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Effects of Oxytocin and Cannabidiol on Alcohol Craving, Neuronal Cue-Reactivity and Negative Affect in Alcohol Use Disorder

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PREFACE

The here presented work is a cumulative dissertation based on the following two peer-reviewed empirical studies:

Publication 1:

Zimmermann, S., Teetzmann, A., Baeßler, J. et al. Acute cannabidiol administration reduces alcohol craving and cue-induced nucleus accumbens activation in individuals with alcohol use disorder: the double-blind randomized controlled ICONIC trial (2024). *Molecular Psychiatry*, 1-8, https://doi.org/10.1038/s41380-024-02869-y.

Publication 2:

Vetter, S., Schnabel, S., Reichl, M. et al. Intranasal oxytocin blunts amygdala response to negative affective stimuli in males and females with alcohol use disorder: a randomized controlled cross-over trial (2025). *Psychopharmacology*, 1-13, https://doi.org/10.1007/s00213-025-06779-x

The text of the manuscripts, tables and figures are identical to the original publications and are presented in section 2.1 (Empirical Study 1) and section 2.2. (Empirical Study 2). Permission for reproduction as part of the cumulative dissertation was obtained from Springer Nature and is marked accordingly under each table and figure. The personal contribution to each task of both publications is listed below.

Tasks	**Publication 1**	**Publication 2**
Conceptualization (%)	90 %	100 %
Literature Review (%)	100 %	100 %
Ethics Application (%)	80 %	100 %
Animal Experimentation Application (%)	-	-
Data Collection (%)	75 %	80 %
Data Analysis (%)	85 %	100 %
Interpretation of Results (%)	100 %	100 %
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TABLE OF CONTENTS

Page

LIST OF FIGURES 1

LIST OF TABLES 3

ABBREVIATIONS 5

1 INTRODUCTION 7

1.1 Alcohol Use Disorder 7

1.1.1 Craving, Relapse and Cue-Reactivity 8

1.1.2 Negative Affect, Craving and Relapse 13

1.1.3 Treatment of Alcohol Use Disorder 14

1.2 Cannabidiol 17

1.2.1 Preclinical Evidence on Cannabidiol 19

1.2.2 Clinical Studies on Cannabidiol 20

1.3 Oxytocin 24

1.3.1 Preclinical Evidence on Oxytocin 27

1.3.2 Clinical Studies on Oxytocin 29

1.4 Research Questions 35

2 EMPIRICAL STUDIES 36

2.1 Empirical Study 1: Acute cannabidiol administration reduces alcohol craving and cue-induced nucleus accumbens activation in individuals with alcohol use disorder: the double-blind randomized controlled ICONIC trial 36

2.1.1 Abstract 36

2.1.2 Introduction 36

2.1.3 Methods 38

2.1.4 Results 43

2.1.5 Discussion 53

2.1.6 Conclusion 56

2.1.7 Supplements 57

2.2 Empirical Study 2: Intranasal oxytocin blunts amygdala response to negative affective stimuli in males and females with alcohol use disorder: a randomized controlled cross-over trial 69

2.2.1 Abstract 69

2.2.2 Introduction 70

2.2.3 Methods 72

2.2.4 Results 80

2.2.5 Discussion 88

2.2.6 Conclusion 90

2.2.7 Supplements 91

3 DISCUSSION 93

3.1 Cannabidiol 93

3.2 Oxytocin 95

3.3 Limitations 101

3.4 Implications 104

3.5 Recommendations for Future Work 107

3.6 Conclusion 109

4 SUMMARY 111

5 ZUSAMMENFASSUNG 113

6 REFERENCES 115

7 CURRICULUM VITAE 131

8 PUBLICATION LIST 132

9 ACKNOWLEDGEMENTS 134

LIST OF FIGURES

Figure 2.1. Schematic overview of the trial design 41

Figure 2.2. Depiction of the activation clusters in the left and right nucleus accumbens and the significant difference in mean alcohol cue-induced brain activation 47

Figure 2.3. Depiction of the mean cue-induced activation in the left and right NAc during presentation of alcohol stimuli and neutral stimuli 48

Figure 2.4. Depiction of the significant effects of cannabidiol on alcohol craving before and after the combined experimental stress- and alcohol cue exposure and on cue-induced alcohol craving during the fMRI cue-reactivity task.....50

Figure 2.5. Depiction of the significant negative correlation between CBD plasma levels and alcohol craving after the combined stress- and alcohol cue-exposure experiment, alcohol cue-induced brain activation in the nucleus accumbens and alcohol craving during the alcohol cue-reactivity task.....52

Figure 2.6. CONSORT-Flowchart.....74

Figure 2.7. Depiction of the study design and procedures.....76

Figure 2.8. Depiction of the activation cluster in the right Amygdala and the difference in mean brain activation between OXY and PLC administration 85

Figure 2.9. Depiction of the significant positive correlation between activation in the right amygdala and alcohol craving after treatment with oxytocin or placebo.....87

List of Supplementary Figures

Supplementary Figure S 2.1. Depiction of the left and right nucleus accumbens region of interest mask 66

Supplementary Figure S 2.2. CONSORT study flow chart. 67

Supplementary Figure S 2.3. Depiction of brain areas that show higher brain activation in response to the presentation of alcohol cues during the alcohol cue-reactivity task 68

Supplementary Figure S 2.4. Depiction of the positive correlation between activation in the right amygdala and alcohol craving in female compared to male individuals ... 92

LIST OF TABLES

Table 2.1. Baseline data on demographic characteristics, alcohol use and severity measures.....44

Table 2.2. Demographic data and substance use patterns.....81

Table 2.3. Depiction of the baseline characteristics, alcohol craving and performance data 82

Table 2.4. Brain regions that show significant condition-dependent activation 83

List of Supplementary Tables

Supplementary Table S 2.1. Baseline data on demographic characteristics, alcohol use and severity measures (N=25).....62

Supplementary Table S 2.2. List of brain areas that show a significant higher activation during the presentation of the alcohol cues compared to the presentation of neutral stimuli during the alcohol cue-reactivity functional magnetic resonance imaging paradigm 64

Supplementary Table S 2.3. Sensitivity analyses.....65

Supplementary Table S 2.4. Demographic data and substance use patterns of all randomized individuals (N = 23).....91

ABBREVIATIONS

5-HT_{1A}	G-Protein Coupled Serotonin 1A Receptor
ADS	Alcohol Dependence Scale
AUD	Alcohol Use Disorder
AUDIT	Alcohol Use Disorders Identification Test
AUQ	Alcohol Urge Questionnaire
BCa	Bias Corrected and Accelerated
BDI-II	Beck Depression Inventory II
BOLD	Blood Oxygenated Level Dependent
BrAC	Breath Alcohol Concentration
CB1	Cannabinoid 1
CB2	Cannabinoid 2
CBD	Cannabidiol
CI	Confidence Interval
CRF	Corticotropin-Releasing Factor
DSM-5	Diagnostic and Statistical Manual of Mental Disorders 5th Version
EPI	Echo-Planar Images
fMRI	Functional Magnetic Resonance Imaging
FTND	Fagerström Test for Nicotine Dependence
FWE	Family-Wise Error Rate Correction
GABA	Gamma-Aminobutyric Acid
GHB	Gamma-Hydroxybutyrate
GLM	General Linear Model
GPR55	G-Protein Coupled Receptor 55
HPA axis	Hypothalamic-Pituitary-Adrenal Axis
LOD	Limit of Detection
LOQ	Limit of Quantification
NAc	Nucleus Accumbens
OCDS-G	Obsessive Compulsive Drinking Scale
OPRM1	Opioid Receptor μ 1
OXTR	Oxytocin Receptor
OXY	Oxytocin

PANAS	Positive And Negative Affect Schedule
PASA	Primary Appraisal Secondary Appraisal
PLC	Placebo
PSS	Perceived Stress Scale
PVN	Paraventricular Nucleus
RCT	Randomized Placebo-Controlled Trial
RM-ANOVAS	Repeated Measures Analysis of Variance Models
ROI	Regions of Interest
SON	Supraoptic Nucleus
SPM	Statistical Parametric Mapping Software
SPSS	IBM Statistical Package for The Social Sciences
STAI	State-Trait-Anxiety Inventory
SUD	Substance Use Disorder
SVC	Small Volume Corrected
THC	Delta-9-Tetra-Hydrocannabinol
TRPV1	Transient Receptor Potential Cation Channel Subfamily V Member 1
TRPV2	Transient Receptor Potential Vanilloid Type 2 Channels
TSST	Trier Social Stress Test
VAS	Visual Analog Scale
VTA	Ventral Tegmental Area

1 INTRODUCTION

1.1 Alcohol Use Disorder

Frequent alcohol use is one of the main risk factors for poor health outcomes, contributing to overall burden of disease, mortality and social harm (World Health Organization, 2024) and can lead to the development of an Alcohol Use Disorder (Rehm & Shield, 2019). According to the fifth version of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5; American Psychiatric Association, 2013) Alcohol Use Disorder (AUD) is defined as "*problematic pattern of alcohol use leading to clinically significant impairment or distress*" (DSM-5, American Psychiatric Association, 2013 , p. 490) which is determined by meeting at least two out of eleven criteria during the last 12 months including alcohol craving, attempts or desire to reduce alcohol use without success, drinking larger amounts of alcohol than intended, neglecting important life responsibilities due to alcohol use, spending an substantial amount of time, continued alcohol use despite physical or psychological harm and interpersonal problems as well as development of tolerance and withdrawal symptoms. Approximately 400 million people worldwide meet the criteria for AUD (World Health Organization, 2024), highlighting its global relevance and the importance to optimize the treatment for people suffering from AUD. According to the theoretical framework of Koob (2015, 2024), AUD can be conceptualized as a cycle consisting of three phases that evolve through adaptations of the brain reward and stress system. The first phase is the *binge/intoxication stage*, in which activation of the basal ganglia plays a central role. The release of dopamine and opioid peptides in the nucleus accumbens (NAc) assigns motivational properties to previously neutral stimuli (i.e., *incentive salience*), promoting repeated behavior and the development of habits that may lead to compulsive alcohol use. In the second phase, the *withdrawal/negative affect stage*, the function of the reward system in the ventral striatum is reduced while the stress system in the extended amygdala is activated. The combination of reduced release of reward neurotransmitters and enhanced stress system activity in the brain creates aversive and stress-like states that increase the motivation for alcohol use. The third phase, the *preoccupation/anticipation stage*, is characterized by impaired control of the prefrontal cortex. The executive function in the prefrontal cortex is controlled by a go-system and a stop-system. The go-system (i.e., anterior cingulate cortex and dorsolateral prefrontal

cortex) promotes habitual behavior, while the stop-system (i.e., ventral prefrontal cortex and orbitofrontal cortex) inhibits impulses. Individuals with AUD develop impairments in decision making and behavioral inhibition, often resulting in a strong desire to drink alcohol (alcohol craving). In this theoretical framework, alcohol craving is divided into two constructs: *reward craving* and *relief craving*. *Reward craving* is triggered by confrontation with alcohol stimuli, while *relief craving* is triggered by acute stress or a state of stress. The constructs are associated with the *binge/intoxication* and *withdrawal negative-affect stage* described above. Both *reward* and *relief craving* can contribute to relapse. Overall, the three stages of the cycle are interconnected and progressively result in the pathological state of AUD.

The following subsections provide a brief overview of the role of craving, cue-reactivity and negative affect in AUD as well as an overview of the pharmacological treatment of AUD, and serve as a basis for the introduction of potentially novel pharmacotherapeutic approaches, i.e. Cannabidiol and Oxytocin, that are presented in section 1.2 and section 1.3.

1.1.1 Craving, Relapse and Cue-Reactivity

Alcohol craving, i.e. a strong compulsion or desire to drink alcohol, is a core symptom of AUD according to the DSM-5 (American Psychiatric Association, 2013) and plays a key role in the maintenance of addictive behaviors (Sinha, 2012). A meta-analysis by Cavicchioli et al. (2020) confirmed that craving represents a significant risk factor for substance use in treatment seeking individuals. Specifically, in individuals with AUD the frequency of craving and the increased reactivity to alcohol cues were associated with increased alcohol use (Cavicchioli et al., 2020). Chronic alcohol use can lead to alterations in reward and motivation pathways in the brain, which in turn increase alcohol craving and cue-related physiological arousal (Sinha, 2012). In the early alcohol abstinence, exposure to alcohol cues lead to emotional distress and increases stress- and alcohol cue-induced craving, which can be attributed to altered stress regulation by alcohol that in turn increases vulnerability to craving, anxiety and risk of relapse, particularly when exposed to alcohol stimuli (Sinha, 2012). Although a pattern of relapse and remission is very common in patients with AUD (Sliedrecht et al., 2019), the exact definition of *relapse* in AUD is still debated and has therefore been systematically examined in a review (Sliedrecht et al., 2022). In the literature, relapse is most commonly used to refer to any use of alcohol, in most cases without specifying a period of

time after which a relapse could potentially occur (Sliedrecht et al., 2022). Other studies also use the term *slip* or *lapse* for a single alcohol use (Sliedrecht et al., 2019). In some more recent studies, *percent heavy drinking days* is increasingly used as an outcome measure (Sliedrecht et al., 2022). In this dissertation, the term *relapse* is used and defined as any use of alcohol.

Relapse is a complex phenomenon that is caused by the interplay of various factors, such as the interindividual characteristics in individuals with AUD, properties of the substance or environmental influences, and is usually characterized by alcohol craving in abstinent individuals prior to relapse (Bottlender & Soyka, 2004). The relationship between craving and relapse during an outpatient program and 12 months after completion of the outpatient program has been investigated in a prospective study in 103 individuals with alcohol dependence (Bottlender & Soyka, 2004). Higher alcohol craving measured by the total score of the Obsessive Compulsive Drinking Scale predicted relapse in the 12 months after the end of treatment. Therefore, patients with elevated craving scores may benefit from intensified aftercare or an anti-craving pharmacotherapy to reduce relapse risk (Bottlender & Soyka, 2004). In a study by Sinha et al. (2011), abstinent alcohol-dependent individuals and healthy controls were exposed to stress, alcohol cues, and neutral contexts. Compared to healthy controls, alcohol-dependent individuals showed a dysregulated hypothalamic-pituitary-adrenal axis (HPA axis), reflected in a blunted cortisol response, higher anxiety, and increased stress- and cue-induced alcohol craving. In addition, higher stress- and cue-induced alcohol craving predicted a shorter time to alcohol relapse (Sinha et al., 2011). In other studies, an association between craving and relapse (Moore et al., 2014; Stohs et al., 2019) as well as stress and craving (Law et al., 2016) has also been shown. Bowen and Neumann (2017) have defined three main triggers for relapse: substance-prime-induced relapse, i.e. hypersensitivity to substance rewards where subliminal substance exposure can lead to reinstatement behavior, substance cues, and stress. A more recent review has specifically examined relapse factors in AUD such as psychiatric comorbidity, addiction severity, craving, negative affect, other substance use, health and social factors (Sliedrecht et al., 2019). Higher AUD severity and craving were significant predictors of relapse and higher negative affect was associated with a higher risk of relapse (Sliedrecht et al., 2019). Another review that has also examined clinical and biological markers associated with relapse, has added that, in addition to AUD severity, acute withdrawal symptoms, chronic stress, genetic effects and sex

differences might also be moderators of relapse (Sinha, 2022). A review of neuroimaging studies has shown that alcohol craving and relapse in individuals with chronic alcoholism is associated with reduced brain volume in the medial prefrontal cortex, orbitofrontal cortex, precuneus, and the amygdala (Seo & Sinha, 2014). Similarly, functional imaging studies revealed altered activation in the medial prefrontal cortex and the orbitofrontal cortex, as well as in the anterior cingulate cortex and ventral striatum which was associated with alcohol craving and relapse in individuals with chronic alcoholism (Seo & Sinha, 2014). Sinha (2013) has further postulated that craving and substance use related neuroadaptations shift along a dimensional continuum as a function of substance use ranging from recreational use to moderate and heavy use and finally leading to dependence. With increasing substance use, a substance use desire is shifting to compulsive drug seeking and craving/wanting, while simultaneously alterations in cortico-striatal and limbic circuits occur (Sinha, 2013). Given the central role of alcohol cues in inducing craving and relapse (Sinha, 2012; Sinha et al., 2011), cue-reactivity has been focused in research for many years (e.g., (Monti et al., 1987; Rohsenow et al., 1991). Cue-reactivity arises from conditioned responses that are implicit or explicit learned through repeated exposure to alcohol-related stimuli associated with alcohol use and can be measured on physiological (i.e., heart rate, cortisol, skin conductance), psychological (i.e., alcohol craving) and neurobiological (i.e., brain activation) levels (Schacht et al., 2013). In early studies, alcohol dependent individuals were exposed with images and the smell of their preferred beverage and a control drink, and responses to alcohol stimuli were compared with those of non-dependent individuals, with increased salivation observed when alcohol dependent individuals were confronted with alcohol stimuli (Monti et al., 1987). Kwako et al. (2015) have combined the *Trier Social Stress Test* (Kirschbaum et al., 1993), an established paradigm in which psychosocial stress is induced, with a cue-reactivity task in which participants were instructed to hold and smell a glass of water for 3 minutes, followed by 3 minutes of relaxation before finally being exposed to the preferred alcoholic beverage for 3 minutes, which they were instructed to prepare and smell, but not to drink. Venegas and Ray (2020) started with a 3-minute relaxation period, after which participants were instructed to hold and smell a glass of water in their hand before holding a glass of their preferred alcoholic beverage for 3 minutes and recalling memories associated with alcohol consumption. Interestingly, this study found that treatment-seeking and non-treatment-seeking individuals with mild to moderate AUD did not differ in cue-

reactivity, leading the authors to conclude that non-treatment-seeking samples are informative for cue-reactivity as a potential biomarker for the investigation of pharmacological treatments (Venegas & Ray, 2020).

In another study, the effect of visual alcohol stimuli on physiological cue-reactivity, craving and relapse was investigated (Witteman et al., 2015). Alcohol stimuli predicted cue-reactivity and craving, and cue-reactivity was positively associated with craving, but neither craving nor cue-reactivity predicted relapse in the natural environment (Witteman et al., 2015). A systematic review and meta-analysis investigated whether cue-exposure, physiological cue-reactivity, cue-induced craving and self-reported craving are associated with drug use and relapse (Vafaie & Kober, 2022). Cue-exposure, physiological cue-reactivity, cue-induced craving und self-reported craving showed a significant association with drug use and relapse across all studies. Drug cues and craving could be considered suitable predictors for estimating the risk of drug use and relapse and may represent key mechanisms underlying drug use (Vafaie & Kober, 2022). Cue-reactivity was also investigated at a neuronal level by presenting alcohol cues and examining the brain's response using functional magnetic resonance imaging (fMRI). For this purpose, the *ALCUE task* was developed, in which alcohol stimuli and neutral stimuli are presented in blocks during fMRI which is used to assess the brain response and participants are asked to rate alcohol craving on a visual analog scale from 0 to 100 after each block (Vollstadt-Klein et al., 2012). It has been shown that the anterior cingulate cortex, the thalamus, prefrontal areas, the ventral and dorsal striatum, and the insula were activated by alcohol-associated stimuli during the fMRI cue-reactivity task (Vollstadt-Klein et al., 2012). A review summarized preliminary findings suggesting that relapse is associated with neural activity in medial prefrontal brain regions, anterior and posterior cingulate, striatal and posterior insula regions, while altered function in these regions was associated with stress- and cue-induced craving (Sinha & Li, 2007). Consistently, a meta-analysis indicated that alcohol cues elicit robust activation of the ventral striatum, anterior cingulate and ventromedial prefrontal cortex in heavy drinkers and individuals with AUD (Schacht et al., 2013). Cue-induced activation of the ventral striatum correlated with AUD severity, alcohol craving, loss of control and drinking outcomes and is most commonly reduced by treatment (Schacht et al., 2013). It was shown that the cue-induced response of the NAc to a pharmacological intervention serves as a predictor for the clinical efficacy of a drug for treating AUD (Bach et al., 2020b; Schacht et al., 2017). Courtney et al. (2016) have specified

that pharmacological treatment was related most frequently to a reduction in cue-induced ventral striatum activation, while psychosocial treatment was most frequently related to reductions in cue-induced dorsal prefrontal cortex and amygdala activation. Additionally, it was demonstrated that fMRI cue-reactivity in the insula and in the dorsal striatum may serve as biomarker for risk of relapse, as cue-induced higher activation in these areas was associated with a shorter time to relapse (Karthik et al., 2017). A more recent voxel-wise meta-analysis revealed higher alcohol cue-induced activity in the medial prefrontal cortex and anterior cingulate cortex as well as in the middle part of the cingulate cortex and lower cue-reactivity in the parietal and temporal regions in individuals with AUD compared to healthy controls (Zeng et al., 2021). AUD treatment resulted in reduced activation in the bilateral caudate nucleus, insula, right dorsolateral prefrontal cortex, and left superior frontal gyrus and increased activation in the precentral gyrus. Contrary to previous studies, however, this meta-analysis did not show a significant association between alcohol craving and relapse and cue-reactivity (Zeng et al., 2021). A recent study in which psychosocial stress was induced and subsequent neural cue-reactivity was examined using fMRI revealed that stress led to an increased alcohol cue-induced activation in the left anterior insula and that increased cue-induced activation of the insula was associated with higher craving during fMRI and higher alcohol use during follow up (Bach et al., 2024). This is in line with the findings of Kirsch et al. (2024), who have shown that individuals with AUD who exhibited higher cue-reactivity consumed more alcohol and reduced their alcohol use less compared to individuals with AUD who showed no cue-reactivity in the laboratory. A worldwide consortium of experts in the field of fMRI cue-reactivity, the *Enhanced NeuroImaging Genetics through Meta-Analyses* (ENIGMA) consortium, has conducted a systematic review on cue-reactivity and published an expert consensus (Ekhtiari et al., 2022). The consortium systematically reviewed 108 fMRI cue-reactivity studies and developed reporting standards, which were published as a checklist with the aim of improving the transparency and harmonization of methodological details in fMRI cue-reactivity studies (Ekhtiari et al., 2022). The same consortium has conducted another systematic review including 415 fMRI cue-reactivity studies to reflect the state of the field and to investigate the value of fMRI cue-reactivity as a potential biomarker (Sangchooli et al., 2024). According to the systematic review, in particular striatal fMRI cue-reactivity might be a promising regional biomarker, considering that individuals with AUD differ from individuals without AUD in striatal fMRI cue-reactivity, a shift from ventral to dorsal

striatum with increasing compulsive alcohol use has been shown, and striatal fMRI cue-reactivity was associated with alcohol use and relapse. Both cue-exposure therapy and pharmacotherapy, such as naltrexone or nalmefene, were shown to alter the striatal fMRI cue-reactivity, and striatal fMRI cue-reactivity before initiating naltrexone administration predicts treatment response, suggesting that this marker might be suitable to evaluate treatment effects in AUD. Another particularly promising marker discussed was a multivariate whole-brain marker identified using machine learning that was associated with craving, distinguished individuals with substance use disorder (SUD) from healthy controls, and detected treatment responses. According to this systematic review of the large ENIGMA consortium, these fMRI cue-reactivity biomarkers could be used diagnostically, prognostically and to evaluate treatment effects as well as to develop new interventions (Sangchooli et al., 2024). Consistently, a recent commentary concluded that the assessment of neural drug cue-reactivity using fMRI offers potential as a diagnostic and predictive biomarker as well as for investigating treatment response in AUD (Pfisterer et al., 2025).

