L)	-0.140	-0.083	-0.218
	(0.723)	(0.858)	(0.625)
Putamen (L)	-0.083	-0.067	-0.172
	(0.858)	(0.921)	(0.729)
NOW – LATER	-0.146	-0.298	-0.325
(craving rating)	(0.927)	(0.691)	(0.724)

Note: NP = non-planning; MI = motor inhibition; AI = attentional impulsivity. Missing values: 5
Missing values were treated with listwise deletion.

Original publication of Experiment IV

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ORIGINAL ARTICLE

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Aversive drug cues reduce cigarette craving and increase prefrontal cortex activation during processing of cigarette cues in quitting motivated smokers

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Abstract

Aversive drug cues can be used to support smoking cessation and create awareness of negative health consequences of smoking. Better understanding of the effects of aversive drug cues on craving and the processing of appetitive drug cues in abstinence motivated smokers is important to further improve their use in cessation therapy and smoking-related public health measures. In this study, 38 quitting motivated smokers underwent functional magnetic resonance imaging (fMRI) scanning while performing a novel extended cue-reactivity paradigm. Pictures of cigarettes served as appetitive drug cues, which were preceded by either aversive drug cues (e.g., smokers' leg) or other cues (neutral or alternative reward cues). Participants were instructed to rate their craving for cigarettes after presentation of drug cues. When aversive drug cues preceded the presentation of appetitive drug cues, behavioural craving was reduced and activations in prefrontal (dorsolateral prefrontal cortex) and paralimbic (dorsal anterior cingulate cortex [dACC] and anterior insulae) areas were enhanced. A positive association between behavioural craving reduction and neurofunctional activation changes was shown for the right dACC. Our results suggest that aversive drug cues have an impact on the processing of appetitive drug cues, both on a neurofunctional and a behavioural level. A proposed model states that aversive drug-related cues activate control-associated brain areas (e.g., dACC), leading to increased inhibitory control on reward-associated brain areas (e.g., putamen) and a reduction in subjective cravings.

KEYWORDS
control network, craving, cue reactivity

1 | INTRODUCTION

Smoking is a leading cause for cancer, respiratory and cardiovascular diseases and related to an estimated 12% of deaths in the adult population worldwide.[1,2] These approximately 4.8 million cases of premature death each year are preventable, highlighting the importance to identify new and enhance already existing prevention and treatment strategies. One promising approach to target smoking in

public health measures and cessation therapy is the use of aversive drug cues that show negative consequences of smoking in the form of an image or text (e.g., on cigarette packets).

Aversive drug cues have been proven to reduce craving in smokers,[3] to curtail the number of smoking initiators,[4] to augment quitting rates and to raise awareness for health issues related to tobacco consumption.[5] To investigate the neurofunctional mechanisms involved in these beneficial effects, previous functional magnetic resonance imaging (fMRI) studies[6–8] investigated aversive drug cue reactivity. They showed activation of a brain network including regions involved in executive control (e.g., dorsolateral prefrontal cortex [DLPFC]), motor planning regions (e.g., supplementary motor area), limbic regions involved in memory and affect (e.g., hippocampus and thalamus) and visual processing regions (e.g., cuneus and precuneus). These results are complemented by an investigation that found that prefrontal cortex (DLPFC) activation in smokers was associated with increased reward anticipation, poorer learning from errors and decreased attention control.[9] However, while the elucidation of aversive drug cue and punishment processing in smokers is still in its early stages, reactivity towards appetitive drug cues (cigarettes) has already been well investigated.[10,11]

Previous studies, examining cue reactivity in smokers, suggest that the mesolimbic brain reward system (e.g., midbrain, putamen, pallidum, nucleus accumbens [NAc] and ventral striatum) gradually becomes sensitized to drug-related stimuli and desensitized to nondrug-related alternative rewards.[12,13] Increased activation of these reward-associated areas has been directly linked to subjective craving and relapse risk.[14–16]

Neural correlates of resisting craving for tobacco have been linked to prefrontal cortex areas, associated with higher executive functioning and cognitive reward control.[17,18] Importantly, brain regions involved in executive and cognitive reward control processes (e.g., PFC, anterior cingulate cortex [ACC], and anterior insula) possess a rich set of connections to cortical and subcortical areas that are key to emotional and reward processing as well as to craving, and this connectivity is assumed to underlay craving regulation processes.[17,19–21] In line with results of aversive drug cue reactivity and the aforementioned processes, Do and Galván[22] showed negative functional connectivity patterns between prefrontal (DLPFC) and limbic (bilateral amygdala) brain regions in smokers while viewing graphic health warning labels, which was interpreted as improved regulatory control over emotionally responsive brain regions.

However, it is still unclear how aversive drug cues influence the subsequent processing of appetitive drug cues and the related craving. Based on the described prior findings on neurofunctional underpinnings of appetitive and aversive drug cue reactivity as well as craving and its control, an aversive cue model of tobacco use disorder (TUD) can be proposed: aversive drug-related cues change the processing of rewarding drug stimuli by (1) decreasing activation of reward areas (e.g., ventral striatum and putamen), (2) increasing activation of control and self-regulation areas (PFC, ACC and anterior insula) and (3) increasing the down-regulation of reward areas by control areas (Figure 1). In the current study, we aimed to test specific hypotheses derived from the aversive cue model of TUD presented in Figure 1, in quitting-motivated TUD subjects.