1.1.2 Negative Affect, Craving and Relapse

Negative affect refers to a state of emotional distress characterized by negative and unpleasant emotions such as anxiety, fear, anger and sadness (Guinle & Sinha, 2020). It has been shown that exposure to stress and alcohol cues in patients with alcohol dependence induces negative affect and alcohol craving, while reducing positive affect (Fox et al., 2007). In turn, according to a meta-analysis, the experimental induction of negative affect leads to more alcohol craving and alcohol use (Bresin et al., 2018). Chronic alcohol use results in a dysregulation of the HPA axis and impaired function of the prefrontal cortex, with both alterations being associated with negative affect (Pahng et al., 2017). As alcohol craving and negative affect are both associated with an increase in alcohol use and the prefrontal cortex plays a key role in both, fMRI was used to investigate whether individuals with AUD can regulate alcohol craving and negative affect through selective activation of the prefrontal cortex using cognitive-behavioral treatment strategies (Suzuki et al., 2020).

This study demonstrated that individuals with AUD were able to effectively regulate craving and negative affect using cognitive-behavioral treatment strategies. It was found that the regulation of craving and negative affect involved shared as well as distinct regulatory systems, i.e. increased activity in the dorsolateral, ventrolateral and

dorsomedial prefrontal cortex was associated with the regulation of craving and negative affect, while decreased activity in the ventral striatum and parts of the anterior cingulate cortex as well as the ventromedial prefrontal cortex and the orbitofrontal cortex was linked to the regulation of craving, and decreased activity in the amygdala was linked to the regulation of negative affect (Suzuki et al., 2020). In addition to the assumed link between negative affect and craving, negative affect is also assumed to be one of the risk factors for relapse (e.g., Guinle & Sinha, 2020; Koob, 2009; Sliedrecht et al., 2019). An early study which analyzed outpatient data from a large sample of individuals undergoing alcohol treatment has shown that a change in alcohol use after treatment was associated with current or prior alterations of negative affect, thus a treatment targeting the relationship between negative affect and alcohol use was recommended (Witkiewitz & Villarroel, 2009). In contrast to the studies that examined individuals with alcohol dependence, a more recent study conducted daily assessments in heavy social drinkers and found that more negative affect was associated with less alcohol use, while more positive affect was associated with more alcohol use (Bresin & Fairbairn, 2019). A recent meta-analysis that analyzed the relationship between affect and alcohol use on a daily basis in 69 studies revealed that individuals did not drink more on days when experiencing high negative affect, but tended to drink more on days with high positive affect, which challenges the hypothesis that negative affect is related to more alcohol use (Dora et al., 2023). However, it should be noted that the meta-analysis by Dora et al. (2023) examined individuals of clinical samples in treatment for alcohol and substance use in only 7 of the 69 studies analyzed and that the majority of the analysis referred to college samples and community samples. In addition, Guinle and Sinha (2020) highlighted that there are gender differences in negative affect in AUD, such as females reporting more negative affect than males and that males are more likely to use alcohol to increase positive affect while females are more likely to use alcohol in response to negative affect. According to Guinle and Sinha (2020) negative affect and stress are main factors contributing to increasing rates of AUD in females.

1.1.3 Treatment of Alcohol Use Disorder

Although there are several interventions for the treatment of AUD, i.e. behavioral interventions and pharmacotherapies (Donato & Ray, 2023) and despite the fact that many people suffer from the symptoms of AUD and resulting alcohol-associated diseases,

there is still a huge treatment gap (Koob, 2024). According to the theoretical framework of the addiction cycle postulated by Koob (2015, 2024) key neurobiological mechanisms underlying AUD include altered reward processing, heightened cue-reactivity, dysregulated craving, and stress-related negative affect, particularly in the *withdrawal/negative affect stage*. According to the German S3 Treatment Guidelines (2020) individuals with AUD should be provided with pharmacotherapeutic treatment with acamprosate or naltrexone in addition to psychotherapeutic interventions. However, those pharmacotherapies primarily target alcohol craving (Marin et al., 2023) and have only limited efficacy with regard to alcohol-related outcomes (Jonas et al., 2014; Köhne et al., 2024). This may be due to the fact, that approved medications fail to sufficiently address stress-induced negative affect and withdrawal-related symptoms which may, in addition to side-effects of approved medications including anxiety, diarrhea, dizziness, nausea and vomiting (Jonas et al., 2014), contribute to poor treatment outcomes and patient compliance. Indeed, less than 8% of adult individuals with AUD receive any therapeutic or pharmacological treatment (Koob, 2024). Therefore, novel effective and well-tolerated pharmacotherapeutic approaches are required (Köhne et al., 2024; Nona et al., 2019; Walker & Lawrence, 2018). Several novel compounds have been discussed in this context, including monoamine modulators (e.g., doxazosin, varenicline, tolcapone, psilocybin), modulators of the neuroimmune system (e.g., apremilast), GABA/glutamate modulators (e.g. baclofen, zonisamide, gabapentin, ketamine), nalmefene, topiramate, semaglutide and the neuropeptides Oxytocin (OXY) and Cannabidiol (CBD) (Köhne et al., 2024; Walker & Lawrence, 2018; Witkiewitz et al., 2019). The potential effects of OXY and CBD in the treatment of AUD symptoms are the main focus of this dissertation. OXY and CBD have recently emerged as promising candidates due to their potential effects on central mechanisms implicated in AUD pathophysiology. Preclinical and early clinical findings suggest that OXY may attenuate stress-induced negative affect and craving, particularly during the *withdrawal/negative affect stage*, whereas CBD appears to reduce cue-induced reactivity and craving during the *binge/intoxication* and *preoccupation/anticipation stages*. Thus, targeting negative affective states and cue-reactivity in addition to craving with these novel compounds may improve pharmacotherapeutic treatments while aligning more closely with the theoretical framework postulated by Koob (2015, 2024). The characteristics of both neuropeptides, as well as the preclinical and clinical evidence

regarding the administration of OXY and CBD in AUD, are presented in detail in section 1.2 and 1.3.

1.2 Cannabidiol

An innovative pharmacotherapeutic target for the treatment of AUD could be the endocannabinoid system (Burnette et al., 2022; De Ternay et al., 2019; Köhne et al., 2024; Walker & Lawrence, 2018). The endocannabinoid system is mainly distributed in the central and peripheral nervous system and regulates brain functions via cannabinoid receptors, endocannabinoids and other mechanisms (Manzanares et al., 2018). Preclinical studies have shown that activation of cannabinoid receptors in the mesocorticolimbic system, particularly cannabinoid 1 (CB1) receptors, enhance dopamine release in the NAc and other limbic structures which is associated with the rewarding effects of substance use and as well as initiating and maintaining substance use (Manzanares et al., 2018). Conversely, modulation of these pathways by blocking CB1 receptors (Manzanares et al., 2018) or activating cannabinoid 2 (CB2) receptors (Navarrete et al., 2021) has been related to reduced substance use and reduced relapse rates. Therefore, preclinical studies suggest that the endocannabinoid system plays a key role in the neurobiological mechanisms underlying SUDs and may represent a promising target for therapeutic intervention. In initial attempts to achieve beneficial effects via the endocannabinoid system on SUDs, synthetic CB1 receptor antagonists have been investigated, but these have led to severe adverse events and the related research was therefore stopped and the CB1 receptor antagonists withdrawn from the commercial market (Manzanares et al., 2018). Since then, more recent studies have focused on approaches involving the CB2 receptor or the allosteric negative modulation of CB1 receptors (Burnette et al., 2022; De Ternay et al., 2019; Manzanares et al., 2018; Navarrete et al., 2021). In particular, the administration of cannabinoid compounds from the *Cannabis sativa* plant has generated considerable interest and is currently being investigated (Navarrete et al., 2021). Apart from terpenes and phenolic components, various cannabinoids can be extracted from the *Cannabis sativa* plant (Andre et al., 2016; Navarrete et al., 2021). The most prevalent phytocannabinoids are delta-9-tetra-hydrocannabinol (THC) and cannabidiol (CBD), with THC having psychoactive properties and being associated with side effects such as anxiety (Andre et al., 2016). CBD, the non-psychoactive component of the *Cannabis sativa* plant, has no addictive potential and is associated with anxiolytic, antipsychotic, antidepressant and anti-inflammatory effects and interacts with multiple targets within the central nervous system i.e., G-protein coupled cannabinoid receptors CB1 and CB2, the G-protein coupled receptor 55 (GPR55), the transient receptor potential cation

channel subfamily V member 1 (TRPV1), the G-protein coupled serotonin 1A receptor (5-HT_{1A}), the anandamide hydrolyzing enzyme or the adenosine transporter (Navarrete et al., 2021). The effects of CBD are not limited to cannabinoid receptors and the interaction of CBD with the CB1 receptor has been debated, but current evidence suggests that CBD is rather acting as negative allosteric modulator than a direct antagonist (Laprairie et al., 2015; Navarrete et al., 2021). In addition, CBD acts as an allosteric modulator on the CB2 receptor (Martínez-Pinilla et al., 2017), which is associated with the alcohol reward system and with alcohol-addictive behavior and has also been shown in post-mortem analyses to be associated with genetic alterations of the CB2 receptor in patients with alcohol dependence (García-Gutiérrez et al., 2022). Beneficial effects of CBD have already been studied in neurological disorders such as epilepsy, multiple sclerosis and Alzheimer's disease (Elsaid et al., 2019; Navarrete et al., 2021) as well as depression, ischemia and pain (Karimi-Haghighi et al., 2022). Due to the properties of CBD including the good safety profile (World Health Organization, 2019; Wright et al., 2020), the potential of CBD has been explored in different psychiatric disorders (Yau et al., 2023) and beneficial effects have been reported in social anxiety disorder, schizophrenia and SUDs (Elsaid et al., 2019), although larger randomized controlled trials and the involvement of both sexes are important to determine the therapeutic potential of CBD (Elsaid et al., 2019; Wright et al., 2020). The potential of CBD in the treatment of SUDs has been subject of several investigations, particularly since the endocannabinoid system plays a role in substance craving, drug-seeking behavior, withdrawal of substance use, cognitive and emotional processes (Kirkland et al., 2022). In preclinical studies, positive effects of CBD have been shown for alcohol, opioids, and methamphetamine, while preliminary human studies have shown that CBD reduces the number of cigarettes smoked, the pleasure associated with tobacco smoking and opioid-craving, suggesting that CBD could be a promising treatment approach for SUDs (Paulus et al., 2022). A review suggests that the positive effect on SUDs is due to the influence of CBD in the central nervous system (Navarrete et al., 2021). Preclinical studies in AUD suggest that CBD has not only positive effects on ethanol intake, relapse and anxiety symptoms and impulsivity, but also reduces alcohol-related changes in the liver such as steatosis and fibrosis as well as alcohol-related brain damage and could therefore be a promising treatment option to reduce the negative effects of alcohol on the liver and brain in terms of a harm reduction approach if a reduction of alcohol use or abstinence has not been successful (De Ternay et al., 2019). CBD

has demonstrated neuroprotective effects that may prevent alcohol-related damage of the hippocampus. Combined with its good safety profile and favorable tolerability, the lack of abuse liability and the lack of interaction with alcohol effects in humans, these characteristics suggest that CBD could be a promising compound in the treatment of AUD. However, further clinical studies are required to determine whether the predominantly preclinical positive findings can be translated to human individuals with AUD (Turna et al., 2019). More recent reviews continue to discuss CBD as a potential novel agent in the treatment of AUD (Burnette et al., 2022; Köhne et al., 2024), even though to date still only a few findings from randomized controlled trials in human individuals with AUD or other SUDs have been published.

The following sections provide an overview of the experimental preclinical research of the effects of CBD on alcohol related behavior (section 1.2.1) and clinical research of the effects of CBD on AUD and other SUDs as well as effects of CBD on neural processes investigated by using neuroimaging methods (section **Fehler! Verweisquelle konnte nicht gefunden werden.**).

1.2.1 Preclinical Evidence on Cannabidiol

In a preclinical study it was shown that the application of CBD reduced reinforcement and motivational effects of ethanol in mice, preventing relapse to ethanol (Viudez-Martínez et al., 2018b). Additionally, this study showed that the observed effects of CBD on ethanol use were associated with alterations in gene-expression in both cannabinoid receptors CB1 and CB2, GPR55 and Opioid receptor μ 1 (OPRM1) receptors in the NAc and tyrosine hydroxylase in the ventral tegmental area, which are linked to alcohol dependence. A preclinical proof-of-principle study in rats that had a history of either alcohol or cocaine self-administration aimed to investigate context- and stress-induced reinstatement behavior as well as anxiety symptoms and impulsive behavior after administration of 15.0 mg/kg CBD every 24 hours for 7 days (Gonzalez-Cuevas et al., 2018). It was shown that CBD dampened context- and stress-induced reinstatement behavior without causing sedative effects. CBD influenced behavior without leading to tolerance for CBD, and the resumption of drug use remained attenuated for up to five months. In addition, a reduction in anxiety symptoms and impulsive behavior was observed in rats with alcohol dependence. Furthermore, this study examined CBD levels in blood plasma and in the brain for two dosages (15 mg/kg and 30 mg/kg). CBD was detected up to three days after the last CBD administration. The authors

concluded that the results demonstrate the potential of CBD in relapse prevention and suggest lasting behavioral effects for up to five months after seven days of CBD treatment, even though CBD-levels were not detectable in blood plasma or in the brain during this period (Gonzalez-Cuevas et al., 2018). In a study with Sardinian Alcohol-preferring rats, acute administration of CBD led to a reduction of alcohol self-administration, but none of the tested CBD dosages had any effect on chocolate self-administration, suggesting that the effect of CBD on the reinforcing effects of alcohol is substance-specific (Maccioni et al., 2022).

1.2.2 Clinical Studies on Cannabidiol

In two preliminary studies, the interaction of cannabidiol and alcohol in healthy volunteers has been investigated (Belgrave et al., 1979; Consroe et al., 1979). Ten healthy post-graduate volunteers were administered placebo (PLC), 200mg CBD, alcohol (1g/kg) and CBD (200mg) plus alcohol (1g/kg) in a double-blind, randomized crossover study at one-week intervals and motor and psychomotor performance as well as attention, concentration and perceptual function were examined. It was shown that alcohol and alcohol plus CBD led to an impaired performance while the performance was not impaired when CBD was administered without alcohol. In addition, the administration of CBD plus alcohol led to a reduction in blood alcohol levels (Consroe et al., 1979). In another double-blind within-subjects study, fifteen healthy social drinkers were administered at four study visits either CBD (0.32mg/kg, i.e., a range between 17.5 mg – 30 mg CBD) or CBD-PLC, 60 minutes before they drank an alcoholic beverage (containing 0.54g/kg alcohol) or PLC. Participants were tested using a test battery consisting of a standing steadiness test, reaction time tasks, coordination test, pursuit rotor task, concentration and attention task and a word construction test at baseline and 100min, 160min and 220min after administration of CBD or CBD-PLC. Alcohol led to a reduced performance of psychomotor coordination and cognition, including the pursuit rotor task, coordination test and word construction test while no effect of CBD or CBD in combination with alcohol was demonstrated between 100 and 220min after CBD administration (Belgrave et al., 1979). To date, except our own study (see section 2.1), no studies have reported results on the effects of CBD in individuals with AUD (Kirkland et al., 2022). Results of our own randomized controlled pilot trial in individuals with AUD, which was designed to investigate the effect of acute CBD administration on alcohol craving and cue-induced activation of the NAc in an fMRI alcohol cue paradigm,

are presented in section 2.1. In addition, a study protocol was recently published aiming to examine neurobehavioral effects of 800mg CBD per day on three consecutive days compared to PLC in non-treatment seeking individuals with AUD (Hurzeler et al., 2024a). Effects of CBD on cue-reactivity and fear response during fMRI, psychophysiological response to alcohol stimuli (i.e., heart rate variability and skin conductance), neurometabolite levels, functional connectivity (resting state fMRI), executive functioning, craving, anxiety and sleep will be compared to PLC to improve knowledge of the mechanisms underlying CBD's effects and examine its potential efficacy as a treatment for AUD. Further studies in AUD have been registered, but have not reported results yet (see Kirkland et al., 2022). Regarding other SUDs, results of CBDs effects on craving, relapse and anxiety symptoms have been reported for opioid use disorder (Hurd et al., 2019), cannabis use disorder (Freeman et al., 2020) and cocaine use disorder (Mongeau-Pérusse et al., 2022; Mongeau-Pérusse et al., 2021). In a double-blind randomized controlled trial acute (160min and 24 hours after first CBD administration), short-term (directly after third administration of CBD) and protracted (7 days after last CBD administration) effects of either 400mg or 800mg per day on three consecutive days were compared to PLC in male and female abstinent individuals with heroin use disorder (Hurd et al., 2019). Cue-induced craving, anxiety, positive and negative affect, cognition as well as cue-induced heart rate and salivary cortisol levels were examined. Drug cue-induced craving and anxiety were reduced immediately after the first assessment that was obtained 160 min after the first administration of CBD (400mg or 800mg), compared to PLC. These effects lasted until 7 days after the final CBD administration. Drug cue-exposure increased heart rate and salivary cortisol levels, with CBD attenuating the increase in salivary cortisol and tending to reduce cue-induced heart rate, but no effects of CBD on positive and negative affect and cognition were observed and during the study, only mild adverse events were reported by participants (Hurd et al., 2019). In another double-blind randomized controlled trial, individuals with cannabis use disorder were orally administered 200mg, 400mg or 800mg CBD or a PLC daily over a period of four weeks to determine the appropriate dose for reducing cannabis use (Freeman et al., 2020). While 200mg CBD was ineffective, both 400mg and 800mg CBD significantly reduced cannabis use. In another sample consisting of individuals with cocaine use disorder effects of CBD on craving and relapse (Mongeau-Pérusse et al., 2021) and anxiety and stress response (Mongeau-Pérusse et al., 2022) were investigated. In this randomized placebo-controlled trial individuals

with cocaine use disorder received either 800mg CBD or PLC daily during ten days of inpatient detoxification and twelve weeks outpatient follow up phase including self-administration of 800mg CBD or PLC per day. Administration of 800mg CBD did not reduce drug-cue-induced craving or relapse (Mongeau-Pérusse et al., 2021) nor did it reduce anxiety symptoms or decrease cortisol levels (Mongeau-Pérusse et al., 2022) in individuals with cocaine use disorder. Recently, a study protocol of a planned multicenter randomized controlled trial was published, which aims to examine the efficacy, safety and effects on quality of life after CBD compared to PLC administration in individuals with moderate to severe cannabis use disorder (Bhardwaj et al., 2024).

The effects of CBD on the brain have already been investigated in several samples of healthy participants using fMRI, positron emission tomography and magnetic resonance spectroscopy (Hurzeler et al., 2024b). In the task-based fMRI, which was also used in the empirical studies presented in this thesis in section 2, the effect of CBD on response inhibition and emotion processing has been investigated. In a double-blind, placebo-controlled, within-subject trial, fifteen healthy volunteers were administered 10 mg THC, 600 mg CBD and PLC at three different timepoints one month apart before participating in a go/no go task during fMRI, in which a motor response was either executed or inhibited depending on the stimuli (Borgwardt et al., 2008). The results showed that THC attenuated activation in the right inferior frontal and the anterior cingulate gyrus, i.e., regions that are associated with response inhibition, while CBD inhibited activation in the left temporal cortex and the insula, i.e., regions that are not associated with response inhibition. The same sample with the same design and treatment, completed a fMRI emotion processing experiment, where different faces with neutral and fearful facial expressions, ranging between mildly and intensely fearful, were presented after participants had received THC, CBD or PLC, respectively (Fusar-Poli et al., 2010). CBD, in contrast to THC, reduced the connectivity between the anterior cingulate cortex and the amygdala during processing of fearful facial expressions and the authors concluded that this change in connectivity may be associated with the anxiolytic properties of CBD. A review summarizing the results of studies using fMRI, positron emission tomography or magnetic resonance spectroscopy in healthy volunteers found that CBD alters activity in brain regions associated with the reward system and in networks that play a key role in reward anticipation and reward processing, emotion regulation and executive functions, and also alters neurotransmitters such as the γ-aminobutyric acid and glutamate signaling pathways in the basal ganglia and

prefrontal cortex (Hurzeler et al., 2024b). CBD thus influences brain areas and neurotransmitter systems that play a significant role in AUD, therefore, the authors consider CBD to have therapeutic potential in the treatment of individuals with AUD (Hurzeler et al., 2024b).

1.3 Oxytocin

Besides the endocannabinoid system, the OXY system might also represent a potential target of an innovative pharmacotherapeutic approach for the treatment of AUD (Köhne et al., 2024; Walker & Lawrence, 2018), due to its effects on stress regulation, attenuation of negative affective states, and potential reduction of craving. OXY is a neuropeptide consisting of nine amino acids that is synthesized in the magnocellular neurons in the paraventricular nucleus (PVN) and the supraoptic nucleus (SON) in the hypothalamus (Bowen & Neumann, 2017; Martinetz & Neumann, 2016). OXY is either released locally at the axonal terminals or released at the dendrites into the extracellular space and reaches more distant oxytocin receptors (OXTR) in the brain by passive diffusion (Lee & Weerts, 2016; Meyer-Lindenberg et al., 2011). As a peripheral hormone, it primarily regulates uterine contractions during childbirth and plays a key role in lactation (Martinetz & Neumann, 2016). OXY is released during birth, suckling or breastfeeding, sexual stimulation or stress (Bowen & Neumann, 2017). As a neurotransmitter, OXY acts in the hypothalamic and limbic brain areas such as the amygdala, the NAc and the hippocampus and modulates social and emotional behaviors (Martinetz & Neumann, 2016). Accordingly, OXTRs are found both in the central nervous system and in peripheral tissues (Lee & Weerts, 2016). The OXTRs are G-protein receptors that are coupled with the proteins Gq, Gi or Go (Cid-Jofré et al., 2021; Lee & Weerts, 2016). Most OXTRs in the brain are located in the hypothalamus, the prefrontal cortex, the hippocampus and the amygdala (Jurek & Neumann, 2018) as well as in the ventral tegmental area, NAc and anterior cingulate cortex (Cid-Jofré et al., 2021). According to Jurek and Neumann (2018), OXTR expression in the respective brain areas is associated with specific behaviors and disorders, i.e., the amygdala with fear and autism, anterior cingulate with decision making and post-traumatic stress disorder and schizophrenia, the hypothalamus and sexual behavior (Jurek & Neumann, 2018). Apart from the G-protein coupled receptors, behavioral effects by OXY are also associated with transient receptor potential vanilloid type 2 channels (TRPV2), which are located in the amygdala and PVN and thought to be mediators of the anxiolytic effect (Martinetz & Neumann, 2016; Van Den Burg et al., 2015). In contrast, the stress-reducing effect of OXY is attributed to a stress-induced reduction in the expression of the corticotropin-releasing factor (CRF), which attenuates the activity of the HPA axis (Martinetz & Neumann, 2016). The effect of OXY on the stress system (i.e., CRF, HPA axis) is particularly relevant, as stress-related mechanisms are central to behavior

driven by negative reinforcement (King et al., 2020). According to the theoretical framework of Koob (2015, 2024), the recruitment of the stress system plays a critical role in the progression of AUD, particularly during the *withdrawal/negative affect stage,* where negative affective states emerge due to a dysregulation of the reward and stress system. Furthermore, it is assumed that OXY interacts with other neurotransmitter systems, such as the gamma-aminobutyric acid (GABA)ergic, dopaminergic and serotonergic systems (King et al., 2020; Martinetz & Neumann, 2016). The interaction with the GABAergic system is linked to the regulation of inhibitory control and anxiety and may contribute to the modulation of stress and drug-seeking behavior in the context of AUD (King et al., 2020). OXY's modulatory effect on the dopaminergic system, in particular in the NAc, is associated with a reduction of the rewarding effects of alcohol, as OXY has been shown to inhibit alcohol-induced increases in dopamine levels in the NAc (King et al., 2020). The interaction with the serotonergic system is due to OXY's enhancing effects on the serotonergic signaling by increasing serotonin release (Yoshida et al., 2009), which is associated with decreasing effects on negative affect and alcohol use (Ketcherside et al., 2013). In addition, alterations of the OXY mRNA levels and alterations of the receptor expression in the NAc, the amygdala, the hippocampus in the ventral tegmental area and particularly in the prefrontal cortex were shown in the rodent model and in post-mortem analyses of human brains of individuals with AUD, while the increase in OXY mRNA levels in the prefrontal cortex in particular was correlated with the extent of alcohol consumption (Lee et al., 2017).

Due to the good safety profile (see **Fehler! Verweisquelle konnte nicht gefunden werden.** for more detail) and the simple and non-invasive application by nasal spray as well as the potential beneficial effects, the intranasal application of OXY has attracted extensive clinical interest (Quintana et al., 2021). In some studies, OXY is considered as a potential novel treatment strategy in psychiatric disorders which are characterized by social dysfunction and has therefore been used for its prosocial effects in autism spectrum disorder, schizophrenia, depression, anxiety disorders (Cid-Jofré et al., 2021; Quintana et al., 2021; Wigton et al., 2015). In addition to the prosocial effects, research has shown that OXY may also reduce anxiety symptoms and alleviate the stress response (Lee & Weerts, 2016; Martinetz & Neumann, 2016; Takayanagi & Onaka, 2021). This is especially relevant in AUD, as the theoretical framework of Koob (2015, 2024) highlights, that negative affect arises due to an imbalance in the reward and stress system during the *withdrawal/negative affect stage*. By reducing stress and

anxiety, OXY may stabilize these systems and may therefore also lower the risk of relapse. OXY affects the stress response by interacting with the HPA axis, dopamine mesolimbic reward system and CRF stress pathway while also attenuating inflammation (Lee & Weerts, 2016; Takayanagi & Onaka, 2021). While OXY reduces HPA axis activity and thereby decreasing the release of the stress hormone cortisol and thus attenuating the stress response, acute stress is associated with an increase in OXY plasma levels, which indicates that OXY plays a modulating role in allostasis, i.e. adaptation to environmental demands, and may provide a protective effect in the maintenance of homeostasis (Lee & Weerts, 2016; Takayanagi & Onaka, 2021). Previous research has shown that repeated exposure to substances such as alcohol, cocaine, gamma-hydroxybutyrate (GHB), ecstasy, morphine or THC leads to alterations in the OXY system which include the loss of oxytocinergic function, e.g. by the degeneration of OXY associated magnocellular neurons in the hypothalamus as well as an increase in OXY mRNA and OXTR expression, while intranasal administration of OXY might have the potential to reduce substance-induced damaging effects (McGregor & Bowen, 2012). Given these alterations and the potential of OXY to counteract the substance-induced damage, along with the assumed overlap between addiction pathways, the stress system and the OXY system in the brain (Bowen & Neumann, 2017), the effects of intranasal OXY administration of have been investigated using fMRI. In a review of intranasal OXY fMRI studies, it was shown that OXY attenuates the response of the amygdala to emotional faces and leads to alterations in the activation of the left inferior orbitofrontal cortex and ventromedial prefrontal cortex during fear processing (Bethlehem et al., 2013). Another meta-analysis, which included 66 fMRI studies with intranasal OXY application found increased activation of the bilateral amygdala, caudate and superior temporal activity in healthy controls while patients showed increased dorsal activation of the anterior cingulate cortex, which the authors interpreted as supporting the anxiolytic and prosocial effects of OXY at the neuronal level (Wang et al., 2017). A more recent review of 17 clinical trials that have administered intranasal OXY to reduce alcohol, cannabis, nicotine and opioid craving and intake of substances such as cannabis, cocaine and nicotine or food has added that drug-related cues lead to hyperactivation of the amygdala, which may be associated with stress- and cue-induced craving (Houghton et al., 2021). In addition, functional connectivity analysis suggests that OXY increases connectivity between the amygdala, orbitofrontal cortex, anterior cingulate cortex, hippocampus, precuneus, supramarginal gyrus and middle

temporal sulcus (Bethlehem et al., 2013). In contrast, another study examining healthy males after intranasal administration of 24 I.U. OXY observed a reduction in connectivity between the bilateral amygdala and the precuneus, as well as an overall reduction in connectivity between the precuneus and the right amygdala (Kumar et al., 2014). In heavy drinkers, attenuated connectivity of the NAc during the processing of alcohol stimuli was found after OXY administration ((Bach et al., 2019); see **Fehler! Verweisquelle konnte nicht gefunden werden.** for more detail). In conclusion, the effects of OXY on cortico-striatal and prefrontal circuits, the attenuating effect on the amygdala, stress-modulating effects i.e. on the HPA axis and the CRF, the effects on neurotransmitters, i.e. dopamine, serotonine, glutamate, provide evidence for the potential of OXY administration in SUDs due to the overlap of OXY's effects and the underlying mechanisms of stress- and cue-induced craving (Che et al., 2021; Houghton et al., 2021). Consequently, several reviews have concluded that OXY may have potential as a treatment for cue-induced, stress-induced or withdrawal-related craving in SUDs and should therefore be further investigated (Che et al., 2021; Cid-Jofré et al., 2021; Houghton et al., 2021; Lee & Weerts, 2016).

The following sections provide an overview of the experimental preclinical research of the effects of OXY on alcohol related behavior (section 1.3.1) and clinical research of the effects of OXY on AUD (section **Fehler! Verweisquelle konnte nicht gefunden werden.**).

1.3.1 Preclinical Evidence on Oxytocin

In one of the first preclinical studies, male Wistar rats were administered OXY (1 mg/kg) intraperitoneally for 10 consecutive days during adolescence, which led to a reduction in generalized and social anxiety and more social interaction, as well as to a lower motivation to consume alcohol, which was reflected in a reduced ad libitum intake of alcohol (Bowen et al., 2011). Another administration of OXY (1mg/kg, intraperitoneal) after 25 days of ad libitum intake of beer significantly reduced alcohol intake without affecting fluid intake in general. In addition, rats that were administered OXY during adolescence, showed a trend towards increased OXY plasma levels and an upregulation of OXTR mRNA in the hypothalamus, suggesting that the exogenous administration of OXY influences the endogenous OXY system (Bowen et al., 2011). Another preclinical study investigated the effects of a 14-day psychosocial stress paradigm (i.e., chronic subordinate colony housing) on ethanol intake and the effect of treatment with

baclofen (2.5 mg/kg) and OXY (10 mg/kg, intraperitoneal) in male mice (Peters et al., 2013). Chronic psychosocial stress increased ethanol consumption compared to single housed mice and the administration of baclofen reduced ethanol self-administration in both single housed mice (i.e., controls) and in the mice exposed to chronic psychosocial stress, whereas the administration of OXY reduced ethanol self-administration only after peripheral administration and only in single housed mice but not in the mice exposed to chronic psychosocial stress (Peters et al., 2013). Consistent with earlier findings (Bowen et al., 2011), MacFadyen and colleagues (2016) demonstrated that peripherally administered OXY reduced ethanol consumption in rats. Lower OXY doses were tested (0.05, 0.1, 0.3, and 0.5 mg/kg, intraperitoneal), which led to less sedative effects. The results suggest that OXY may inhibit rewarding effects of alcohol and the authors hypothesized that this might be due to a specific effect in the NAc (MacFadyen et al., 2016). In another study, male and female prairie voles were administered 1.0, 3.0, and 10.0 mg/kg OXY peripherally (Stevenson et al., 2017). OXY administration reduced alcohol use in male and female prairie voles, with the strongest effect in the first hour of alcohol access after OXY administration. OXY did not affect locomotor activity, while alcohol administration increased locomotor activity and induced anxiolytic effects in female prairie voles only. In mice showing binge-drinking behavior, OXY (0, 0.3, 1, 3, or 10 mg/kg, intraperitoneal) dose-dependently reduced ethanol consumption across different self-administration paradigms, with the results of the study suggesting that these effects were not due to sedative effects and that the response to other natural reward stimuli remained unaffected (King et al., 2017). In another study, rats with alcohol dependence induced by chronic alcohol inhalation were injected intracerebroventricularly with 10 nM OXY or a vehicle after a 3-week abstinence phase and cue-induced reinstatement of alcohol use was compared. It was shown that OXY reduced cue-induced reinstatement of alcohol use only in rats with alcohol dependence while it had no effect on reinstatement behavior in rats without alcohol dependence (Hansson et al., 2018). In addition, the examination of the expression of the OXY system revealed that OXTR mRNA and protein levels in frontal and striatal brain areas were increased in abstinent rats with alcohol dependence, whereas OXY mRNA and OXY peptide levels were reduced in the PVN and SON (Hansson et al., 2018). Aiming to further investigate the mechanisms underlying the effects of OXY, Peters and colleagues (2017) injected either OXY (1 µg/5 µl) or a vehicle (5 µl Ringer solution) intracerebroventricularly in male rats that had chronic intermittent access to alcohol and

found that OXY administration attenuated self-administration of ethanol. Additionally, it was also shown that intraperitoneal application of ethanol (1.5 g/kg) increased dopamine release in the NAc, while OXY blocked the ethanol-induced release of dopamine in the NAc (Peters et al., 2017). In contrast to previous studies that reported effects only in rats with alcohol dependence (e.g., Hansson et al., 2018), the effects of OXY were observed both in rats with chronic access to ethanol and in rats without previous alcohol exposure. To examine the effects of OXY on alcohol use and motivation for alcohol use as well as GABAergic transmission in the central nucleus of the amygdala, male rats exhibiting alcohol-dependent behavior were administered OXY intraperitoneally (0, 0.125, 0.25, 0.5, and 1 mg/kg) or intranasally (0, 0.25, 0.5, and 1 mg/kg/20 µl) and were compared to non-dependent rats in a further preclinical study (Tunstall et al., 2019). It was shown that only in alcohol-dependent rats, but not in non-dependent rats, OXY blocked excessive alcohol use (after 0.25, 0.5 and 1 mg/kg interperitoneally or 1 mg/kg intranasally OXY) and motivation for alcohol use (after 0.125 and 0.5 mg/kg interperitoneally or 0.5 and 1 mg/kg intranasally OXY) in alcohol-dependent male rats and reduced GABAergic transmission in the central nucleus of the amygdala by inhibiting alcohol-induced GABA release (Tunstall et al., 2019). A more recent study investigated the effects of intranasal administration of OXY (5.0 mg/kg and 10.0 mg/kg) on alcohol consumption in socially housed male and female prairie voles, as well as the brain penetrance of OXY and examined the impact of an intraperitoneal administered OXTR agonist (i.e., LIT-001) on the ability to modulate alcohol intake (Potretzke et al., 2023). Intranasal OXY and the synthetic OXTR agonist reduced alcohol intake in male, but not female prairie voles, which led the authors to the conclusion that OXTRs may represent a potential target in the pharmacological treatment of AUD. The observed sex-specific effect was attributed to differences in OXTR distribution between sexes, as well as the potentially higher resilience of female prairie voles to fluctuations in OXY signaling, given the relevance of OXY in reproductive and maternal behavior (Potretzke et al., 2023).

1.3.2 Clinical Studies on Oxytocin

Besides endogenous OXY, which naturally occurs in the body, exogenous OXY can be synthetically produced and administered intranasally by using nasal spray in human individuals (Quintana et al., 2021). Intranasal administration has repeatedly been demonstrated to enable OXY to cross the blood-brain barrier via the olfactory system,

reaching the brain in sufficient concentrations to induce relevant effects on the brain and on behavior (Bethlehem et al., 2013; Quintana et al., 2021). In most studies, doses ranging from 20 to 48 IU were administered (Bethlehem et al., 2013; Quintana et al., 2021), with the majority having administered 24 IU of OXY and having observed behavioral and neuronal effects within 20-90 minutes after intranasal administration (Quintana et al., 2021). Pharmacodynamic studies have shown that OXY plasma levels peak 15 minutes after intranasal administration, while decreased OXY plasma levels are observed after 75 minutes (Striepens et al., 2013) and OXY plasma levels return to baseline on average within 90 minutes after intranasal administration (Gossen et al., 2012). In some individuals, OXY plasma levels are still elevated 150 minutes after intranasal administration (Gossen et al., 2012), suggesting that there may be inter-individual differences such as body weight, sex, or mental status that may have an impact on the effects and that there may also be differences in the effects between studies due to varying schedules of OXY administration or administration devices (Quintana et al., 2021). Other factors that may lead to inter-individual differences in OXY's effects are the context, including task difficulty and valence of the stimuli, or character traits such as attachment anxiety or symptoms of borderline personality disorder (Bartz et al., 2011). With regard to sex effects some studies found differences in brain activation between males and females, for example in the response to emotional stimuli (Bethlehem et al., 2013; Lieberz et al., 2020). Lieberz et al. (2020) have demonstrated that intranasal administration of OXY in females enhances the sensitivity of the amygdala and the striatum to social stimuli, while in males a reduction in amygdala activation in response to social signals was shown. In addition, differences in OXTR were found in the NAc due to sex and estrus cycle, which could explain differences in the inhibitory effect of OXY on alcohol reward (Peris et al., 2020). Hansson and Spanagel (2020) have added that although reduced endogenous OXY and increased OXTR binding sites suggest intranasal application of exogenous OXY, these differences are specific to males, while females show no differences in the OXY. Therefore, OXY may not be effective in the treatment of AUD symptoms in females according to Hansson and Spanagel (2020). Due to the observed sex differences some authors recommend to investigate OXY interventions for AUD in males only (Hansson & Spanagel, 2020), others recommend to consider sex differences in the effects in the analyses (Bethlehem et al., 2013). With the increasing application of intranasal OXY, the safety profile was analyzed (Cai et al., 2018; MacDonald et al., 2011). One review showed

that no subjective changes in individuals, no reliable side-effects, as the side-effects did not differ between OXY and PLC administration, and no adverse reactions were caused by the intranasal application of OXY at dosages between 18 and 40 I.U. OXY in short-term use (MacDonald et al., 2011). In a further review, the side-effects after long-term administration of intranasal OXY in dosages between 16 and 48 I.U. OXY per day over 5 to 12 weeks in individuals with autism spectrum disorder were investigated and also showed that OXY was well-tolerated and safe (Cai et al., 2018).