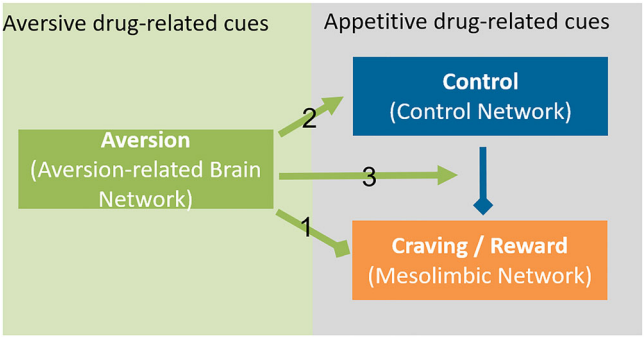

FIGURE 1 Aversive cue model of tobacco use disorder. The model highlights the effects of aversive drug-related cues on the processing of appetitive drug cues in tobacco use disorder subjects (pointed arrows indicate activation; blunt arrows indicate inhibition). It proposes three pathways through which aversive drug-related cues impact on appetitive drug cue reactivity: drug-related aversive cues (1) directly reduce craving and decrease reactivity of the mesolimbic reward network towards appetitive drug cues, (2) increase activation in control areas during the processing of appetitive drug cues and (3) increase down-regulation of reward areas by control areas

On the basis of the above framework, we hypothesized a reduction of cigarette-cue-induced craving in TUD subjects by prior presentation of aversive drug cues reflected in subjective craving ratings. On a neurofunctional level, we expected reduced activation of the mesolimbic brain circuit (e.g., ventral striatum and putamen) during the processing of appetitive drug cues after presentation of aversive drug cues. On the other hand, we hypothesized greater activations in craving-regulating control areas (e.g., PFC, ACC and anterior insula). Finally, examining functional connectivity patterns, using significantly activated brain regions identified in the group-level analysis as seed regions, we hypothesized negative functional connectivity between control and reward areas after presentation of aversive drug cues.

2 | MATERIALS AND METHODS

2.1 | Participants

The study was part of the German Collaborative Research Center (TRR 265: losing and regaining control over drug intake), a consortium comprising three German universities funded by the German research foundation (DFG).[23] Here, we present data from one of the main projects of the consortium from Berlin, focusing on understanding specific neural underpinnings underlying human TUD. Whereas previous analyses of the project focused on drug versus alternative reward cue reactivity, the present study investigated the impact of aversive drug stimuli on drug cue reactivity in TUD subjects. Thirty-nine TUD subjects (21 female) were included in the study. One participant had to be excluded due to technical issues with the

autoalignment process during fMRI acquisition, resulting in 38 analysed datasets. Participants were recruited in Berlin through (online and subway) advertising and flyers. Inclusion criteria were current DSM-5 diagnosis of TUD using a structured clinical interview for DSM-5[24] and an age range between 18 and 65 years. Exclusion criteria were comorbid DSM-5 mental disorders within the last 12 months, a lifetime history of any substance use disorder other than TUD, bipolar disorder or psychotic disorder according to DSM-5, current suicidal intent, concurrent psychopharmacological treatment, or psychotherapeutic/psychiatric treatment, a history of brain injury and pregnancy. Additionally, MRI-related exclusion criteria (e.g., ferromagnetic mental implants) were applied. Participants received financial compensation (50 euros) and a 6-week smoking cessation intervention, as all of them were motivated to quit. Additionally, half of the participants were randomized to a sport intervention, of which they were informed before the assessment. The study was approved by the local ethics committee, and all subjects gave written informed consent before participating in the study.

2.2 | Clinical assessments

The Fagerstroem Test for Nicotine Dependence (FTND; range 0–10)[25] was used to measure severity of nicotine dependence. To assess participants' global level of intelligence, subjects completed a 35-item multiple choice vocabulary test (MWT; range 0–37).[26] Furthermore, the Alcohol Use Disorder Identification Test (AUDID; range 0–40)[27] was applied to measure everyday alcohol intake and drinking behaviours. Additionally, participants answered five questions assessing their therapy expectancies, evaluating their motivation to take part in the programme and their assessment of its success (range 0–50), and formulated three individualized goal attainments, using the goal attainment scaling.[28]

2.3 | fMRI paradigm

A novel fMRI paradigm was established to compare self-reported craving ratings and brain responses to cigarette cues preceded by aversive drug cues with those preceded by other cues (neutral cues or alternative rewarding cues). TUD subjects were instructed to refrain from smoking and eating for 3 h prior to the session. Conventional photographs displaying smoking-related items were used as appetitive drug cues, pictures of attractive food were used as alternative reward cues, pictures showing long-term consequences of smoking (e.g., smokers' leg and lung cancer) were used as aversive drug cues and pictures displaying neutrally valenced items were presented during the neutral control condition. Before the assessment, 140 pictures of each category (appetitive drug cues, alternative reward and aversive drug cues) were rated, with the questions 'how strong is your desire to consume this now?' (appetitive drug cues and alternative reward) and 'how deterrent do you experience this picture?' (aversive drug cues), by each participant using an 8-point Likert scale. The 50% most rewarding/threatening stimuli were automatically selected for the experiment, so that each of the four categories was composed of 70 pictures. In this investigation, we are focusing on the appetitive drug-related and aversive drug-related condition only. Stimuli were presented in the scanner using back-projection. Four pictures of one category were presented per block. Each block lasted 16 s and ended with the presentation of a fixation cross (intertrial interval [ITI]), jittered around 2.5 s. In one run, two blocks of each of the four categories were presented. Within each run, the two blocks with appetitive drug-related cues were preceded once by the aversive drug-related condition and once by one of the other two conditions (either alternative reward or neutral condition). Subjects were instructed to attend to all stimuli and were once per run asked to rate their current desire to consume a cigarette after presentation of the appetitive drug condition and to rate their desire to consume the food after presentation of the alternative reward condition by pressing one of eight buttons covering an 8-point scale ranging from *not at all* to *very strongly*. At the end of each run, participants were additionally asked to rate how strongly they desire to smoke a cigarette, using the same rating scale. In total, the task consisted of nine runs, which altogether lasted maximal 38 min (for an example run, see Figure 2).

2.4 | Statistical analysis of behavioural data

Statistical analysis of behavioural data was performed using IBM SPSS statistics 27.0. To quantify the impact of aversive drug cues on subjective desire for cigarettes, we calculated the difference between craving ratings when appetitive drug cues were preceded by aversive drug cues in comparison with other categories (alternative rewards or neutral cues) using a paired-samples *t*-test. In the following, we will refer to this difference as craving reduction induced by aversive drug cues.

2.5 | fMRI data acquisition and analysis pathway

Scanning was carried out on a 3T MRI scanner (Siemens Magnetom Prisma) using a 64-channel head coil. Functional images were acquired using a Siemens simultaneous multislice T2*-weighted gradient-echoplanar imaging (EPI) sequence (TR = 869 ms, TE = 38 ms, 60 slices, slice thickness = 2.4 mm, voxel size 2.4 × 2.4 × 2.4 mm, no interslice gap, field of view [FoV] = 210 mm, matrix size 88 × 88, acquisition orientation T > C, interleaved slice order, acceleration factor slice = 6, flip angle = 58°, bandwidth = 1832 Hz/Px, prescan normalize, weak raw data filter, fat sat). Field map images were obtained using a Siemens dual gradient-echo sequence (TR = 698 ms, TE1 = 5.19 ms, TE2 = 7.65 ms, 64 slices, slice thickness = 2.4 mm, no slice gap, voxel size 2.4 × 2.4 × 2.4 mm, FoV = 210 mm, matrix size 88 × 88, acquisition orientation T > C, interleaved slice order, flip angle = 54°, bandwidth = 279 Hz/Px). High-resolution anatomical images were acquired using a T1-weighted MPRAGE sequence

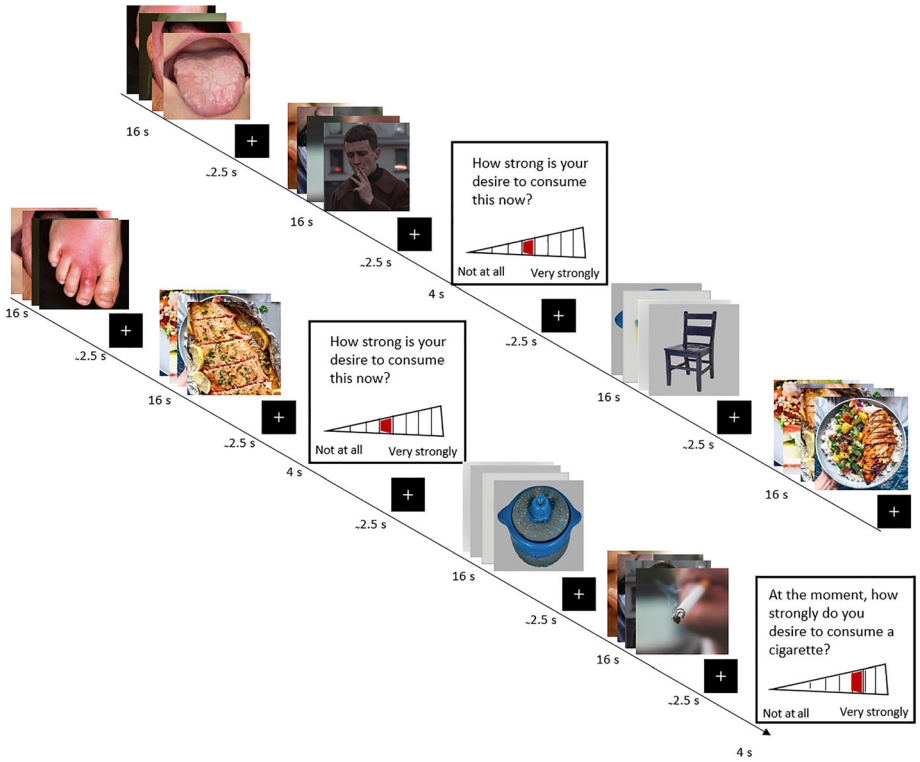

FIGURE 2 Illustration of an example run of the extended cue-reactivity task. Each condition was presented twice per run (aversive drug related, appetitive drug related, neutral and alternative reward). The appetitive drug-related category was preceded once per run by the aversive drug-related condition (see above) and once by either the neutral or alternative reward condition. Once per run, participants were asked how strong they desire to consume this now (referring to the appetitive drug-related condition). At the end of each run, they were asked how strongly they desire to consume a cigarette now. The task consisted of nine runs with a maximum duration of 38 min

(TR = 2000 ms, TE = 2.01 ms, TI = 880 ms, FoV = 256 mm, 208 sagittal slices, voxel size 1 × 1 × 1 mm, flip angle = 8°, GRAPPA factor 2 [PE], 24 ref. lines, prescan normalize, 23.1% slice oversampling, bandwidth = 240 Hz/Px). To minimize movement artefacts, participants' heads were positioned on a pillow and fixated using foam pads surrounding the head. Image preprocessing was performed using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12), implemented in MATLAB R2020a (MathWorks, Sherborn, Massachusetts) and comprised slice timing with reference to the middle slice, SPM12 standard realignment and unwarping including correction for field deformations based on a previously acquired field map, coregistration, normalization to MNI stereotactic space using unified segmentation based on the SPM tissue probability map for six tissue classes, and spatial smoothing with 8-mm full-width at half-maximum isotropic Gaussian kernel (similar to previous studies in our field[29,30]). Following preprocessing, all nine runs were visually inspected, for each subject separately, for a visual quality control.