One of the first randomized, double-blind clinical pilot studies investigated whether OXY can reduce withdrawal symptoms and the amount of benzodiazepines (i.e., lorazepam) needed during inpatient alcohol withdrawal treatment in alcohol dependent patients (Pedersen et al., 2013). Eleven male and female patients were administered either 24 I.U. intranasal OXY or a matched PLC twice daily on three consecutive days and the amount of lorazepam needed, severity of withdrawal symptoms, alcohol craving and mood states were repeatedly measured. Compared to PLC, OXY reduced the amount of lorazepam taken and withdrawal symptoms during withdrawal, as well as alcohol craving and tension/anxiety on the mood scale on day 2, indicating potential beneficial effects of OXY on alcohol withdrawal in patients with alcohol dependence (Pedersen et al., 2013). Another double-blind crossover study aimed to examine the behavioral effects of OXY on social perception, cue-induced alcohol craving, and alcohol and food approach behavior (Mitchell et al., 2016). To this end, 32 non-treatment-seeking individuals with alcohol abuse were administered a single dose of 40 I.U. OXY and PLC one week apart before they participated in a social perception task, a cue-induced craving task consisting of an exposure to the favorite alcoholic beverage and assessment of alcohol craving using the Alcohol Urge Questionnaire, an approach avoidance task including alcohol and appetitive stimuli and general negative and positive stimuli, and completed a questionnaire measuring relationship attachment-associated anxiety and avoidance. It was shown that a single dose of OXY was well tolerated and reduced alcohol craving in individuals with higher attachment anxiety, but increased alcohol craving in individuals with low attachment anxiety, and that OXY reduced the approach bias to food stimuli, while OXY had no effect on social perception and approach bias to alcohol (Mitchell et al., 2016). In another clinical pilot study, neuronal cue-reactivity in heavy social drinkers was examined (Hansson et al., 2018). In this placebo-controlled crossover study, twelve non-treatment seeking heavy social drinkers were administered a single dose of 24 I.U. OXY and PLC intranasally and the

neuronal cue-reactivity during the presentation of alcohol cues in fMRI was assessed and compared between OXY and PLC. Compared to PLC, OXY reduced neuronal cue-reactivity in the insula, the cingulum, the hippocampal and para-hippocampal gyrus, the cuneus, parts of the frontal gyrus and visual and motor regions (Hansson et al., 2018). Additionally, following a translational approach, the study examined 27 post-mortem samples of human brains of alcohol-dependent and non-dependent individuals and analyzed OXTR expression. Similar to the findings in preclinical models (see section 1.3.1), analyses revealed increased OXTR expression and increased OXTR proteins in frontal regions, i.e. in the anterior cingulate cortex and dorsolateral prefrontal cortex, and striatal regions, i.e. ventral striatum and nucleus caudatus (Hansson et al., 2018). In the same sample as reported in Hansson et al. (2018), the functional connectivity of the NAc during the same alcohol cue-reactivity task (Bach et al., 2019) and the neural response during an emotion processing task (Bach et al., 2020a) were investigated. The administration of 24 I.U. OXY attenuated NAc connectivity during the processing of alcohol stimuli, but not during the processing of neutral stimuli during fMRI, while functional connectivity during the processing of alcohol stimuli was associated with alcohol craving measured by visual analog scales during fMRI (Bach et al., 2019). In addition, it was shown that 24 I.U. OXY reduced the response of the bilateral amygdala, parts of the frontal gyrus and the parietal lobe and that the attenuating effect of OXY on the bilateral amygdala was specific for negative facial expressions during the emotion processing task and was associated with lower craving and a reduction of heavy drinking days (Bach et al., 2020a). Another double-blind randomized controlled trial aimed to replicate the findings of Pedersen et al. (2013) in a larger sample of 40 alcohol dependent patients that underwent inpatient alcohol detoxification (Melby et al., 2019). Patients were administered either 24 I.U. OXY or PLC intranasally twice daily on three consecutive days while oxazepam dose, withdrawal symptoms, psychological distress and sleep duration were assessed. The total amount of oxazepam needed, severity of withdrawal symptoms, psychological distress and self-reported duration of sleep during inpatient detoxification did not differ between OXY and PLC treatment. Thus, the initial results of one of the first studies that administered OXY in alcohol dependent individuals could not be replicated by Melby et al. (2019). In another double-blind randomized controlled trial, the effect of intranasal self-administration of 8 I.U. OXY or PLC up to three times daily for four weeks on alcohol intake, self-reported alcohol craving, sleep, psychological distress and phosphatidylethanol blood

concentration as a biological marker of alcohol use, was investigated in 38 patients with alcohol dependence after completion of detoxification (Melby et al., 2021). Compared to the PLC group, the OXY treatment group showed no significant differences in alcohol use, days until relapse, proportion of individuals relapsing, alcohol craving, sleep, phosphatidylethanol blood concentration except a larger decrease of self-reported nervousness after OXY compared to PLC treatment (Melby et al., 2021). In a further exploratory analysis of one of the secondary outcomes from the randomized controlled trial by Melby and colleagues (2019), the processing of emotional stimuli was examined using image excerpts of the eye regions of faces showing emotional expressions (i.e., the *Reading the Mind in the Eyes Test*), after 24 I.U. OXY or PLC was administered intranasally twice daily for three consecutive days in 39 alcohol-dependent patients during inpatient detoxification (Melby et al., 2022). The results of this exploratory analysis indicated that OXY compared to PLC did not improve performance on the emotion processing task, but tended to improve performance on negative emotional stimuli, and the results suggest that OXY might be particularly effective in individuals who have consumed large amounts (i.e., consumption of ≥ 20 alcohol units per day on average) of alcohol (Melby et al., 2022). Another double-blind randomized controlled trial aimed to investigate the effect of OXY on cue-induced alcohol craving after exposure with the individual preferred alcoholic beverage, subjective aggression, task-induced intimate partner aggression using the *Taylor Aggression Paradigm* and salivary cortisol (Flanagan et al., 2022). For this purpose, 100 couples with AUD and co-occurring intimate partner aggression received either a single dose of 40 I.U. OXY or PLC. No significant group differences were found in this study. A single dose of OXY compared to PLC had no beneficial effects on cue-induced alcohol craving, subjective aggression, intimate partner aggression in the laboratory experiment and did not alter salivary cortisol, but was well-tolerated and safe (Flanagan et al., 2022). In two studies OXY treatment was investigated in individuals with comorbid AUD and post-traumatic stress disorder (Flanagan et al., 2019; Stauffer et al., 2019). In order to investigate whether OXY compared to PLC attenuates stress reactivity and alcohol craving in response to a stress-induction task, 67 male military veterans were administered either OXY or PLC intranasally in a double-blind randomized controlled trial (Flanagan et al., 2019). The participants received either 40 I.U. OXY or PLC intranasally before participating in a stress-induction task in the laboratory (i.e., the *Trier Social Stress Test*) and salivary cortisol and alcohol craving were assessed. The results revealed that OXY

compared to PLC led to a reduction in cortisol reactivity compared to PLC, particularly in individuals with high baseline cortisol levels, while alcohol craving in response to stress induction did not differ between the groups (Flanagan et al., 2019). Another double-blind, randomized controlled trial investigated whether intranasal OXY (20 I.U. and 40 I.U.) compared to PLC reduced cue-induced alcohol craving and heart rate in patients with co-occurring AUD and post-traumatic stress disorder (Stauffer et al., 2019). To this end, 56 patients and 45 controls were administered 20 I.U. and 40 I.U. OXY and a PLC intranasally each one-week apart and an alcohol cue exposure was performed 65 minutes after intranasal application, during which self-reported craving and heart rate were measured. It was demonstrated that alcohol cue-exposure induced alcohol craving and a heart rate increase, but neither the dose of 20 I.U. nor 40 I.U. OXY had an effect on self-reported alcohol craving or heart rate, when compared to PLC (Stauffer et al., 2019). A recent review, which included 17 randomized controlled trials investigating the effect of OXY in treating AUD and SUDs, reported that OXY reduced withdrawal symptoms, negative emotional states, (cue-induced) craving and substance use across AUD, opioid use disorder, cocaine and stimulants use disorder, cannabis use disorder and nicotine use disorder in about half of the trials analyzed, but in 16 of these trials an overall risk of bias was identified, so that the results should be considered preliminary (Mellentin et al., 2023). Although OXY has been shown to have promising effects on alcohol outcomes such as alcohol intake, alcohol craving and heavy drinking in rodent models and initial studies in humans have initially reflected promising effects, some more recent studies were not able to replicate the beneficial effects of OXY in AUD (Ryabinin & Zhang, 2022). Differences in study design or the short half-life of OXY may be some of the reasons why the effects are not replicable, although OXY may still have potential in the treatment of AUD, which is why methodologically well-structured and well-powered studies, including clearly defined and precise psychometric outcome measures and the consideration of covariates such as comorbidities or sex are needed to evaluate the potential of OXY in the treatment of AUD (Fischler et al., 2022; Mellentin et al., 2023; Ryabinin & Zhang, 2022).

1.4 Research Questions

Although many individuals suffer from AUD symptoms, only a few receive any therapeutic or pharmacological treatment (Koob, 2024). To close this treatment gap, novel, effective, and well-tolerated pharmacotherapeutic approaches are needed (Köhne et al., 2024; Nona et al., 2019; Walker & Lawrence, 2018). As outlined in section 1.2 and 1.3, preclinical and preliminary clinical evidence indicates potential therapeutic effects of OXY and CBD in the treatment of AUD symptoms. However, clinical studies are still required to investigate the effects of CBD in AUD (Kirkland et al., 2022; Turna et al., 2019). Regarding the effects of OXY in AUD, preclinical and some clinical studies show promising results in reducing alcohol use, craving, and withdrawal symptoms. However, other studies have failed to replicate these effects (Mellentin et al., 2023; Ryabinin & Zhang, 2022). Further research, including larger sample sizes and the consideration of both sexes, is needed to investigate its efficacy in AUD (Fischler et al., 2022; Mellentin et al., 2023; Ryabinin & Zhang, 2022).

This dissertation aims to investigate the potential of intranasal administration of 24 IU of OXY and 800mg of CBD, each compared to a PLC, on the neuronal response to alcohol-related stimuli (i.e., cue-reactivity) and negative emotional stimuli, as well as alcohol-related outcomes such as alcohol craving in individuals with AUD in two separate randomized controlled trials. The following research questions (RQ) will be addressed:

RQ 1: Does the administration of 800mg CBD reduce cue-reactivity to alcohol stimuli and alcohol craving in individuals with AUD and what are the implications of the effects of CBD for the treatment of AUD?

RQ 2: Does the administration of 24 IU OXY reduce the neural response of the amygdala during processing of negative emotional stimuli and alcohol craving in individuals with AUD and what are the implications of the effects of OXY for the treatment of AUD?

2 EMPIRICAL STUDIES

2.1 Empirical Study 1: Acute cannabidiol administration reduces alcohol craving and cue-induced nucleus accumbens activation in individuals with alcohol use disorder: the double-blind randomized controlled ICONIC trial

2.1.1 Abstract

Although alcohol use disorder (AUD) is highly prevalent, only a few medications are approved for its treatment leaving much room for improvement. Cannabidiol (CBD) might be a particularly promising candidate, with preclinical data suggesting that CBD is effective in targeting AUD symptoms and disease processes that drive alcohol use and relapse, due to its anti-craving, stress-reducing and anti-compulsive effects. Here we report data from the double-blind randomized controlled ICONIC trial that compared the effects of a single dose of 800mg cannabidiol against placebo (PLC) in N=28 individuals with AUD. Cue-induced nucleus accumbens (NAc) activation, alcohol craving during a combined stress- and alcohol cue-exposure session, as well as craving during an fMRI alcohol cue-reactivity task and CBD plasma levels served as outcomes. Individuals receiving CBD showed lower bilateral cue-induced NAc activation ($t_{left\_NAc(23)}$=4.906, p<.001, d=1.15; $t_{right\_NAc\,(23)}$=4.873, p<.001, d=1.13) and reported significantly lower alcohol craving after a combined stress- and alcohol cue-exposure session ($F_{group(1,26)}$=4.516, p=.043, eta^2=.15) and during the fMRI cue-reactivity task ($F_{group(1,24)}$=6.665, p=.015, eta^2=.23). CBD levels were significantly higher in the CBD group ($t_{(25)}$=3.808, p<.001, d=1.47) and showed a significant negative association with alcohol craving during the cue-exposure experiment (r=-.394, p_{FDR}=0.030) and during fMRI (r =-.389, p_{FDR}=0.030), and with left and right NAc activation (r_{left_NAc}=-.459, p_{FDR}=0.030; r_{right_NAc}=-.405, p_{FDR}=0.030). CBD's capacity to reduce stress- and cue-induced alcohol craving and to normalize NAc activation – a region critical to the pathophysiology of AUD – contribute to understanding the neurobiological basis of its clinical effects and support its potential as a treatment option for AUD.

2.1.2 Introduction

Alcohol use disorder (AUD) is one of the most prevalent and devastating diseases globally (World Health Organization, 2018). Currently, the majority of AUD patients relapse even if treated with pharmacological relapse-preventive medication, stressing

the need for developing new pharmacological treatments (Fleury et al., 2016). Cannabidiol (CBD) might be a promising candidate, due to its effects on substance use and craving. With regards to the specific effects of CBD on alcohol consumption, preclinical studies demonstrated that administration of CBD reduces the reinforcing properties of alcohol and decreases cue- and stress-induced alcohol self-administration (Gonzalez-Cuevas et al., 2018; Viudez-Martínez et al., 2018a; Viudez-Martínez et al., 2018b), as well as the frequency of impulsive choices (for review, see (Nona et al., 2019; Turna et al., 2019)). Further studies indicated that the effects of CBD on substance use are based on the ability of CBD to modulate the activation of dopaminergic brain circuits – including the ventral tegmental area (VTA) and nucleus accumbens (NAc) – that are closely linked to drug craving and drug seeking (Gonzalez-Cuevas et al., 2018; Hurd et al., 2019). The potential of CBD for the treatment of substance use disorders was supported by two randomized placebo-controlled trials (RCTs) in patients with opioid use disorder (OUD) that showed significant craving-reducing effects of CBD in dosages of 400 & 800mg daily, already a few hours after the first administration (Hurd et al., 2019; Hurd et al., 2015). In addition, an observational study in 120 alcohol and cannabis using adults showed that ad libitum use of CBD-dominant cannabis over 5 days resulted in lower reported drinking days and drinks per drinking day compared to THC-dominant or THC/CBD-balanced cannabis (Karoly et al., 2021). Another RCT in individuals with cannabis use disorder showed higher abstinence rates from cannabis in individuals receiving 400mg and 800mg oral CBD daily versus placebo, supporting CBD's potential for the treatment of substance use disorders (Freeman et al., 2020). For AUD however, clinical evidence is still lacking. Hence, we conducted the randomized, placebo-controlled ICONIC trial ("*Investigation of the effects of Cannabidiol ON cue-InduCed alcohol craving and nucleus accumbens activation*") to determine the effects of CBD on stress- and cue-induced alcohol craving and NAc activation in individuals with AUD. Effects of CBD on craving were examined using a validated experimental stress- and cue-exposure procedure (Bach et al., 2024; Kwako et al., 2015). The effect of CBD on alcohol cue-induced NAc activation was tested using a validated alcohol cue-reactivity functional magnetic resonance imaging (fMRI) paradigm (Bach et al., 2021a; Vollstadt-Klein et al., 2012). We focused on the NAc as region of interest, because it was identified as a key neurobiological substrate of the addiction circuit (Volkow et al., 2007; Volkow et al., 2010), and because neuroimaging studies demonstrated robust effects of alcohol cue presentation on NAc activation (Schacht et al.,

2013) and significant associations of NAc activation, alcohol craving (Schacht et al., 2013) and relapse risk in AUD (Bach et al., 2015). In addition, it was demonstrated, that higher activation in the ventral striatum is associated with the efficacy of naltrexone, supporting the potential of cue-induced brain response as a marker for individual efficacy of pharmacotherapeutic approaches (Bach et al., 2021b). The trial was designed to test the primary hypotheses that CBD reduces (i) cue-induced alcohol craving and (ii) NAc activation in individuals with AUD.

2.1.3 Methods

Study design and procedures

This double-blind RCT was conducted at the Central Institute of Mental Health in Mannheim, Germany. The trial was approved by the local ethics committee (2022-579-AF 5), preregistered (German clinical trials database: DRKS00029993) and conducted in accordance to the Declaration of Helsinki. Enrolled participants were randomized to one of two groups (CBD, PLC) and completed one test session, which was scheduled on one day. On this day, individuals received CBD or PLC 3 hours prior to blood sampling for determining CBD levels, which was followed by a sequential stress- and cue-exposure and an fMRI-based assessment of alcohol cue-induced NAc activation. The study was designed to detect at least medium effects (f >= .25) of CBD on the primary outcomes with a power of at least 80% (see **Supplements** for details).

Participant recruitment

Non-treatment seeking individuals with mild to severe AUD, between 18 and 60 years of age were recruited through online, social media and newsletter advertisements. Potential participants were screened in a brief telephone interview and those who met preliminary criteria were scheduled for an on-site visit, where written informed consent was obtained and individuals were screened to determine their study eligibility. Specifically, the Structured Clinical Interview for DSM-5 was used to confirm the diagnosis of an AUD and to rule out any other substance use disorder (except tobacco use disorder) and severe psychiatric conditions (see **Supplements** for detailed exclusion criteria). Enrolled participants were randomly assigned to receive 800 mg of CBD or matching placebo. The block randomization schedule was produced by the independent study pharmacy and blinding was maintained until the last participant was assessed.

Study drug

The oral CBD capsules (200 mg/capsule, consisting of >99.8% pure, synthetic CBD in a Hydroxypropylmethylcellulose capsule, THC content was < 0.1%) and the matched oral placebo capsules were provided by Endosane Pharmaceuticals (Berlin, Germany). Packaging, blinding and quality assurance were performed by the independent study pharmacy of Heidelberg university hospital (Heidelberg, Germany). The CBD formulation was favoured over other CBD products, because it does not contain ethanol (e.g. Epidiolex) and because regulatory agencies already approved its use in human trials. The dose was chosen in accordance to previous trials, which indicated a dose-response association with higher efficacy of a dosage of 800 mg CBD compared to lower dosages on drug craving (Hurd et al., 2019; Sholler et al., 2020; Turna et al., 2019). The dose corresponded to an average weight adjusted dose of 9.7 (range: 6.3-12.7) mg/kg CBD. The oral placebo capsules were identical in appearance, taste, and composition except for the active ingredient CBD. CBD or placebo (4 capsules) were administered once during the test session, 3 hours prior to the combined stress- and cue-exposure and the following fMRI. This schedule was chosen because previous work demonstrated peak plasma and brain concentrations of CBD 3–6 hours after oral administration (Calapai et al., 2020; Millar et al., 2018).

Procedure

The timeline and design of the experimental session are depicted in **Figure 2.1**. At the beginning of the session. All participants were instructed to remain abstinent for at least 24 hours before starting the experimental session. They were screened for alcohol intoxication by determining breath alcohol concentration and drug use by drug urine screening and withdrawn if they tested positive. Women were additionally screened for pregnancy using urine pregnancy tests. Alcohol craving was assessed using the Alcohol Urge Questionnaire (AUQ) (Bohn et al., 1995) and further questionnaires were administered to assess alcohol use prior to enrolment, AUD severity, nicotine use, stress, positive and negative affect, symptoms of depression and anxiety (see **Supplements** for details). Either CBD or PLC was administered at the beginning of the test session, followed by a rest period. After 170 minutes, venous blood was drawn for determination of CBD levels and a combined stress- and cue-exposure was conducted from minute 180 to 200 after medication administration using a combination of the Trier Social Stress Test (TSST) (Kirschbaum et al., 1993) and an alcohol cue-exposure in a bar laboratory setting, which has been established and validated in previous studies as an

experimental intervention for the induction of alcohol craving (Bach et al., 2024; Kwako et al., 2015) (for details see **Supplements**). Following the experimental craving induction, participants were transferred to the fMRI scanner where neural response to alcohol cues and craving were assessed, along with structural MRI (see **Supplements**). After that participants were debriefed on the TSST, i.e. that their performance during the task was not recorded and/or evaluated. In addition, well-being was assessed by the study personnel and any side effects were noted.

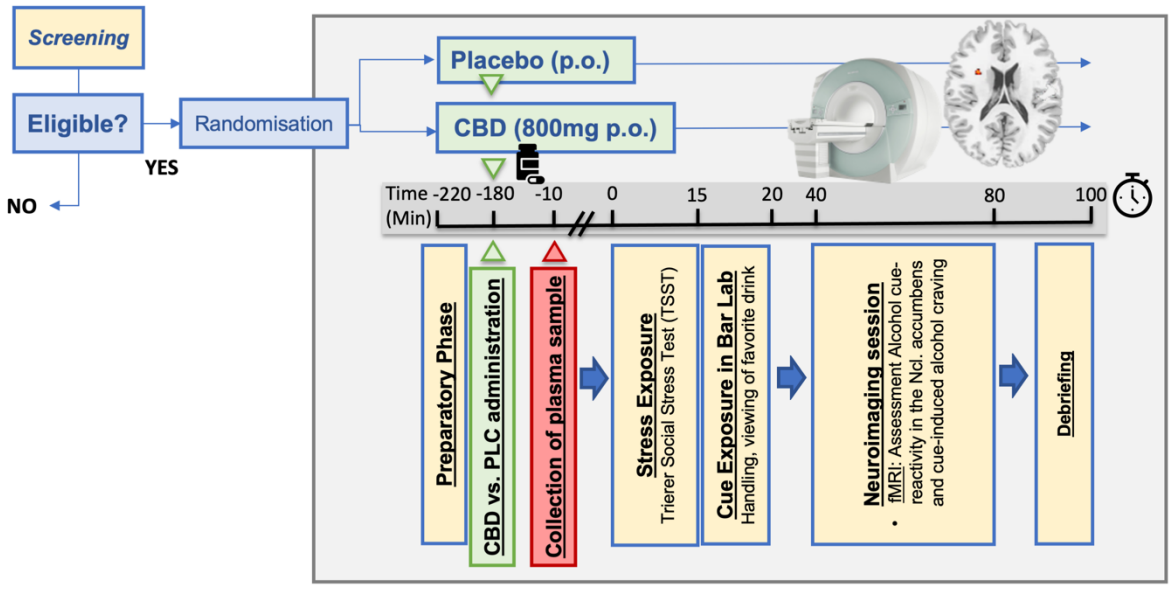

Figure 2.1. Schematic overview of the trial design. Either cannabidiol (CBD) or placebo (PLC) was administered after a preparatory phase at the beginning of the test session, followed by a rest period. 170 minutes after medication administration, venous blood was drawn for determination of CBD levels and a combined stress- and cue-exposure was conducted from minute 220 to 240 (i.e., 180 minutes after medication administration) using a combination of the Trier Social Stress Test (Kirschbaum et al., 1993) and an alcohol cue-exposure in a bar lab setting (for details see text). Directly afterwards, an alcohol cue-reactivity functional magnetic resonance imaging task was performed to investigate cue-induced brain activation and craving.

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Primary and secondary endpoints

Alcohol cue-induced brain response in the NAc, measured using the blood oxygenated level dependent (BOLD) response, during presentation of alcohol cues, was pre-registered as the primary outcome of the study. In addition, alcohol craving before and after the experimental combined stress- and cue-exposure, measured using the AUQ, and cue-induced alcohol craving during fMRI, measured using a visual analogue scale (0 – "no craving" to 100 – "very intense craving"), served as secondary study outcomes together with CBD plasma levels.

Data analysis

The primary analysis included all randomized patients with fMRI data (N=25). The primary endpoint was compared between both treatment arms using a t-test for independent samples (two-tailed), as implemented in the statistical parametric mapping software (SPM, Wellcome Department of Cognitive Neurology, London, UK) version 12 for Matlab (version 2016b, The MathWorks Inc., Natick, Massachusetts, USA), considering the first-level statistical maps that contrast activation during alcohol versus neutral blocks (contrast: "alcohol – neutral") as input. Significance was set to a cluster-level family-wise error rate correction (FWE) of $p<.05$, considering the right and left NAc as pre-specified regions of interest (ROI), defined using a ROI mask from the NeuroVault repository (http://neurovault.org/media/images/12980/MNI_res-epi_label-NAcc_mask.nii.gz, see **Supplementary Figure S2.1**). We performed additional sensitivity analyses considering days since last alcohol use as covariate in the model. To complement the analyses of averaged cue-induced brain activation, we also extracted block-wise activation values from the left and right NAc (see **Supplements** for details) and tested the main effect of treatment group (k=2 groups), time (i=12 alcohol blocks) and the interaction between treatment x time in the framework of a General Linear Model (GLM) in the IBM Statistical Package for the Social Sciences (SPSS) version 29.0. Betas of alcohol and neutral blocks were compared using t-tests for independent samples in SPSS to test specificity of the treatment effect. Alcohol craving was compared between treatment arms using GLMs in SPSS with treatment group (k=2 groups) and time (i=2 before/after combined stress- and cue-exposure) for the AUQ score and time (i=12 block-wise alcohol craving during fMRI) for alcohol craving during the fMRI session respectively. CBD levels were compared between treatment arms using t-test for independent samples. Associations between primary and secondary endpoints were explored using Pearson bivariate correlations in SPSS. Significance was set to

p<.05 and corrected for multiple comparisons using the false discovery rate correction procedure and bootstrapping, using the Bias corrected and accelerated (BCa) bootstrapping procedure. Even though both treatment groups did not differ on any sociodemographic variable and there was no evidence for a significant impact of these variables in CBD levels, we performed additional sensitivity analyses considering sex (male/female) and current smoking status (yes/no) as covariates in the models. The inclusion of smoking status as covariate essentially also captured the individuals reporting recent CBD or THC use.

2.1.4 Results

A total of 28 individuals with AUD were enrolled in the study of whom 25 provided fMRI data for analyses of the primary endpoint (n_{CBD_group}=12, n_{PLC_group}=13; see **Supplementary Figure S2.2**). For the secondary outcomes, data was available for all participants**.** Participants were on average 35.8 (*SD* = 12.1) years old, met an average of 5.4 (*SD* = 2.2) AUD criteria (18% mild, 46% moderate, 36% severe AUD) and drank on average of 46 gram alcohol per day with an average of 36% heavy drinking days during the 90 days prior to the assessment. Prior CBD use was reported by 40% (lifetime) and 11% (last 3 months), and prior THC use was reported by 89% (lifetime) and 18% (last 3 months) respectively, but none of the participants showed a positive drug urine screening at the time of assessment. There were no significant differences between the treatment groups on any sociodemographic or substance use variable, neither when considering the whole sample (*N*=28), nor for the groups contributing to the fMRI analyses (*N*=25)(see **Table 2.1** and **Supplementary Table S2.1**).

Table 2.1. Baseline data on demographic characteristics, alcohol use and severity measures for participants randomized to the cannabidiol and placebo treatment arms.

	1	**2**		
	CBD (n=14) [A]	**PLC (n=14)** [A]	**Statistics**	**Significance**
Demographical variables (self-reported)				
Sex[B] [male/female; number (%)]	11 (78%) / 3 (22%)	8 (57%) / 6 (43%)	Z = 1.47	*p* = .42
Age [years; mean (SD)]	37.29 (7.14)	34.36 (15.86)	*t*(18.06) = 0.63	*p* = .54
Race/ethnicity				
White [number (%)]	14 (100%)	14 (100%)	-	-
European ancestry [number (%)]	14 (100%)	14 (100%)	-	-
Substance use				
AUD criteria [sum; mean (SD)]	5.14 (2.21)	5.57 (2.24)	*t*(26) = 0.51	*p* = .62
Audit [total score; mean (SD)]	7.29 (1.68) 7	7.14 (2.18)	*t*(26) = 0.19	*p* = .85
ADS [total score; mean (SD)]	16.29 (7.60)	12.57 (4.80)	*t*(26) = -1.55	*p* = .13
Mean daily alcohol use last 90 days [gram/day; mean (SD)]	42.50 (35.04)	49.58 (29.57)	*t*(26) = 0.58	*p* = .57
Percent heavy drinking days last 90 days [mean (SD)]	.30 (.24)	.44 (.31)	*t*(26) = 1.32	*p* = .20
Days since last alcohol use [mean (SD)]	3.50 (4.18)	1-8, 2.71 (1.73)	*t*(26) = -0.65	*p* = .52
CBD use lifetime [yes/no; number (%)]	5 (36%) / 9 (64%)	6 (43%) / 8 (57%)	Z = 0.15	*p* = 1.00
CBD use last 30 days [yes/no; number (%)]	1 (7%) / 13 (93%)	2 (14%) / 12 (86%)	Z = 0.37	*p* = 1.00
THC use lifetime [yes/no; number (%)]	12 (86%) / 2 (14%)	13 (93%) / 1 (7%)	Z = 0.37	*p* = 1.00
THC use last 30 days [yes/no; number (%)]	4 (29%) / 10 (71%)	1 (7%) / 13 (93%)	Z = 2.19	*p* = .33
Current cigarette smoker [yes/no; number (%)]	3 (22%) / 11 (78%)	3 (22%) / 11 (78%)	-	-
Psychometric data				
AUQ at baseline (T0) [total score; mean (SD)]	15.36 (5.18)	15.93 (6.03)	*t*(26) = 0.27	*p* = .79
BDI [total score; mean (SD)]	13.21 (7.96)	18.71 (11.98)	*t*(26) = -1.43	*p* = .16
STAI trait [total score; mean (SD)]	43.14 (9.24)	50.57 (10.38)	*t*(26) = 2.0	*p* = .06

	1	2		
	CBD (n=14) [A]	**PLC (n=14)** [A]	**Statistics**	**Significance**
PANAS positive affect [total score; mean (SD)]	32.21 (7.57)	31.07 (5.41)	$t(26) = -0.46$	$p = .65$
PANAS negative affect [total score; mean (SD)]	25.43 (11.10)	26.00 (9.55)	$t(26) = -0.15$	$p = .89$
PASA [stress index score; mean (SD)]	-2.02 (1.03)	-1.79 (1.61)	$t(22^{B}) = -0.44$	$p = .67$
CBD level (ng/ml)	256.76 (242.36)	0.42 (0.28)	$t(13^{C}) = -3.96$	$p = .002$

AUD = Alcohol Use Disorder, AUDIT = Alcohol Use Disorders Identification Test, ADS = Alcohol Dependence Scale, AUQ = Alcohol Urge Questionnaire, BDI = Beck Depression Inventory, STAI = State Trait Anxiety Inventory, PANAS = Positive and Negative Affect Schedule, PASA = Primary Appraisal Secondary Appraisal; [A] remaining sample size for fMRI analysis is n=12 for the CBD and n=13 for the PLC group; [B] Gender and sex were recorded by self-report and were consistent with each other; [C] adjusted degrees of freedom according to standard procedures implemented in IBM SPSS version 29.0, due to unequal variances, indicated by positive Levene test.

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Cue-induced nucleus accumbens activation

Alcohol cues compared to neutral cues induced higher brain activation in several clusters, including parts of the occipital, temporal, parietal and frontal cortices, the orbitofrontal cortex, as well as the NAc, cuneus, middle and posterior cingulum, precuneus, hippocampus, and the cerebellum (see **Supplementary Table S2.2** and **Supplementary Figure S2.3**). Individuals receiving CBD showed lower brain activation in the bilateral NAc compared to those receiving placebo ($p_{FWE\ left\ NAc\ (small\ volume\ correction)}$=.001, $p_{FWE\ right\ NAc\ [small\ volume\ correction]}$=.002, see **Figure 2.2**). The effect remained significant, when considering days since last alcohol use as covariate ($p_{FWE\ left\ NAc\ [small\ volume\ correction]}$=.004, $p_{FWE\ right\ NAc\ [small\ volume\ correction]}$=.003). Analyses of the extracted mean NAc activation for the contrast "alcohol – neutral" showed large effects of the pharmacological intervention on left ($t_{(23)}$=4.906, p<.001, d=1.15) and right NAc activation ($t_{(23)}$=4.873, p<.001, d=1.13, see **Figure 2.2**). No effects of CBD on brain activation in other brain areas were observed, even when considering a lenient uncorrected whole-brain threshold of p<.001. Analyses of block-wise activation values for the twelve alcohol picture blocks corroborated the significant main effect of treatment group on cue-induced brain activation during alcohol cue-presentation blocks in the left and right NAc ($F_{left\ NAc(1,23)}$=3.573, p=.047, partial eta^2=0.12; $F_{right\ NAc(1,23)}$=4.862, p=.034, partial eta^2=0.18). The effects remained significant when considering gender and smoking status in the models (see **Supplementary Table S2.3**). In contrast, the analyses of block-wise activation values for the eight neutral picture blocks showed no significant main effect of group on either left or right NAc activity during neutral picture blocks ($F_{left\ NAc(1,23)}$=0.978, p=.333, partial eta^2=0.041; $F_{right\ NAc(1,23)}$=0.055, p=.816, partial eta^2=0.002), suggesting that the observed effects of CBD were specific for the presentation of salient alcohol cues. Additional analysis of betas support the specificity of CBD-effects in the left and right NAc for the presentation of alcohol cues ($t_{left\ NAc\ (23)}$=4.150, p<.001, mean difference score=2.150, d=1.661, $t_{right\ NAc\ (23)}$=4.281, p<.001, mean difference score=2.195, d=1.714), but not for neutral cues ($t_{left\ NAc\ (23)}$=-1.355, p=.188, mean difference score=-0.125, d=-.543, $t_{right\ NAc\ (23)}$=-0.279, p=.783, mean difference score=-0.023, d=-.112) (see **Figure 2.3**). There was no significant main effect of time or interaction between group and time on left and right NAc activation, neither for the presentation of alcohol pictures, nor for neutral picture blocks (p > .05).

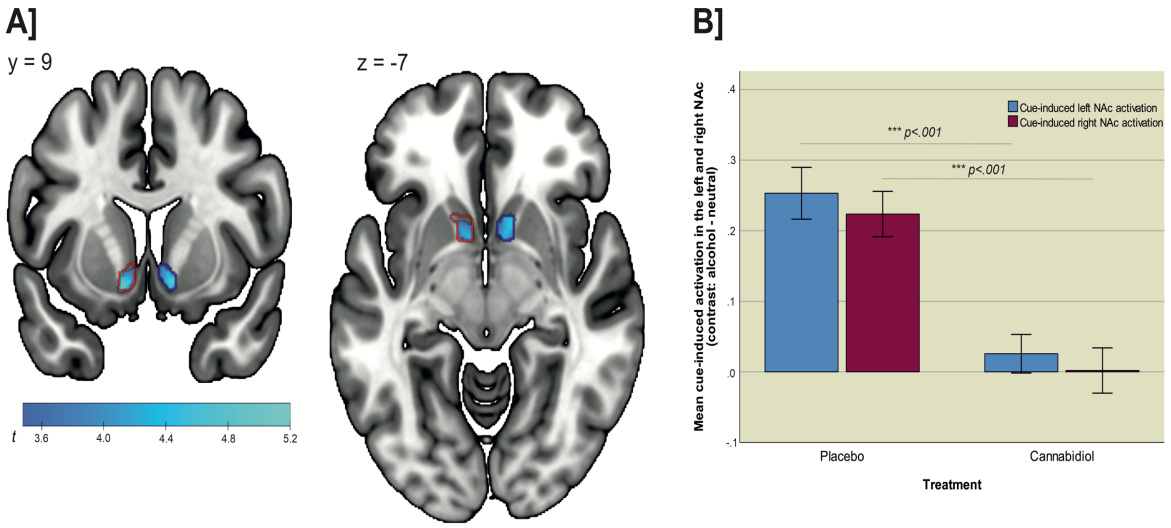

Figure 2.2. Depiction of **A]** the activation clusters in the left and right nucleus accumbens (NAc), which showed a significant treatment effect with significantly lower cue-induced activation in the CBD group (n=12) compared to the PLC group (n=13) (p_{FWE} < .05 small volume corrected for the NAc as pre-specified region of interest; boundaries of the region of interest mask are plotted as red and blue lines) and **B]** the significant difference in mean alcohol cue-induced brain activation in the left and right NAc between the CBD and PLC groups (as part of a post hoc test, mean activation for the contrast "alcohol – neutral" was extracted using the pre-specified region of interest mask for the NAc and the marsbar toolbox for Matlab).

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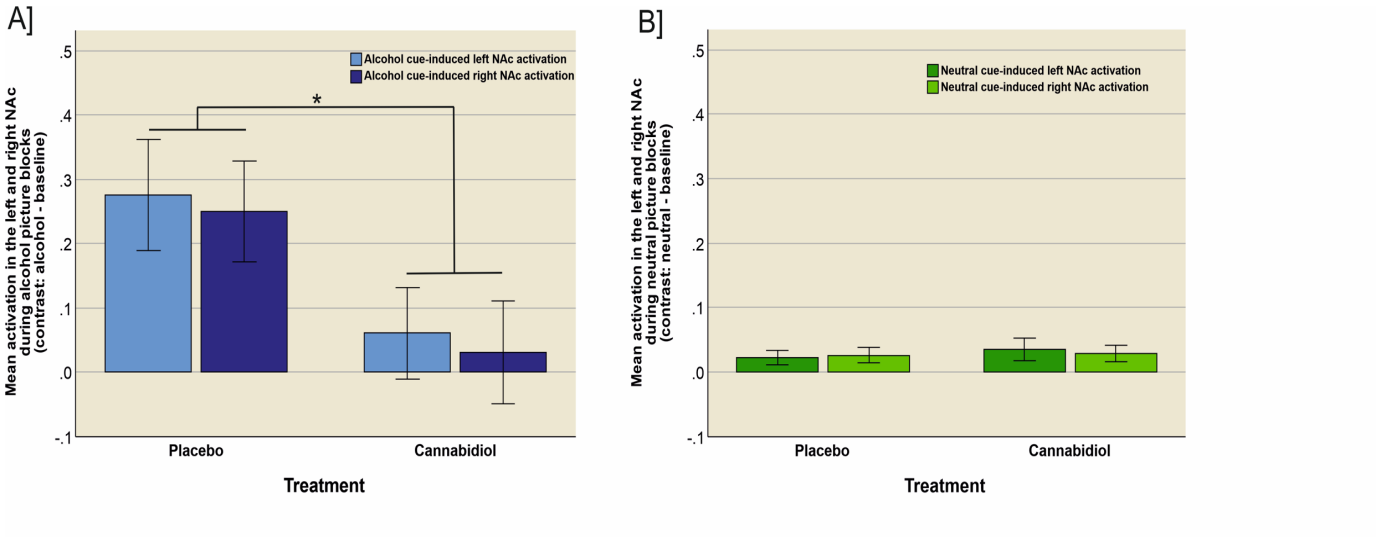

Figure 2.3. Depiction of the mean cue-induced activation in the left and right NAc during presentation of **A]** alcohol stimuli ($t_{left\ NAc\ (23)}$=4.150, p<.001, mean difference score=2.150, d=1.661, $t_{right\ NAc\ (23)}$=4.281, p<.001, mean difference score=2.195, d=1.714) and **B]** neutral stimuli ($t_{left\ NAc\ (23)}$=-1.355, p=.188, mean difference score=-0.12, d=-.543, $t_{right\ NAc\ (23)}$=-0.279, p=.783, mean difference score=-0.023, d=-.112) for cannabidiol compared to placebo treatment.

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Cue-induced alcohol craving

At baseline, there was no significant difference between the groups regarding their AUQ craving scores (see **Table 2.1**). AUQ scores significantly increased from baseline to after combined stress- and alcohol cue-exposure (mean difference score=9.893, $F_{(1,26)}$=21.798, p<.001, see **Figure 2.4**). There was also a significant main effect of treatment group and interaction between treatment and time on AUQ scores ($F_{group(1,26)}$=4.516, p=.043, eta^2=.15, mean difference score=5.607; $F_{group\ x\ time(1,26)}$=5.648, p=.025). Individuals receiving PLC reported significantly greater increase in alcohol craving from before to after the combined stress- and alcohol cue exposure (mean difference score=14.928) compared to participants receiving CBD (mean difference score=4.857). Cue-induced craving during the fMRI alcohol cue-exposure task was higher for alcohol compared to neutral blocks ($t_{(24)}$=4.017, p<.001, mean difference score=12.457, d=.803). There was also a significant main effect of treatment group on craving during alcohol picture blocks ($F_{group(1,24)}$=6.665, p=.015, eta^2=.23, mean difference score=22.028, see **Figure 2.4**). Individuals receiving PLC reported significantly higher alcohol craving during the cue-reactivity fMRI paradigm compared to participants receiving CBD. This effect remained significant when considering gender and smoking status in the models (see **Supplementary Table S2.3**). In contrast, there was no significant group effect on craving during neutral picture blocks ($F_{group(1,24)}$=4.218, p=.052, mean difference score=14.496).

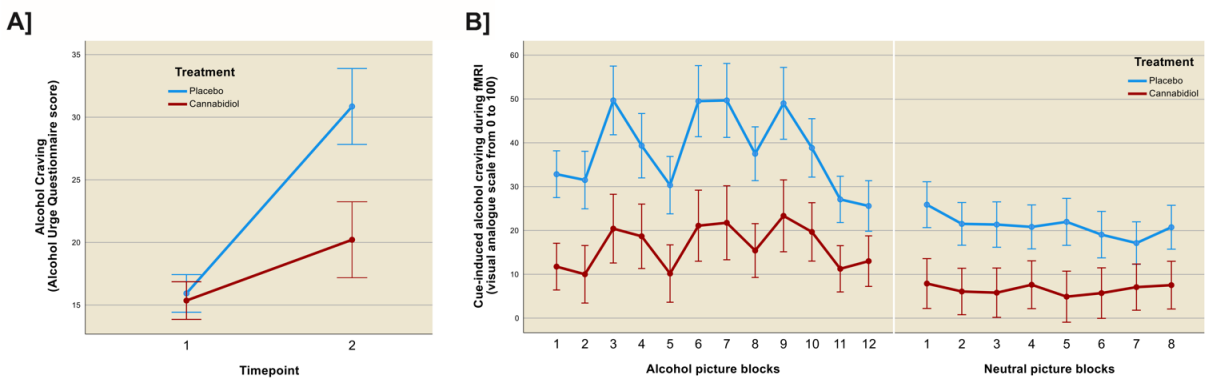

Figure 2.4. Depiction of the significant effects of cannabidiol **A]** on alcohol craving before (timepoint 1) and after (timepoint 2) the combined experimental stress- and alcohol cue exposure ($F_{treatment\_group(1,26)}$=4.516, p=.043, eta^2=.15) and **B]** on cue-induced alcohol craving during the fMRI cue-reactivity task ($F_{treatment\_group(1,24)}$=6.665, p=.015, eta^2=.23, n=12 alcohol picture blocks and $F_{treatment\_group(1,24)}$=4.218, p=.052, n=8 neutral picture blocks, errors bars represent to +/- 1 standard error).

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CBD plasma levels

Mean (M_{CBD} = 256.76 ng/ml, M_{PLC} = 0.41 ng/ml) and median (Med_{CBD} = 158.50 ng/ml, Med_{PLC} = 0.30 ng/ml) CBD plasma levels were significantly higher in the CBD group compared to the PLC group ($t_{(25)}$=3.808, p<.001; U=182, p <.001). Still, we observed substantial variance in CBD plasma levels (SD=215.42 ng/ml), which mirrored findings of previous studies (30). We explored whether age, gender, BMI, smoking status or recent CBD use (during the last 3 months) predicted CBD plasma levels, but neither of these factors showed a significant association with plasma levels (p>.05).