On the subject level, brain activation differences related to presentation of the different stimuli were analysed using the general linear model (GLM) in SPM12. The blood oxygen level-dependent response was modelled by a canonical haemodynamic response function (HRF) for each of seven conditions: neutral cues, alternative reward cues, aversive drug related cues, nicotine cues preceded by aversive drug-related cues ($Nico^+$), nicotine cues preceded by other cues ($Nico^-$), button presses and ratings, resulting in three regressors of interest ($Nico^+$, $Nico^-$ and neutral) for the current analysis. Model parameter estimates and the resulting *t*-statistic images (condition against baseline) were submitted to the group-level analysis.

Within-group differences were assessed using paired-samples *t*-tests on the second level. To test the effects of presentation of aversive drug-related cues on appetitive cue reactivity, we analysed the contrast $Nico^+ > Nico^-$ and vice versa. Whole-brain analyses as well as an anatomical region of interest (ROI) analysis of a priori defined brain areas were conducted. To investigate the mesolimbic brain reward system, same ROIs as described in previous investigations were included (e.g., Lin et al.[11]): the ventral striatum (NAc), thalamus, pallidum, caudate and midbrain (including ventral tegmental area [VTA]). Furthermore, brain areas responsible for executive and cognitive reward control processes were selected for a second ROI of control areas, including the middle frontal gyrus (DLPFC), orbitofrontal gyrus (ventromedial prefrontal cortex [VMPFC]), superior medial frontal gyrus (dorsomedial prefrontal cortex [DMPFC]), ACC and insula (e.g., Brandl et al.[17] and Morawetz et al.[31]). Combining the definitions from the Automated Anatomical Labeling Atlas,[32] which is implemented in the toolbox 'Wake Forest University PickAtlas'[33] in SPM12, the bilateral ROIs were investigated using one mask. To further specify the regions, we report the corresponding Brodmann areas. Small volume correction was applied using this ROI mask and a family-wise error (FWE) corrected threshold of $p_{fwe} < 0.05$ with a minimum cluster size of $k = 10$ continuous voxels. ROI analyses were followed by whole-brain analyses, thresholded at $p < 0.001$ uncorrected. Furthermore, as sensitivity analysis and to ensure that the experimental manipulation worked, we investigated an activation of the selected ROIs of the reward system in response to appetitive drug cues not preceded by aversive drug cues in comparison with the neutral control condition ($Nico^-$ > neutral).

2.6 | Correlation analysis

To test associations between behavioural and neurofunctional effects of aversive drug cues, we computed the Pearson correlations between beta values at significant peaks of the ROIs activated in the group-level analysis and craving reduction induced by aversive drug cues (craving rating after $Nico^-$ minus craving rating after $Nico^+$), implicating that higher values reflect an increased influence of aversive drug cues on subjective cravings. Beta values of the significant ROIs were extracted using the toolbox marsbar[34] with a 5-mm sphere around the peak voxel.

2.7 | Generalized psychophysiological interaction analysis (gPPI analysis)

The Functional Connectivity Toolbox (CONN toolbox v18.4)[35] for Matlab and SPM12 was used to perform functional connectivity analyses using the implemented gPPI procedure. This analysis was conducted post hoc to explore the connectivity profile of seed regions identified in the former group-level analysis (bilateral anterior insulae and bilateral dorsal anterior cingulate cortex [dACC] and left DLPFC). A gPPI analysis allows the description of connectivity alterations between brain regions due to an experimental context. The selected seed regions were created as 5-mm spheres around the peak voxel identified in our group-level analysis in the contrast $Nico^+ > Nico^-$ (for MNI coordinates, see Table 2). We used a seed-to-voxel approach to conduct gPPI analyses on the $Nico^+ > Nico^-$ condition. In the first-level analysis, the BOLD time course of all seeds was extracted from each participant and condition, and then, a seed-to-voxel beta map was calculated including the interaction between the seed regions BOLD time series and the $Nico^+ > Nico^-$ contrast condition. Afterwards, the seed regions and beta images were entered into a regression model at the second level. Our goal was to investigate possible stronger negative relationships between control and reward areas. Therefore, we used a one-sided FWE corrected $p < 0.05$ at the cluster level and an uncorrected $p < 0.001$ at the voxel level (as implemented in the CONN toolbox software) to guard against false-positive findings. Cerebrospinal fluid, white matter and six rigid-body parameters were regressed out of the whole-brain grey matter activity.

TABLE 1 Sociodemographic and psychometric characteristics of the TUD sample

Sample characteristic	TUD subjects $N = 38$
Age (M [SD])	35.18 (10.57)
Female gender (*n* [%])	21 (55.26)
Right-handedness (*n* [%])	38 (100)
Level of education	
A level[a] (*n* [%])	30 (78.95)
Monthly income in € (*n* [%])	
<1000	7 (18.42)
1000–2000	12 (31.58)
2000–3500	16 (42.11)
3500–4500	2 (5.26)
>4500	1 (2.63)
MWT (M [SD])	28.32 (4.67)
TE (M [SD])	40.92 (6.91)
FTND (M [SD])	4.03 (2.27)
Pack years (M [SD])	10.75 (9.55)
Cigarettes/day (M [SD])	14.40 (6.05)
AUDID	5.92 (4.69)

Note: Missing values = monthly income: 1.
Abbreviations: AUDID, Alcohol Use Disorder Identification Test; FTND, Fagerstroem Test of Nicotine Dependence; MWT, Mehrfachwahl-Wortschatz Test; TE, therapy expectancies; TUD, tobacco use disorder.
[a]Abitur.