Testing associations between CBD levels and response on the primary and secondary outcomes, we found that CBD levels were negatively correlated with cue-induced left and right NAc activity during fMRI ($r_{left\ NAc}$=-.459, Bias corrected and accelerated 95% confidence interval [95% BCa CI]=-.704 to -.220, p=0.012, p_{FDR}=0.030; $r_{right\ NAc}$=-.405, 95% BCa CI=-.642 to -.161, p=0.025, p_{FDR}=0.030, see **Figure 2.5**). Further, we observed a significant negative correlation between CBD levels and AUQ craving scores after the combined stress- and alcohol cue exposure session (r_{AUQ}=-.394, 95% BCa CI=-.655 to -.025, p=0.028, p_{FDR}=0.030, see **Figure 2.5**) and with alcohol cue-induced craving during the fMRI alcohol cue-reactivity task ($r_{craving\ fMRI}$=-.389, 95% BCa CI=-.624 to -.075, p=0.030, p_{FDR}=0.030, see **Figure 2.5**). These findings suggest a plasma level-response association for the primary and secondary outcomes.

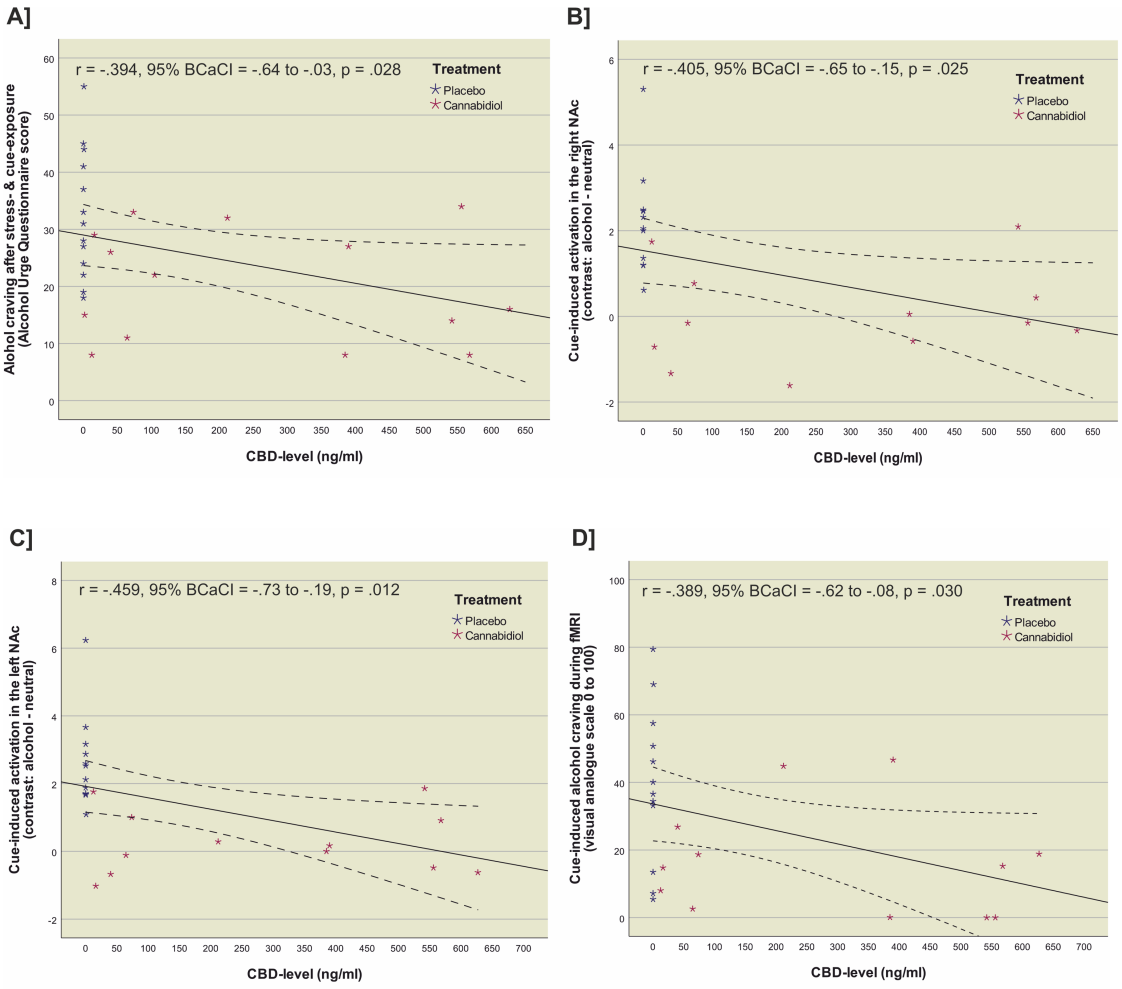

Figure 2.5. Depiction of the significant negative correlation between CBD plasma levels and **A]** alcohol craving after the combined stress- and alcohol cue-exposure experiment (r_{AUQ}=-.394, Bias corrected and accelerated 95% confidence interval [95% BCa CI] =-.655 to -.025, p=0.028, p_{FDR}=0.030), **B]** alcohol cue-induced brain activation in the right nucleus accumbens (NAc) ($r_{right\ NAc}$=-.405, 95% BCa CI=-.642 to -.161, p=0.025, p_{FDR}=0.030) and **C]** left NAc ($r_{left\ NAc}$=-.459, 95% BCa CI=-.704 to -.220, p=0.012, p_{FDR}=0.030), and **D]** alcohol craving during the functional magnetic resonance imaging alcohol cue-reactivity task ($r_{craving\ fMRI}$=-.389, 95% BCa CI=-.624 to -.075, p=0.030, p_{FDR}=0.030).

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Safety

No adverse events or serious adverse events were reported by the participants of the study during the test session.

2.1.5 Discussion

The results of this randomized double-blind placebo-controlled trial show that administration of 800 mg CBD reduces alcohol cue-induced bilateral NAc activity and alcohol craving in individuals with AUD. These results suggest that CBD can modulate central neurobiological mechanisms underlying alcohol craving and alcohol use and alleviate disease symptoms, such as craving. These effects were observed 3 hours after acute administration of CBD, indicating rapid onset of CBD's actions when peak plasma levels are expected (Millar et al., 2018). These findings are in line with preclinical data (Nona et al., 2019) and also with a previous RCT in individuals with OUD that reported that effects of CBD on drug cue-induced craving can be observed a few hours after administration of CBD (Hurd et al., 2019). Effects of CBD on alcohol use and relapse-like behavior in animals have been reported by several preclinical studies with body-weight adapted doses that fall in the range of the here investigated dose of 800mg CBD (Nona et al., 2019; Turna et al., 2019). For the first time, the current RCT provides evidence for significant effects of CBD on neurobiological disease mechanisms and symptoms in AUD. Effects of CBD on gene expression in the NAc were indicated by preclinical studies (Viudez-Martínez et al., 2018a; Viudez-Martínez et al., 2018b), providing the basis for our focus on the NAc as region of interest. In addition, previous work showed close associations between NAc activity and AUD symptoms, such as alcohol craving (Kühn & Gallinat, 2011; Schacht et al., 2013). Further, our own group and others have repeatedly shown that the response of cue-induced NAc activation to pharmacological intervention is a predictor for the clinical efficacy of a drug for treating AUD (Bach et al., 2020b; Schacht et al., 2017), stressing its relevance in investigation novel pharmacological interventions in AUD. Thus, the observed effects of CBD on cue-induced NAc activation, which was specific for the presentation of alcohol cues, indicate CBD's potential to target central neurobiological disease mechanisms in AUD. Our findings are in line with previous RCTs in individuals at high risk for psychosis that showed significant effects of a single dose of 600mg CBD on striatum activation during an fMRI verbal memory task (Bhattacharyya et al., 2018) and processing of fearful faces during fMRI (Davies et al., 2020). Further studies in individuals with psychosis

also indicated that a single dose of 600mg CBD, added to antipsychotic therapy, can significantly modulate mediotemporal-striatal connectivity (Annibale et al., 2021). The specificity of the effects of CBD and a plasma level-response association are supported by the significant correlation between CBD plasma levels and NAc activation. This is also in line with preclinical data indicating dose-dependent effects of CBD on alcohol use in animals (Nona et al., 2019) and evidence suggesting that plasma and brain levels of CBD are linked with about four hours to maximum brain concentrations after oral administration (Calapai et al., 2020). This corresponds closely to the schedule of the current RCT in which effects of CBD on NAc activation were investigated about four hours after its administration.

CBD also showed significant effects on alcohol craving during an experimental combined stress- and cue-induction session and also during a following fMRI cue-reactivity task. The negative correlation between CBD levels and alcohol craving support the specificity and plasma level-dependency of the observed effects. CBD's craving-reducing effects were specific to alcohol cue-induced craving, suggesting that CBD – via its effects on NAc activation – might attenuate alcohol-specific motivational salience in AUD and not appetitive behavior per se, leading to lower cue-induced "wanting" or craving (Robinson & Berridge, 1993). This idea is in line with the finding that CBD attenuates attentional bias to cigarette cues in in tobacco smokers, suggesting that CBD impacts on attentional salience of drug cues (Hindocha et al., 2018). It also harmonizes with studies in individuals with psychosis, which showed that CBD attenuated the activation of salience networks during an fMRI monetary incentive delay task (Gunasekera et al., 2023; Wilson et al., 2019). CBD's effects on craving appear clinically meaningful, as alcohol craving is a core symptom of AUD (Gauld et al., 2023) and potential marker for predicting the transition from moderate to severe AUD (Miller et al., 2023), emphasizing its role in AUD. Thus, pharmacotherapies targeting craving-related pathologies in AUD might be effective in reducing symptom burden and progression of the disease.

Limitations

Several aspects of this RCT should be considered for the interpretation of the results. The RCT was designed to provide first proof of CBD's effects in AUD and thus investigated the effects of an acute single administration of CBD, as previous RCTs in other substance use disorders (Hurd et al., 2019) and preclinical data indicated effects of CBD – if any –already after the first administration (Nona et al., 2019). Presented

results can thus not answer the question, whether CBD's effect in AUD are robust over time, but preclinical (Nona et al., 2019) and clinical data in other substance use disorders (Freeman et al., 2020; Hurd et al., 2019) indicate that effects are not transient and persist even after CBD is discontinued, suggesting that the here observed effects of CBD might translate to continued clinical effects in AUD. Higher activation during the presentation of the alcohol cues compared to neutral stimuli during the fMRI paradigm was evident in multiple brain regions, however CBD reduced alcohol cue-induced activity only in the NAc. This could be due to the small sample size. Besides that, the number of tests was also reduced to prevent from effects due to multiple testing. Both study groups were exposed to the same validated combined stress- and alcohol cue-exposure paradigm (Bach et al., 2024; Kwako et al., 2015). Thus, the effects of the intervention on the investigated outcomes are comparable between both study groups. However, it needs to be considered that stress- and cue-exposure prior to the fMRI session mostly likely influenced outcome measures collected during fMRI. The AUQ score after the stress- and cue-exposure session showed a significant difference between both study groups and we could not determine whether craving returned to baseline prior to the following fMRI cue-reactivity task. Thus, the here observed effects of CBD on neural cue-reactivity and craving during fMRI cannot be fully separated from effects on the prior stress- and cue-exposure, i.e. the effects of CBD on cue-reactivity and cue-induced craving during the fMRI could be partially due to its effects on prior stress- or cue-exposure or a combination of both. Hence, presented results cannot be generalized to cue-induced nucleus accumbens activation without prior stress- and cue-exposure. Still, results seem meaningful as they inform about CBD's effects on stress- and cue-reactivity in AUD and outcomes are comparable between both study arms as procedures were the same. We observed substantial inter-participant variability in CBD plasma levels, despite similar weight-adjusted doses across participants, which were not explained by either sex, age, BMI, smoking status or recent THC/CBD use, potentially owing to the complex metabolism and high lipophilicity of CBD, which was reported by preclinical studies (Calapai et al., 2020; Millar et al., 2018). The here observed plasma level-response association for the primary and secondary outcomes of the study suggest that individuals might respond to the same CBD dose, highlighting the importance to consider CBD plasma levels and its determinants, when investigating CBD's effects. The sample size of the presented RCT mirrored the sample sizes of previous RCTs (Freeman et al., 2020; Hurd et al., 2019) and was based on a-priori

calculations for the primary outcome, assuming that only at least medium effects of CBD on the primary outcome are clinically meaningful and warrant further larger confirmatory trials. These trials should also systematically investigate effects of sex and determinants of individuals' plasma CBD levels.

The current study provides evidence for the effects of CBD in non-treatment seeking individuals with AUD. However, results might not generalize to treatment-seeking individuals with severe AUD. Further, the sample included predominantly white individuals with European ancestry, reflecting the national and European health care context and limiting heterogeneity. Still, results might not generalize to other populations, demanding for subsequent studies in these populations.

2.1.6 Conclusion

In summary, the observed potential of CBD to reduce cue-induced NAc activity and alcohol craving, together with its good safety profile, support the potential of CBD to treat individuals with AUD. New pharmacological treatment options that target central neurobiological disease mechanisms and core symptoms of AUD, such as craving, could complement existing treatment options and reduce relapse risk and the enormous disease burden inflicted by AUD.

2.1.7 Supplements

Supplementary Methods

Sample Size Estimation

The study was designed to detect at least medium effects (f >= .25) of CBD on the primary outcomes with a power of at least 80%. This decision was taken, because the probability that smaller effects translate to clinically meaningful effects seems low. A priori sample sizes estimation indicated that *n* = 13 patients per group are needed to yield a power of at least 80% for a repeated measures analysis of variance with twelve repeated assessments testing the main effect of treatment group (f >= .25, α = 0.05, two-sided, correlation among repeated measures = .11, based on prior data (Bach et al., 2024). Even though this number was not fully met for the imaging analyses (25 out of 28), power estimations still indicated a power of 81% to detecting at least medium effects (f >= .25, α = 0.05, two-sided, correlation among repeated measures = .10, based on current data).

Inclusion and Exclusion criteria

Participants were excluded when they tested positive for (i) alcohol (breath alcohol concentration [BrAC] > 0.0‰, alcohol consumption was not allowed in the last 24 hours before starting the test session) or (ii) illegal drugs (nal von minden - Drug-Screen Multi 5B test) or (iii) they presented withdrawal symptoms (Clinical Institute Withdrawal Assessment Scale > 3 points), or (iv) when DSM-5 interview indicated presence of a psychotic disorder, a bipolar disorder, or severe depressive symptoms with acute suicidality, or when they reported (v) pregnancy, lactation or breastfeeding, or (vi) any severe somatic comorbidities (e.g. liver cirrhosis or severely impaired renal function, severe heart insufficiency, pre-existing epilepsy), (vii) when contraindication for functional magnetic resonance imaging (e.g. metal implants) existed, or (viii) when participants reported hypersensitivity to Cannabidiol or to any excipient present in the pharmaceutical form of the investigational medicinal product.

Psychometric assessments and questionnaires

At the beginning of the testing session, participants were screened for excessive drinking using the Alcohol Use Disorders Identification Test (AUDIT) (Saunders et al., 1993), severity of alcohol dependence was assessed using the Alcohol Dependence Scale (ADS) (Kivlahan et al., 1989), subjective stress was assessed using the Primary and Secondary Appraisal Scale (PASA) (Gaab, 2009), positive and negative affect and depressive symptoms were assessed using the Positive and Negative Affect Schedule

(PANAS) (Thompson, 2007) and the Beck Depression Inventory (BDI-II) (Hautzinger, 2009), alcohol use during the 90 days prior to enrolment was assessed using the Form-90 semi-structured interview (Miller, 1996), anxiety was assessed using the State Trait Anxiety Inventory (STAI) (Spielberger, 1983) , and nicotine use and nicotine dependence severity were assessed using the Fagerström Test for Nicotine Dependence (FTND) (Heatherton et al., 1991).

Combined stress- and alcohol cue-exposure session

The combined stress- and alcohol cue-exposure session was a combination of the Trier Social Stress Test (TSST) (Kirschbaum et al., 1993) and an alcohol cue-exposure, which has been established and validated in previous studies (Bach et al., 2024; Kwako et al., 2015). The TSST is an established procedure for induction of psychosocial stress. The stressor includes a social evaluative part (public speaking in front of a panel) and a performance task (serial subtraction). Participants were informed that they will soon have an interview for their "dream job". They had five minutes to prepare a five-minute presentation. After this, participants entered a room, where a panel, consisting of two interviewers (male and female) that were unfamiliar to the participant, sat a table. These interviewers instructed the participant to give their presentation. During the presentation, the interviewers remained neutral, took notes and asked the participant to continue their presentation or give more information if they stopped for more than 20 seconds. After these five minutes, participants were instructed by the panel to perform a serial subtraction task for five minutes and received feedback on errors and were repeatedly told to perform subtractions quicker, even if performing well. Directly afterwards, participants entered a bar environment (Bar Lab), consisting of a mock-up bar, chairs and a bottle of their favorite drink positioned at the bar counter. In the Bar Lab, participants were exposed to their preferred drink. They were instructed to hold the drink in their hand for three to five minutes and smell it and swirl the glass, but without drinking any of it. Participants were alone in the Bar Lab, but were observed by clinical staff via a room monitor to ensure they were not drinking. The duration of the procedure was 20 minutes (15 minutes stress-exposure and 5 minutes alcohol cue-exposure) and both groups underwent the same procedure to sensitize cue-reactivity before the fMRI examination.

Alcohol Cue-Reactivity Paradigm

Functional and structural MRI data was collected using a 3 Tesla SIEMENS Magnetom PRISMA^{FIT} MRI scanner (Siemens Healthcare, Erlangen, Germany). During the 12-

minute alcohol cue reactivity paradigm (12), 840 T2*-weighted images (echo planar imaging sequence) were captured for each participant using the standardized image parameters (TR = 0.89 s, TE = 25 ms, flip angle = 80°, 42 slices, slice thickness = 2 mm, 1-mm gap, voxel dimensions 3 x 3 x 3 mm3, FOV = 192 x 192 mm2, 64 x 64 in-plane resolution). During the fMRI examination twelve blocks of alcohol-associated images and nine blocks of neutral images were presented (5 images per block) in a pseudo-randomized order using a screen and an MRI-compatible mirror. Each image was displayed for four seconds. Between each block, participants were asked to rate their current alcohol craving on a visual analog scale (VAS) ranging from 0 (no craving at all) to 100 (very intense craving). Image presentation and craving data collection were performed using Presentation® software (version 16.0, Neurobehavioral Systems Inc., Albany, CA, USA). The entire alcohol stimulus reactivity paradigm consists of 20 image blocks and takes twelve minutes to complete.

Processing and statistical modelling of functional MRI data

In order to reduce artefacts due to magnetic saturation effects, the first five scans were removed from subsequent processing. The remaining fMRI data was pre-processed using the statistical parametric mapping software (SPM, Wellcome Department of Cognitive Neurology, London, UK) version 12 and Matlab version 2016b (The MathWorks Inc., Natick, Massachusetts). A phase map correction was applied, in order to correct for any geometric distortions, using a voxel displacement map that was computed from a grey field mapping sequence using the VDM utility in SPM12. All images were spatially realigned, corrected for micro-movements in the scanner and normalized to a standard MNI [Montreal Neurological Institute, Quebec, Canada] EPI template. Subsequently, all data were smoothed using an isotropic Gaussian kernel for group analysis [8 mm Full Width at Half Maximum]). First level statistics were computed for each participant, modelling the different experimental conditions (alcohol, neutral, rating phase) in a general linear model using the FAST method for temporal autocorrelation modelling (Olszowy et al., 2019), considering craving ratings and movements parameters (n=6) as nuisance variables. Resulting contrast images (“alcohol – neutral”) were imputed in second-level analyses SPM12. In addition, following the approach established in previous studies (Bach et al., 2024; Gerhardt et al., 2023), we computed additional first level statistics, modelling each picture block of the fMRI cue-reactivity task separately (n=12 alcohol blocks, n=8 neutral blocks), in order to investigate block-wise brain activation. To this end, we used a custom toolbox to extract parameter estimates

from individual beta maps for each stimulus block using the left and right nucleus accumbens mask from the Neurovault repository (http://neurovault.org/media/images/12980/MNI_res-epi_label-NAcc_mask.nii.gz) and exported these values into the IBM Statistical Package for the Social Sciences (SPSS) version 29.0 for further analyses.

Blood samples

Venous blood (plasma) was drawn 210 minutes after medication intake. Blood samples for analyses of cannabidiol levels were transported on ice and immediately transferred to the local biobank, where samples were centrifuged at 4000 rounds per minute (rpm) and plasma aliquots were stored at – 80°C and sent via express transport service in special cooling container for batch-analyses in a specialized laboratory (MVZ Labor Dessau GmbH, Dessau, Germany) to reduce inter-assay variation.