3 | RESULTS

3.1 | Sample characteristics and subjective craving ratings

Demographic and smoking characteristics of the TUD sample are shown in Table 1. Craving ratings within the task were significantly lower in the $Nico^+$ condition compared with the $Nico^-$ condition, $t(37) = -4.03$, $p < 0.001$, $d = 0.922$ (see also Table 1 and Figure 3). Participants showed a high motivation to quit smoking and expected the therapy to be helpful to reach this goal (see also Table 1 and Text S1).

3.2 | fMRI results

Contrasting the $Nico^+ > Nico^-$ condition, we found greater activations in the bilateral anterior insulae and dACC and in the left DLPFC in the ROI analysis. No significant activations in the VMPFC and DMPFC were observed. On the whole-brain level, significant activations were found in the left middle frontal gyrus (DLPFC), insula, superior frontal gyrus (pre-supplementary motor area, SMA), precentral gyrus, SMA, angular gyrus and caudate as well as in the right calcarine sulcus, cerebellum, SMA, angular gyrus, middle occipital gyrus, insula, middle fontal gyrus (DLPFC), ACC and thalamus (Table 2 and Figure 3). The contrast $Nico^- > Nico^+$ revealed no significant results, neither in the ROI analysis nor in the whole-brain approach.

3.3 | Correlation results

We found a positive correlation between craving reduction induced by aversive drug cues (Figure 3B) and right dACC activation

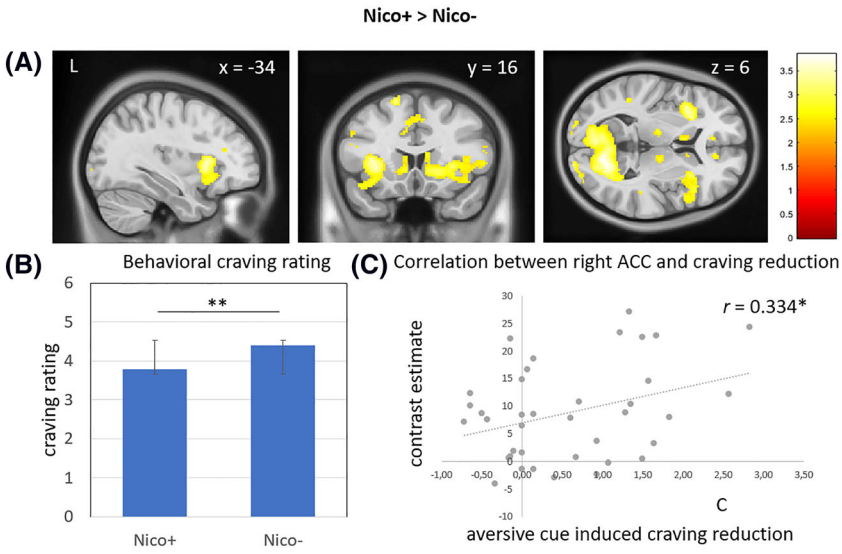

FIGURE 3 (A) Significantly activated brain regions during the processing of appetitive drug cues, preceded by aversive drug cues in the whole-brain analysis ($p < 0.001$ unc.). (B) Behavioural craving ratings after Nico$^+$ and Nico$^-$. (C) Positive correlation between significantly activated right anterior cingulate cortex (ACC) in the contrast Nico$^+$ > Nico$^-$ and behavioural craving reduction induced by aversive cues. *$p < 0.05$, **$p < 0.001$

($r = 0.386$, $p = 0.040$) and a trend for the left DLPFC ($r = 0.334$, $p = 0.056$) for the contrast Nico$^+$ > Nico$^-$ (Figure 3C). No other correlations reached significance (left dACC and bilateral insulae).

3.4 | Sensitivity results

To ensure that the experimental manipulation worked, we investigated the contrast Nico$^-$ > neutral as sensitivity analysis. We could show an activation of the brain reward system to appetitive drug cues not preceded by aversive drug cues in the ROI analysis (left ventral striatum [NAc] and caudate as well as bilateral pallidum, midbrain and thalamus) and that these activations could be positively associated with behavioural craving ratings (see Tables S1 and S2).

3.5 | gPPI results

To exploratively examine functional connectivity patterns of significantly activated brain regions identified in the group-level analysis (left DLPFC as well as bilateral dACC and bilateral anterior insulae), a gPPI analysis was conducted for the contrast Nico$^+$ > Nico$^-$. We found a stronger negative functional connectivity between the left DLPFC and the right supramarginal gyrus, fusiform gyrus, superior occipital gyrus and the left cerebellum. The right anterior insula showed a significant negative functional connectivity to the right nucleus caudatus and the left anterior insula to the right superior occipital gyrus. The right dACC showed a significant negative functional connectivity to the left putamen and the left dACC to the right brain stem (see Table 3). These stronger inverse couplings point towards an aversive cue-induced down-regulation process on mesolimbic brain reward areas (putamen and caudate) by prefrontal and paralimbic control areas (dACC and anterior insula) in TUD subjects.