Chemical and reagents

Acetonitrile (ULC/MS-CC/SFC), methanol (ULC/MS-CC/SFC) and water (ULC/MS-CC/SFC) was purchased from Biosolve BV (Dieuze, France), ammonia solution (32%) and hydrochloric acid (37%) was from Merck KGaA (Darmstadt, Germany), formic acid (≥ 99.9%) from VWR (Leuven, Belgium), ethylene glycol (ROTIPURAN ≥ 99.5%) from Carl Roth (Karlsruhe, Germany), PBS tablets pH 7.2 (for 1 L) from AppliChem (Darmstadt, Germany). The serum quality control samples Medidrug® DOA-I S low and DOA-I S high were from Medichem (Steinenbronn, Germany) and a whole blood control sample STUP 07/20-B WH was from ACQ Science (Rottenburg-Hailfingen, Germany). The certified reference material cannabidiol (CBD) and the corresponding deuterated internal standard CBD-d3 was obtained from LGC Standards GmbH (Wesel, Germany).

Blood sample preparation

To 20 µL of plasma or control samples 200 µL MeOH/ACN (50/50, v/v) containing 2 ng/mL CBD-d3 was added. The mixture was vortexed and centrifuged at 13000 rpm for 5 min. Subsequently 55 µL of the supernatant was transferred to a 96-well plate and concentrated into 10 µL ethylene glycol at 60 °C in a vacuum evaporation centrifuge. The residue was mixed with 15 µL of mobile phase A (20 mM ammonium formate, 0.1% formic acid) and 2 µL was injected into the UPLC system.

UPLC-MS/MS analysis

CBD was quantified using an DIN EN ISO 17025 accredited routine UPLC-MS/MS method. Data were acquired with a Waters® Acquity® UPLC® connected to a Xevo®

TQ-XS detector with an UniSpray™ ion source (Waters®, Eschborn). The detector operated in the positive ionization mode. Chromatographic separation was performed at 60 °C on a Waters 2.1 mm x 150 mm, 1.7 µm, BEH Phenyl column with pre-filter. Mobile phase A consisted of 20 mM ammonium formate plus 0.1% formic acid (pH 3.0) while mobile phase B was 0.1% formic acid in methanol. Gradient separation was performed within 11 min at a flow rate of 0.3 mL/min. The gradient program initiated with 10% mobile phase B, increased to 100% at 10 minutes and remained for 0.5 minutes before re-equilibration. During data acquisition, 0.0037% HCl was infused post-column with a flow rate of 5 µL/min to enhance ionization. The autosampler temperature was maintained at 8 °C.

Compound-specific transitions monitored

	Retention time [min]	Parent-ions [m/z]	Daughter-ions [m/z]
CBD-d3	9.25	318.1	196.1, 262.1
CBD	9.25	315.1	193.1, 123.1, 259.1

Quantification was based on the response ratio of the target ion and the corresponding deuterated internal standard. Ten concentrations for CBD calibrators were prepared in EDTA whole blood ranging from 0.5 ng/mL to 120 ng/mL. The coefficient of determination (r2) was ≥ 0.99. Samples with concentrations exceeding 120 ng/mL were diluted 1:10 with PBS and re-analysed.

The method was validated following the GTFCh validation guidelines for quality assurance in forensic-toxicological analysis. Parameters evaluated include selectivity, linearity, limit of detection and quantification, precision, accuracy, matrix effect and stability. Full validation was performed using EDTA whole blood and the method was also cross-validated for plasma. Limit of detection (LOD) and limit of quantification (LOQ) were 0.3 ng/mL and 0.8 ng/mL, respectively.

Supplementary Tables

Supplementary Table S 2.1. Baseline data on demographic characteristics, alcohol use and severity measures for participants randomized to the cannabidiol and placebo treatment arms with available neuroimaging data (N=25).

	1	**2**		
	CBD (n=12)	**PLC (n=13)**	**Statistics**	**Significance**
Demographical variables (self-reported)				
Sex[A] [male/female; number (%)]	9 (75%)/ 3 (25%)	8 (62%)/ 5 (38%)	Z = 0.52	*p* = .67
Age [years; mean (SD)]	36.75 (7.38)	32.85 (15.43)	*t*(17.51) = 0.82	*p* = .43
Race/ethnicity				
White [number (%)]	12 (100%)	13 (100%)	-	-
European ancestry [number (%)]	12 (100%)	13 (100%)	-	-
Substance use				
AUD criteria [sum; mean (SD)]	4.75 (1.87)	5.38 (2.22)	*t*(23) = 0.77	*p* = .45
Audit [total score; mean (SD)]	7.33 (1.61)	7.00 (2.20)	*t*(23) = 0.43	*p* = .67
ADS [total score; mean (SD)]	15.33 (5.93)	12.38 (4.94)	*t*(23) = 1.36	*p* = .19
Mean daily alcohol use last 90 days [gram/day; mean (SD)]	37.06 (15.71)	48.39 (30.43)	*t*(23) = 1.15	*p* = .26
Percent heavy drinking days last 90 days [mean (SD)]	.27 (.14)	.41 (.29)	*t*(23) = 1.48	*p* = .15
Days since last alcohol use (mean (SD)]	3.75 (4.48)	2.77 (1.79)	*t*(23) = -0.73	*p* = .47
CBD use lifetime [yes/no; number (%)]	4 (33%)/ 8 (67%)	6 (46%)/ 7 (54%)	Z = 0.43	*p* = .69
CBD use last 30 days [yes/no; number (%)]	1 (8%)/ 11 (92%)	2 (15%)/ 11 (85%)	Z = 0.29	*p* = 1.00
THC use lifetime [yes/no; number (%)]	10 (83%)/ 2 (17%)	12 (92%)/ 1 (8%)	Z = 0.48	*p* = .59
THC use last 30 days [yes/no; number (%)]	3 (25%)/ 9 (75%)	1 (8%)/ 12 (92%)	Z = 1.39	*p* = .32
Current cigarette smoker [yes/no; number (%)]	3 (25%)/ 9 (75%)	3 (23%)/ 10 (77%)	Z = 0.01	*p* = 1.00
Psychometric data[a]				
AUQ at baseline (T0) [total score; mean (SD)]	15.58 (5.45)	15.69 (6.21)	*t*(23) = 0.46	*p* = .96

	1	2		
	CBD (n=12)	**PLC (n=13)**	**Statistics**	**Significance**
BDI [total score; mean (SD)]	13.58 (8.21)	18.08 (12.22)	$t(23) = 1.07$	$p = .30$
STAI trait [total score; mean (SD)]	43.42 (8.48)	49.85 (10.43)	$t(23) = 1.68$	$p = .11$
PANAS positive affect [total score; mean (SD)]	32.17 (7.74)	25.85 (9.92)	$t(23) = 0.21$	$p = .84$
PANAS negative affect [total score; mean (SD)]	25.08 (10.98)	25.85 (9.92)	$t(23) = 0.18$	$p = .86$
PASA [stress index score; mean (SD)]	-2.18 (.99)	-1.94 (1.57)	$t(23^B) = 0.44$	$p = .66$
CBD level (ng/ml)	290.63 (245.31)	0.36(0.20)	$t(11^B) = -4.10$	$p = .002$

AUD = Alcohol Use Disorder, AUDIT = Alcohol Use Disorders Identification Test, ADS = Alcohol Dependence Scale, AUQ = Alcohol Urge Questionnaire, BDI = Beck Depression Inventory, STAI = State Trait Anxiety Inventory, PANAS = Positive and Negative Affect Schedule, PASA = Primary Appraisal Secondary Appraisal;

[A] Gender and sex were recorded by self-report and were consistent with each other;

[B]adjusted degrees of freedom according to standard procedures implemented in IBM SPSS version 29.0, due to unequal variances, indicated by positive Levene test.

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Supplementary Table S 2.2. List of brain areas that show a significant higher activation during the presentation of the alcohol cues compared to the presentation of neutral stimuli during the alcohol cue-reactivity functional magnetic resonance imaging paradigm (One sample t-test in SPM12 with sex (male/female), current smoking status (yes/no) and days since last alcohol use as covariates, considering all N = 25 participants contrast "alcohol – neutral", all results cluster-level whole-brain corrected at p_{FWE} < .05 and p_{FWE} < .05 small volume corrected for the nucleus accumbens as pre-specified region of interest).

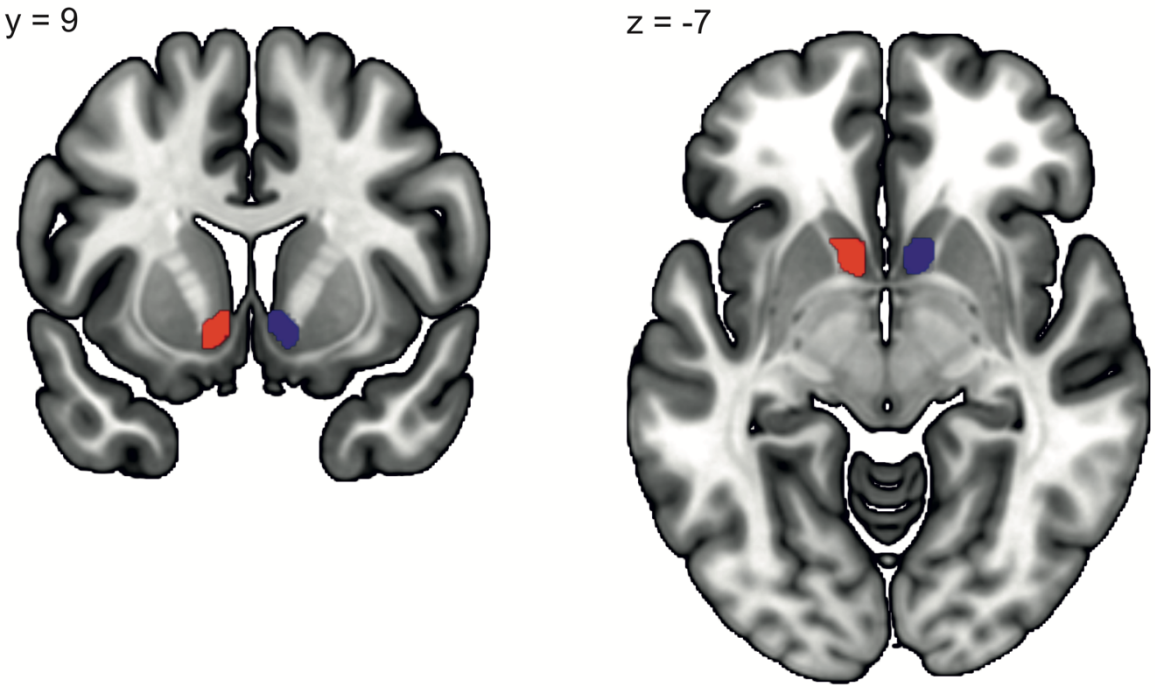
Side	Lobe	Brain areas	Cluster size (voxel)	MNI coordinates (x, y, z)			t_{max}
Alcohol > Neutral							
L	Occipital	Calcarine Gyrus, Fusiform Gyrus, Superior/Middle/Inferior Occipital Gyrus, Cerebellum	5651	-38	-88	-6	11.12
R	Occipital	Fusiform Gyrus, Superior/ Inferior Occipital Gyrus, Cuneus, Calcarine Gyrus	2458	32	-90	-6	10.69
L	Frontal	Superior/Middle Frontal Gyrus, Supplementary Motor Area	675	-22	30	64	7.75
R	Frontal	Inferior/ Middle/ Superior Frontal Gyrus,	1094	48	42	32	6.63
L & R		Middle/Posterior Cingulum, Precuneus, Hippocampus	1339	2	-32	34	5.95
R	Parietal	Angular Gyrus, Superior/Inferior Parietal Gyrus	495	38	-62	48	5.94
L	Parietal/ Occipital	Angular Gyrus, Inferior Parietal Gyrus, Middle Occipital Gyrus, Superior Parietal Gyrus	1020	-34	-60	46	5.85
R		Cerebellum	372	54	-64	46	5.59
L	Frontal	Middle/Inferior Frontal Gyrus orbital parts, Middle Frontal Gyrus, Precentral Gyrus	1187	-46	16	32	5.39
R		Cerebellum	351	38	-58	-20	5.29
L		Nucelus Accumbens[#]	71	-12	10	-12	5.52
R		Nucleus Accumbens[#]	56	12	14	-6	5.25

Note. MNI = Montreal Neurological Institute; t_{max} = maximum t-value; p_{FWE} <.05; [#]= Region of interest (ROI) based small volume corrected (SVC) analysis.

Supplementary Table S 2.3. Sensitivity analyses – Repeated measures analysis of variance models (RM-ANOVAS) with alcohol picture blocks (k=12) as within subject factor and treatment group (i=2) as between-subject factor and gender (male/female) and current smoking status(yes/no) investigating the main effect of treatment group on **A]** cue-induced activation in the left nucleus accumbens (NAc), **B]** right nucleus accumbens, and **C]** cue-indcued alcohol craving during the fMRI alcohol cue-reactivity paradigm.

A] left NAc activation			
	$F_{(1,21)}$	Significance	Partial Eta^2
Constant	2,242	,149	,096
Treatment group	4,279	,050*	,169
Gender	1,854	,188	,081
Smoking status	,591	,451	,027
B] right NAc activation			
	$F_{(1,21)}$	Significance	Partial Eta^2
Constant	1,607	,219	,071
Treatment group	4,321	,049*	,171
Gender	1,637	,215	,072
Smoking status	,116	,736	,006
C] Cue-indcued craving			
	$F_{(1,20)}$	Significance	Partial Eta^2
Constant	5,389	,032	,221
Treatment group	7,748	,011*	,279
Gender	,259	,617	,013
Smoking status	2,209	,153	,099

Supplementary Figures



Supplementary Figure S 2.1. Depiction of the left (red) and right (blue) nucleus accumbens region of interest mask obtained from (http://neurovault.org/media/images/12980/MNI_res-epi_label-NAcc_mask.nii.gz)

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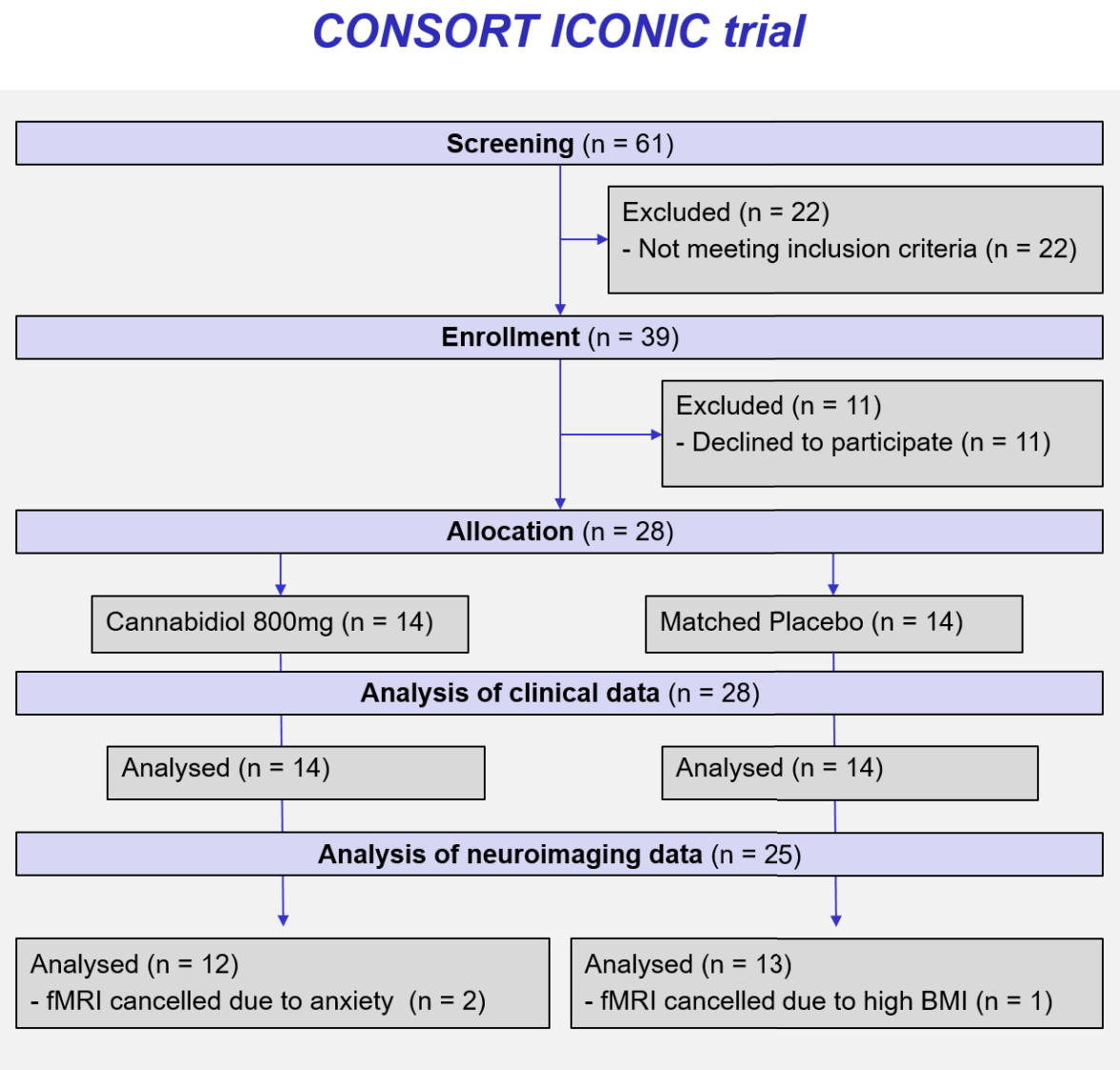

Supplementary Figure S 2.2. CONSORT study flow chart.

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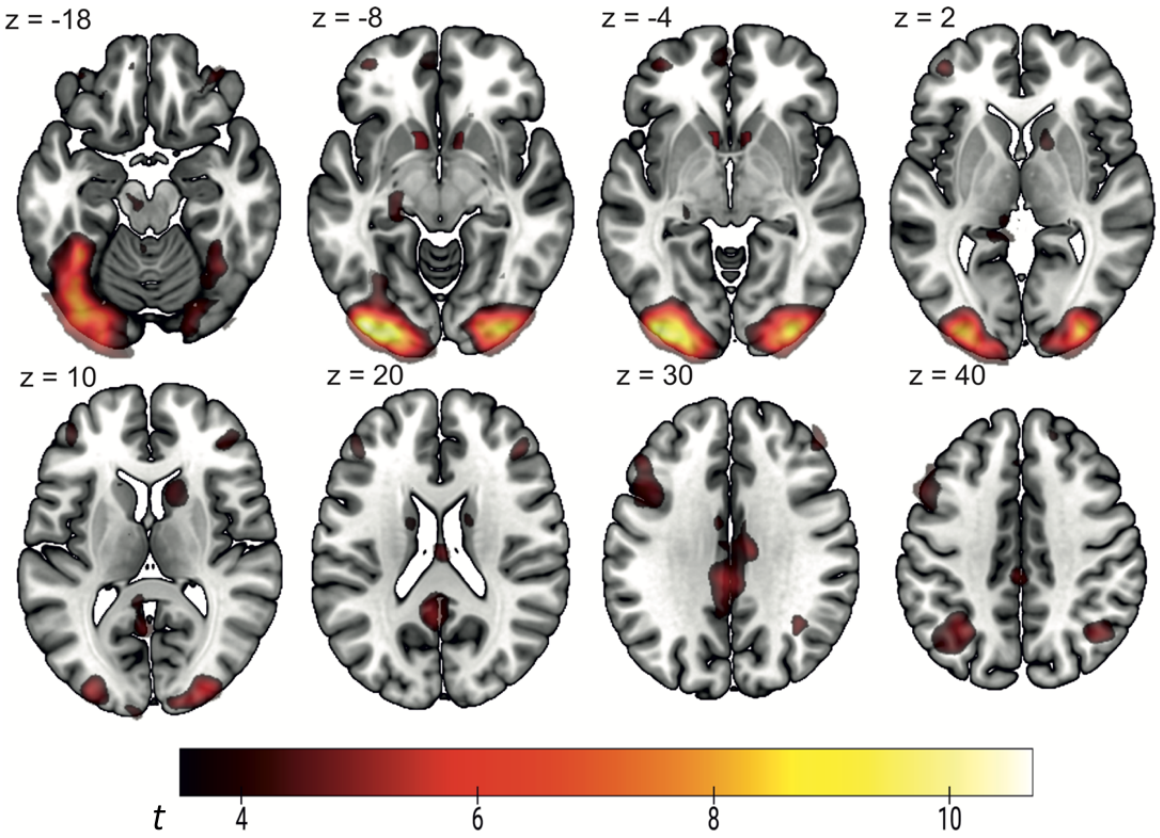

Supplementary Figure S 2.3. Depiction of brain areas that show higher brain activation in response to the presentation of alcohol cues versus the presentation of neutral stimuli during the alcohol cue-reactivity task (One sample t-test with sex (male/female), current smoking status (yes/no) and days since last alcohol use as covariates in SPM12, considering all n = 25 participants contrast “aclohol – neutral”, all results cluster-level whole-brain corrected at $p_{FWE} < .05$ or $p_{FWE} < .05$ small volume corrected for the nucleus accumbens as pre-specified region of interest).