4 | DISCUSSION

The present study proposed an aversive cue model of TUD (Figure 1), describing different pathways through which drug-related aversive cues impact on the processing of appetitive drug cues, based on the literature. According to this aversive cue model of TUD, aversive drug-related cues modulate subsequent drug cue reactivity by (1) reducing subjective craving and neural responsivity in mesolimbic reward areas, (2) enhancing activation in prefrontal control areas and (3) increasing prefrontal top-down-regulation of mesolimbic reward areas. To test our hypotheses, derived from this model in quitting motivated TUD subjects, we employed a novel extended cue-reactivity paradigm, where aversive drug cues (displaying negative consequences of tobacco consumption) preceded the presentation of appetitive drug cues. When appetitive drug cues were preceded by aversive drug cues, we found (1) reduced cigarette craving, but not reduced reactivity in mesolimbic reward areas towards appetitive drug cues; (2) enhanced activation of prefrontal and paralimbic control areas (DLPFC, dACC and anterior insulae), with a positive association between aversion-related reduction of craving and prefrontal activation; and (3) down-regulation of mesolimbic reward areas (putamen and caudate) by prefrontal and paralimbic control areas (dACC and anterior insula). Overall, these findings support our hypotheses referring to all three pathways proposed by the model.

4.1 | Impact on craving and mesolimbic reward areas

The craving reduction induced by aversive drug cues is in accordance with our hypothesis (Pathway 1 in Figure 1), consistent with findings from a previous investigation that used graphic health warning labels with different emotional contents[5] and was expected a priori. However, contrary to our hypothesis, we found no corresponding activation reduction of reward-associated brain areas (e.g., NAc and

TABLE 2 Significant activated brain regions during the processing of appetitive drug cues preceded by aversive drug cues ($Nico^+$) or other cues ($Nico^-$)

Contrast/region	Side	Voxels	*x*	*y*	*z*	*t*	BA	*p* < 0.001
$Nico^+$ > $Nico^-$								
Region of interest analysis								
Insula	L	460	−34	16	6	4.92	13	0.013*
Insula	R	241	32	16	4	4.02	13	0.049*
ACC	L	59	−10	42	10	4.14	32	0.026*
ACC	R	92	8	34	22	4.32	32	0.017*
DLPFC	L	59	−40	36	18	4.77	46	0.030*
Whole-brain analysis								
Calcarine sulcus	R	3503	14	−70	8	5.44	17	<0.001
Insula	R	938	31	18	4	4.65	13	<0.001
Cerebellum	R	678	6	−44	−16	4.80	-	<0.001
Insula	L	650	−34	16	6	4.92	13	<0.001
Cerebellum	R	311	2	−46	−16	4.59	-	<0.001
Supplementary motor area	R	286	16	4	62	4.56	6	<0.001
Angular gyrus	L	230	−58	−42	26	4.30	39	<0.001
Angular gyrus	R	211	54	−44	22	4.40	39	<0.001
Supplementary motor area	L	191	−18	10	66	4.83	6	<0.001
ACC	R	192	10	34	20	4.32	32	<0.001
Middle occipital gyrus	R	177	20	−100	0	4.68	18	<0.001
Caudate	L	174	−10	16	0	4.03	-	<0.001
Precentral gyrus	L	143	−16	−32	66	4.70	4	<0.001
Middle frontal gyrus (DLPFC)	L	134	−40	36	18	5.36	46	<0.001
Thalamus	R	129	12	−20	14	4.07	-	<0.001
Superior frontal gyrus (pre-SMA)	L	127	−14	14	70	4.74	6	<0.001
Middle frontal gyrus (DLPFC)	R	105	30	42	38	4.45	9	<0.001
$Nico^-$ > $Nico^+$								
Region of interest analysis					No differential activation			
Whole-brain analysis					No differential activation			

Abbreviations: ACC, anterior cingulate cortex; BA, Brodmann area; DLPFC, dorsolateral prefrontal cortex; L, left; $Nico^+$, appetitive drug-related cues preceded by aversive drug-related cues; $Nico^-$, appetitive drug-related cues preceded by other cues; R, right; SMA, supplementary motor area; voxels, number of voxels per cluster; *x*, *y*, *z*, MNI coordinates.
*$p < 0.05$ family-wise error (FWE) corrected: For ROI analyses, an FWE corrected threshold of $p_{fwe} < 0.05$ with $k > 10$ voxels on the peak level was applied. For whole-brain analyses, an uncorrected threshold of $p < 0.001$ was applied.

TABLE 3 Results of the seed-based generalized psychophysiological interaction analysis for the contrast $Nico^+$ > $Nico^-$

Seed	Region	Side	Voxels	*x*	*y*	*z*	*t*	*p* < 0.05 FWE*
DLPFC (L)	Supramarginal gyrus	R	227	56	−30	56	−4.52	0.023
	Cerebellum	L	137	−26	−82	−22	−4.58	0.031
	Fusiform gyrus	R	87	22	−82	−12	−4.16	0.049
	Superior occipital gyrus	R	82	16	−66	64	−4.10	0.010
Insula (R)	Caudate	R	76	10	6	16	−5.17	<0.001
Insula (L)	Superior occipital gyrus	R	57	16	−66	64	−4.11	0.045
ACC (R)	Putamen	L	66	−26	0	−8	−4.99	0.027
ACC (L)	Brainstem	R	30	22	−28	−32	−4.80	0.041

Abbreviations: ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; FWE, family-wise error; L, left; R, right.
*One-sided, multiple testing correction of $p_{fwe\ (cluster\ level)} < 0.05$.

caudate). This result suggests that activation of the brain reward system through appetitive drug cues is not directly weakened by previous presentation of the negative consequences of smoking, at least not in quitting motivated TUD subjects. Former investigations[36,37] found that a quit interest of smokers modulates smoking cue-reactivity responses in brain reward areas. Thus, it can be assumed that an effect in the mesolimbic reward circuit is more difficult to detect in quitting motivated smokers and may require a lager sample of TUD subjects.

4.2 | Impact on prefrontal control areas

Our finding of increased activation of prefrontal control areas related to aversive drug cues complements previous studies on drug cue reactivity, which found appetitive drug cues to activate prefrontal control areas in addition to mesolimbic reward areas.[18] The present findings also add to studies that have linked cognitive control of craving for hedonic stimuli with activations in lateral fronto-parietal cortices (DLPFC and anterior insulae).[17] In accordance with Pathway 2 of the aversive cue model of TUD (Figure 1), we here demonstrate that aversive drug cues enhance the activity of prefrontal control areas during subsequent processing of appetitive drug cues in quitting motivated TUD subjects. The areas identified here (DLPFC, dACC and anterior insulae) are known to play a role in executive functions and different strategies and goals of emotion regulation and cognitive reward control.[17,31,38–40]

Previous studies found the dACC to be involved in cognitive reappraisal and cognitive modulation of emotion as well as in quitting motivated smokers when instructed to actively suppress their urge for cigarettes.[41,42] These results suggest that dACC activation represents an important substrate of inhibition of cue-induced craving in smokers. On the other hand, the DLPFC was found to be involved in different aspects of (cognitive) emotion regulation[31] such as the down-regulation of different kinds of appetitive desires.[43,44] Furthermore, Kober et al.[21] showed DLPFC activation during cognitive down-regulation of craving for cigarettes in smokers when explicitly applying cognitive strategies to regulate craving. These findings suggest that the DLPFC is involved in deliberate regulation of automatic responses to various kinds of affective cues, including drug cues. In terms of emotion regulation, the anterior part of the insula has been suggested to control activity in other brain regions, to initiate and adjust cognitive control mechanisms.[31,45]

Summarizing the above and integrating our own results, two ways of activating the control network can be distinguished. First, previous studies found that the usage of explicit instructions to exert deliberate control over different kinds of stimuli (positive, negative emotions or drug cues) activates the prefrontal control network. Second, we could demonstrate an indirect, implicit activation of prefrontal control areas through aversive drug-related cues, without the instruction to actively apply any strategies, suggesting a rather subsidiary increase of the control network. This second way, which is consistent with our aversive cue model of TUD, may be relevant for smoking prevention programmes and cessation therapy. Based on the knowledge that explicitly targeting the control system in smokers through cognitive interventions (e.g., cognitive behavioural therapy) is limited in its success,[40] alternative strategies are clearly needed. Our results suggest an indirect and automatic activation of control processes through the presentation of (unknown) aversive drug cues, preceding appetitive drug cues. Such strategies could complement explicit cognitive approaches through different forms of application. As a novel part of smoking cessation therapy, (unknown) aversive drug cues could be paired with (individualized) appetitive drug cues of quitting motivated smokers in a conditioning paradigm, maybe inducing decreased craving for these favourite drug cues through enhanced cognitive control. Furthermore, it could be beneficial to make aversive drug cues more visible in different places where smokers are used to consume cigarettes (e.g., smoking areas in public places). By applying such strategies, prevention efforts and cessation success may be enhanced, at least in quitting motivated TUD subjects.

4.3 | Impact on top-down control processes

Confirming our hypothesis, we found stronger negative functional connectivity of activated prefrontal control areas to parts of the mesolimbic reward system. Together with the observation of a positive association between right dACC activation and craving reduction induced by aversive drug cues, these findings suggest that aversive drug cues may induce down-regulation processes,[21,22] as proposed by our model (Pathway 3 in Figure 1). A reduction of the overall motivational appeal of smoking may be achieved through balancing the value of cigarettes with the value of the anticipated reward. The value of anticipated reward in mesolimbic brain regions may be down-regulated by prefrontal/paralimbic control areas when aversive drug cues were presented before. However, we only found increased down-regulation of reward areas to appetitive drug cues immediately preceded by aversive drug cues, which might suggest a short-lasting effect of aversive drug cues. This underlines that prevention strategies or cessation interventions, which use aversive drug cues, may benefit from the immediate and contingent presence of aversive drug cues during drug consumption (e.g., on cigarette packets or in novel conditioning paradigms).

4.4 | Impact on extended visual system and (pre-) SMA

In addition to prefrontal control areas, we found activation of the extended visual system (e.g., calcarine sulcus and occipital gyrus) as well as in the SMA and pre-SMA in our whole-brain analysis. While the SMA and pre-SMA have been associated with cognitive reward control across a wide range of rewarding stimuli,[17] the extended visual system has consistently been more responsive to smoking cues than neutral cues in previous investigations (e.g., Engelmann et al.[10]). Former fMRI studies, comparing emotionally arousing stimuli with

neutral stimuli, have found that emotionally arousing stimuli consistently evoke larger responses than neutral stimuli in these brain regions, a finding that has been interpreted as increased allocation of attentional resources to the processing of the arousing stimuli.[46,47] In our study, stronger activation of the (extended) visual system during appetitive cue reactivity, when aversive drug cues preceded the presentation, may suggest that those cues are processed as emotionally arousing, particularly in quitting motivated TUD subjects.

5 | LIMITATIONS AND CONCLUSION

Our findings should be interpreted within the limitations of this study. Our sample consists of quitting motivated TUD subjects who are medium nicotine dependent according to the FTND scores. Including strong, nonquitting motivated smokers may have changed the results and led to other implications. To specify and extend the effects of this investigation, it would be desirable to study a sample of strong smokers who are not intended to quit smoking. Furthermore, we recruited participants through online or subway advertising, which could possibly have led to a selection bias (e.g., recruiting those who are actually working) and therefore probably limit the external validity of the study.

In conclusion, we assume that cues displaying the negative consequences of smoking have an impact on cigarette cue reactivity and craving in TUD subjects who are motivated to quit. On the basis of previous studies, we proposed an aversive cue model of TUD including three different pathways of the impact of aversive drug-related cues on the processing of appetitive drug-related cues through a reduction of subjective craving and neural responsivity in mesolimbic reward areas, enhanced activation in prefrontal control areas and increased prefrontal top-down-regulation of mesolimbic reward areas. Derived from this model, specific hypotheses were tested. We found a reduction of craving for cigarettes in TUD subjects on a behavioural level. The pattern of brain areas activated when aversive drug cues preceded the presentation of appetite drug cues suggests increased cognitive control (of reward), as well as down-regulation of brain reward areas. Thus, from a neurofunctional perspective, TUD subjects automatically and implicitly applied self-regulation and control strategies. Implications for prevention programmes and smoking cessation interventions include the application of aversive drug cues in different ways (e.g., as conditioning paradigm in cessation interventions). Further research is clearly needed to specify the effect and to investigate the applicability of negative drug-associated stimuli in cessation therapy.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

The following authors have approved the final article and have participated in the research. Stefanie L. Kunas, Heiner Stuke, Andreas Ströhle and Felix Bermpohl designed the study. Stefanie L. Kunas collected the data. Stefanie L. Kunas and Heiner Stuke analysed the data. Stefanie L. Kunas, Heiner Stuke, Irene S. Plank, Andreas Ströhle and Felix Bermpohl interpreted the results. Stefanie L. Kunas wrote the first draft. All authors revised the article critically.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on reasonable request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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Supplementary Materials

Aversive drug cues reduce cigarette craving and increase prefrontal cortex activation during processing of cigarette cues in quitting motivated smokers

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Supplementary Text 1: Therapy expectancies (TE)

Questions which were asked regarding therapy expectancies and motivation can be translated as following:

1. How logical does the therapy appears to you, as it was described to you?
2. How successful does the therapy appears to you?
3. Would you recommend the therapy to a friend of you?
4. How strong is your motivation to take part in the program?
5. How successful do you think will the therapy be in your case?

The questions were answered using a 10-point Likert-scale ranging from 1 (not at all) to 10 (very much). The question regarding therapy motivation (4) reached a mean of 9.61 with a standard deviation of 0.83 implicating a very high therapy motivation of the participating TUD subjects.

Table S1. Significantly activated brain regions during the processing of appetitive drug-related cues preceded by other cues ($Nico^-$) compared to neutral control cues ($Nico^-$ > neutral)

Contrast/ Region	Side	Voxels	x	y	z	*t*	$p_{fwe} < 0.05$
$Nico^-$ > Neutral							
Region of interest analysis							
Pallidum	R	22	16	2	2	3,87	0.006
Pallidum	L	12	-12	-2	0	3.66	0.011
Ventral Striatum (NAc)	L	18	-10	12	-2	3.51	0.020
Caudate	L	15	-4	16	4	3.40	0.045
Midbrain (incl. VTA)	L	15	-18	-16	-12	3.86	0.012
Midbrain	R	10	4	-34	-24	3.40	0.043
Thalamus	R	12	20	-32	1	3.72	0.016
Thalamus	L	11	-6	-12	18	3.57	0.030

L: left; R: right; voxels: number of voxels per cluster; x, y, z: MNI coordinates; $Nico^-$ = appetitive drug-related cues preceded by other cues;
$p_{fwe} < 0.05$: for ROI analyses a family wise error corrected threshold of $p_{fwe} < 0.05$ with k > 10 voxels on the peak level was applied.

Table S2. Correlations between significantly activated brain regions and subjective craving ratings within the task after the Nico$^-$ condition. Brain areas were activated in the ROI analysis during the processing of appetitive drug-related cues compared to neutral cues in TUD subjects (see Table S1). Craving ratings refer to ratings after appetitive drug-related cues not preceded by aversive drug-related cues. Beta weights were extracted using the toolbox marsbar and a sphere around the significant peak voxel of 5 mm.

	Pallidum L	Pallidum R	NAc L	Caudate L	Midbrain L	Midbrain R	Thalamus R	Thalamus L
Craving Rating Nico$^-$	0.382 * (0.035)	0.074 (0.331)	0.038 (0.411)	0.135 (0.213)	0.065 (0.351)	0.243* (0.042)	0.300* (0.040)	0.106 (0.265)

Note: R = right, L = left; Missing values for carving rating = 1
*$p < 0.05$

B Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt,

- dass ich die vorliegende Arbeit selbstständig und ohne unerlaubte Hilfe verfasst habe,

- dass ich mich nicht bereits anderwärts um einen Doktorgrad beworben habe und keinen Doktorgrad in dem Promotionsfach Psychologie besitze, und

- dass ich die zugrunde liegende Promotionsordnung vom 08.08.2016 kenne.

Berlin, den 14.10.2021

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Stefanie L. Kunas

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CURRICULUM VITAE

For reasons of data protection, the Curriculum Vitae is not included in the public version of this thesis.