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2.2 Empirical Study 2: Intranasal oxytocin blunts amygdala response to negative affective stimuli in males and females with alcohol use disorder: a randomized controlled cross-over trial

2.2.1 Abstract

Rationale

Negative affect plays a prominent role in the maintenance of alcohol use disorder (AUD) and has been identified as a risk factor for relapse to alcohol. To date, however, treatment options that target negative affective states and consecutive relapse risk in AUD are insufficient. Oxytocin (OXY) might be a promising approach for addressing negative affective states and resulting motivation to use alcohol.

Objectives

We aimed to investigate the acute effects of 24 I.U. OXY, administered intranasally, compared to matched placebo (PLC) on central processing of negative emotional stimuli in the amygdala in individuals with AUD.

Methods

We conducted a randomized double-blind placebo-controlled crossover study in *N*=24 individuals with AUD. Amygdala response to emotional stimuli served as primary outcome and was assessed using a validated functional magnetic resonance imaging emotion-processing task. Alcohol craving served as secondary outcome.

Results

OXY versus PLC attenuated right amygdala reactivity to fearful and angry emotional face stimuli during the fMRI task ($t(33)=3.32$, $p_{FWE}=.035$), while no effect of OXY on amygdala activation was observed during the presentation of geometric figures. In addition, right amygdala reactivity to fearful and angry emotional face stimuli was positively associated with alcohol craving ($r=.332$, Bias corrected and accelerated 95% confidence interval [95% BCa CI]=-.044 to .624, $p=.042$).

Conclusions

OXY's effects on the neurocircuitry underlying negative affect and craving in AUD support its potential for dampening alcohol craving induced by negative affective states and implicate OXY as a potential future treatment option for AUD.

Clinicaltrials Registry: DRKS00026218

Keywords: Alcohol Use Disorder; Negative Affect; Oxytocin; Amygdala; Faces-Task; fMRI

2.2.2 Introduction

Despite the high prevalence of AUD worldwide and its tremendous impact on global health (World Health Organization, 2018), only a few medications are approved for its treatment that overall show only limited efficacy (Litten et al., 2018; Rosner et al., 2010). Especially negative affective states, which have been identified as a major driving force of excessive goal-directed drug choice and risk factor for exacerbated craving and relapse risk in AUD (Heilig et al., 2019; Oslin et al., 2009; Witkiewitz & Villarroel, 2009), are only insufficiently addressed by approved medications. Previous research indicated that negative affect and relapse to alcohol are dynamically linked, suggesting that targeting negative affect in AUD could decrease craving and relapse risk (Oslin et al., 2009; Witkiewitz & Villarroel, 2009). Still, effective treatment options are lacking. In this context, oxytocin (OXY), an endogenous neuropeptide, might be a potential candidate, due to its effects on emotion processing neurocircuits (Grinevich & Neumann, 2021; Quintana & Guastella, 2020). Specifically, the amygdala has been identified as a brain region relevant for the processing of negative affective states in addiction (Koob & Volkow, 2010), in particular with respect to the oxytocinergic system (Koob, 2021; Koob & Vendruscolo, 2023). Within the amygdala, a substantial number of oxytocinergic fibers and oxytocin receptors have been localized (Gimpl & Fahrenholz, 2001), suggesting OXY is a potential mediator of the responses to negative emotional stimuli and occurrence of negative affective states. Indeed, direct manipulation of OXY receptors and OXY levels within the amygdala resulted in altered affective states and reduced distress in animal models (Bale et al., 2001; Ebner et al., 2005).

Preclinical studies reported that OXY attenuated anxious behavior and alcohol use in rats (Bowen et al., 2011) and that application of OXY reduced self-administration of alcohol in mice (King et al., 2017), prairie voles (Potretzke et al., 2023; Stevenson et al., 2017) and rats (Hansson et al., 2018; MacFadyen et al., 2016). These effects are centrally mediated. In this context, a recent animal study demonstrated significant effects of OXY on alcohol-induced GABA release in the central nucleus of the amygdala and a corresponding reduction of alcohol consumption (Tunstall et al., 2019).

In humans, effects of OXY on alcohol-related phenotypes are heterogenous. A pilot study in alcohol dependent patients reported a reduction of withdrawal symptoms, after administration of 24 IU OXY per day for three days (Pedersen et al., 2013) and we found reduced reactivity to alcohol cues in the insular cortex, the hippocampus / parahippocampal gyrus, the cingulate gyrus, the inferior and medial frontal gyrus, and in

visual and motor regions after a single administration of 24 IU OXY (Hansson et al., 2018). On the other hand, a following study failed to replicate these findings (Melby et al., 2019) and a recent randomized controlled trial showed no significant effects of OXY (40 IU up to three times per day) versus placebo on alcohol consumption, relapse risk and craving, thus questioning direct effects of OXY on these phenotypes (Melby et al., 2021). Still, the latter trial indicated significant effects of OXY on subjective nervousness, pointing towards a potential effect on negative affective states. The idea that OXY's effects in AUD might be indirect, via effects on emotion processing and the underlying neurocircuits, is also in line with another clinical trial that showed a significant moderation of OXY's effects by attachment anxiety. For example, OXY reduced craving only in subjects with higher attachment anxiety (Mitchell et al., 2016). Effects of OXY on the amygdala, as a central hub of emotion-processing neurocircuits, were consistently reported across a large number of studies that used an emotion-processing task to probe the response of the amygdala to emotional stimuli (Domes et al., 2007; Kirsch et al., 2005; Tully et al., 2018), supporting the general potential of OXY to modulate emotion-processing neurocircuits. Regarding the effects of OXY in populations that regularly use alcohol, a pilot study by our group in male social drinkers could show that OXY attenuated amygdala response to negative face expressions, which was associated with lower subjective alcohol craving and a lower percentage of heavy drinking days (Bach et al., 2020a). However, OXY effects on emotion-processing neurocircuits and craving in individuals with AUD has so far not been studied. To address this question, we conducted a randomized placebo-controlled crossover study in males and females with AUD to investigate the acute effects of OXY on amygdala response to emotional stimuli and alcohol craving using a validated functional magnetic resonance imaging emotion processing paradigm. The single-dose design enabled us to examine the immediate effects of OXY within a randomized-controlled crossover design, providing insights into whether OXY could be used as an acute, on-demand medication during high craving states resulting from negative affective states. We hypothesized that OXY reduces amygdala activation during processing of negative emotional stimuli, and that higher amygdala activation during processing of negative emotional stimuli is associated with higher subjective alcohol craving after the cue-exposure, i.e. before the imaging session, and higher subjective alcohol craving after the imaging session.

2.2.3 Methods

The project (Target-OXY) as a whole was designed to investigate the effects of OXY compared to PLC on experimental models for two central stages of the addiction cycle, specifically striatal alcohol cue-reactivity and amygdala response to emotional stimuli that were chosen, due to their close links with alcohol craving. Results on the alcohol cue-reactivity paradigm represent a different pre-registered analysis that are reported elsewhere (Vetter et al., 2025a). In short, we found that OXY compared to PLC increased alcohol cue-induced brain activation in parts of the frontal gyrus, supplementary motor area, hippocampus, parts of the temporal gyrus, fusiform gyrus, parahippocampal gyrus, precuneus, the superior parietal gyrus (p_{FWE}<.05 whole-brain cluster-level corrected). We found no significant effect of OXY in the ventral striatum and OXY increased cue-induced craving that was measured via visual analogue scales during the fMRI alcohol cue-reactivity task. However, we observed a significant interaction of treatment x sex on cue-induced craving during the fMRI alcohol cue-reactivity task. Female participants reported higher while males reported lower cue-induced alcohol craving during fMRI after OXY compared to PLC application.

Here we focus on the analyses of OXY's acute effects on emotion processing, i.e. amygdala response to emotional stimuli, and its association with alcohol craving measured via the Alcohol Urge Questionnaire (AUQ) after cue-exposure with the favorite alcoholic drink, i.e. directly before fMRI measurement (AUQ second assessment), and after fMRI session (AUQ third assessment).

Study design

We conducted a randomized, double-blind, placebo-controlled crossover study (clinical trial registry: DRKS00026218) to investigate the effects of intranasal OXY on amygdala activation during the presentation of fearful and angry face stimuli in 24 individuals with AUD. After screening and enrolment, individuals were randomized to one of two assessment sequences (OXY-PLC, PLC-OXY) to control for sequence effects. The two study visits were scheduled at an interval of 7 to 14 days, to reduce risk of any carryover effects. Participants received OXY either at the first or at the second study visit and a matched PLC during the other visit, according to their sequence group. Matched PLC sprays were identical in odor and appearance, containing the same ingredients except for the active ingredient Oxytocin. Both nasal sprays were blinded and packaged in identical brown glass bottles with a spray nozzle by the study

pharmacy. Neither study personnel nor participants were able to notice any differences between OXY and PLC. A single dose of 24 IU OXY (Syntocinon, Oxytocin, ATC code: H01BB02) was administered intranasally (6 puffs in each nostril) 45 minutes prior to the functional magnetic resonance imaging (fMRI) session. The intranasal application, the dose and timing were chosen in line with previous studies, which reported a maximum effect of OXY on brain response and behaviour between 20-90 minutes after intranasal application (Quintana et al., 2021).

Study sample

In total, 139 non-treatment seeking participants with AUD that took part in a national cohort study, as part of the German collaborative research center TRR265 (Heinz et al., 2020; Spanagel et al., 2024), were re-contacted and screened for inclusion and exclusion criteria after completing the observational study. Of those, 24 individuals with AUD, according to DSM-V (at least two AUD criteria met and exclusion of individuals with withdrawal symptoms), between 18 and 65 years were eligible for participating in the trial and have provided written informed consent (see **Supplementary Table S2.4**). In addition, participants needed to have normal vision. Contraindications for MRI, pregnancy, lactation and breastfeeding, as well as severe somatic (Long QT-syndrome or other severe heart disease, liver cirrhosis) or psychiatric comorbidities (psychotic disorders, mania, bipolar disorder, severe depression with suicidal ideation) were exclusion criteria and assessed by trained study personnel via structured assessment of the medical history. Intake of psychotropic medication (except antidepressants from the group of serotonin re-uptake inhibitors or mirtazapine and pipamperone), was not permitted. Positive urine drug screening (amphetamines, opiates, benzodiazepine and cocaine) or breath alcohol > 0.0 ‰ at either one of the two treatment visits led to exclusion from the study.

Of the 24 participants, one did not attend the second scanning session (drop out), and four participants had to be excluded from further analysis due to heavy movement during the fMRI session or technical issues with the MRI device (see CONSORT flow chart, **Figure 2.6**).

According to statistical analysis plan, only complete datasets were considered in the analysis of the primary endpoint. In total datasets of 19 participants were analysed.

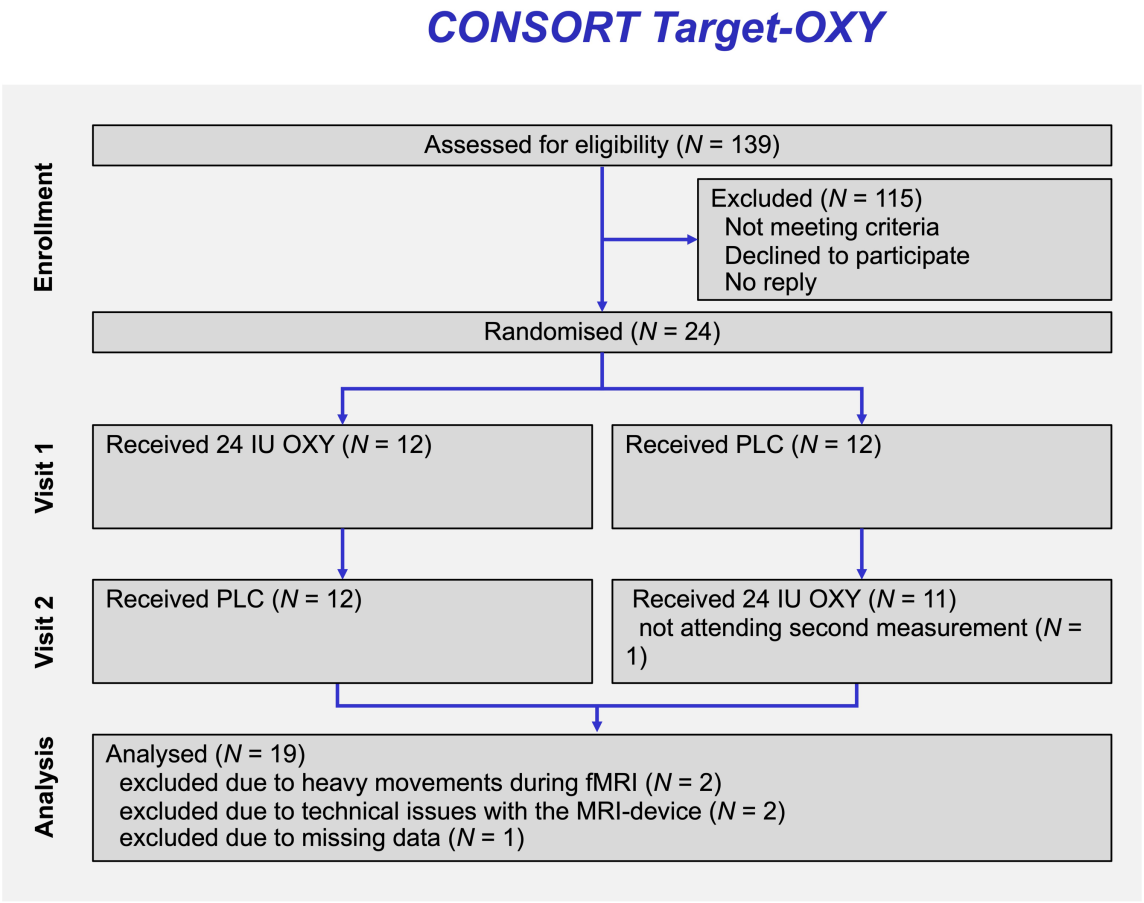

Figure 2.6. CONSORT-Flowchart.

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Study procedure

Apart from the treatment with OXY or PLC, both study visits followed the same procedures. Each study visit started with a verification of the absence of any exclusion criteria. To this end, participants underwent drug urine screening, breath alcohol test, and in women with childbearing potential, a pregnancy test was performed. Afterwards, baseline alcohol craving was assessed using the Alcohol Urge Questionnaire (AUQ; (Bohn et al., 1995)). Further, the Obsessive Compulsive Drinking Scale (OCDS-G; (Mann, 2000)), the Beck Depression inventory (BDI-II; (Hautzinger, 2009)), the State-Trait-Anxiety inventory (STAI; (Laux et al., 1981)) and the Perceived Stress Scale (PSS; (Cohen et al., 1994)) were administered to assess craving in the last days, depressive symptoms, anxiety and subjective stress levels as baseline characteristics. Alcohol cue exposure with the preferred alcoholic beverage was conducted in a laboratory bar setting for three minutes in line with an established procedure (Kwako et al., 2015; Monti et al., 1987) to induce alcohol craving directly before OXY or PLC administration. Twenty minutes after intranasal application of OXY or PLC, a second measurement of the AUQ, was completed. Afterwards participants were prepared for fMRI measurement, then the alcohol cue-reactivity task (Vollstadt-Klein et al., 2012) started, followed by the face matching task (Hariri et al., 2002), a resting state measurement and a structural measurement. Participants underwent a third measurement of the AUQ after completing MRI. Study procedures are depicted in **Figure 2.7**.

The ethics committee of the medical faculty Mannheim of the University of Heidelberg approved all experimental procedures (AZ 2021-515).

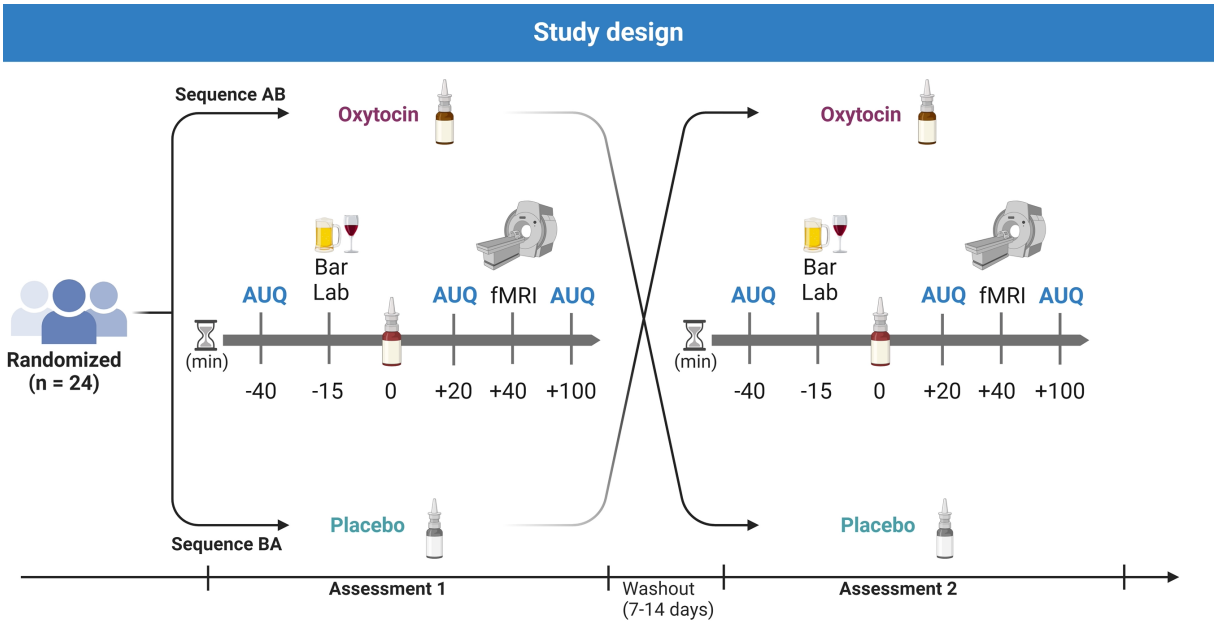

Figure 2.7. Depiction of the study design and procedures. Either 24 I.U. Oxytocin (OXY) or Placebo (PLC) were administered intranasally after an alcohol cue-exposure in a bar lab setting. 40 minutes after administration of OXY or PLC an emotion processing face-matching task was performed during functional magnetic resonance imaging (fMRI) to investigate Amygdala response. Alcohol craving was measured at three timepoints by using the Alcohol Urge Questionnaire (AUQ).

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Primary and secondary endpoints

The parent study was designed to investigate the effects of OXY compared to PLC on alcohol cue-reactivity and Amygdala response to emotional stimuli. Here we focus on the amygdala response to emotional stimuli in a validated fMRI emotion-processing task measured by the blood oxygenated level dependent (BOLD) response, that was defined as primary outcome in this analysis. Situational alcohol craving measured by the AUQ (second and third assessment, directly before and after the fMRI) and performance data i.e., accuracy and reaction times during fMRI served as secondary endpoint. For the here presented analyses, the AUQ score following the alcohol exposure in the Bar Lab setting (second AUQ assessment) was of particular interest as a secondary endpoint, as it represents situational craving in an ecologically valid drinking scenario, which may be relevant for the context of craving triggered by negative affective states.

fMRI emotion processing face-matching task

During fMRI all participants performed a face-matching task adapted from Hariri and colleagues (Hariri et al., 2002). The task consisted of 4 shape- and 4 faces-blocks with 6 trials per block. In each block either shapes or faces with angry or fearful expressions were presented. Participants had to decide whether the left or right face or shape at the bottom matches with the face or shape at the top of the screen. They were instructed to choose the matching face or shape by pressing the left or right button on a MRI-compatible response pad. Reaction times and accuracy (correct vs. incorrect button presses) were recorded during the task using the Presentation® software (Version 16.0, Neurobehavioral Systems Inc., Albany, CA, USA). The task took approximately five minutes in total.

Assessment and pre-processing of fMRI data

The fMRI measurements were conducted using a Siemens MAGNETOM 3 Tesla whole-body-tomograph (MAGNETOM PRISMA^{fit}, Siemens, Erlangen, Germany). During the faces task a total of 306 T2*-weighted echo-planar images (EPI) were acquired during the alcohol cue-reactivity task using the CMRR multi-band EPI sequence (Moeller et al., 2010; Setsompop et al., 2012) (TR=0.869 s, TE=38 ms, flip angle=58°, 60 interleaved slices, slice thickness=2.4 mm, voxel dimensions=2.4x2.4x2.4 mm^3, FOV=210x210mm^2, 88x88 matrix, AP phase-encoding, multi-band factor 6, bandwidth 1832 Hz/Px, MB LeakBlock Kernel, weak raw filter, prescan normalization, excite pulse

duration 7ms). Field map images were acquired with a standard Siemens dual gradient echo sequence (TR = 0.698 s, TE1 = 5.19 ms, TE2 = 7.65 ms, flip angle=54°, 64 interleaved slices, slice thickness=2.4 mm, voxel dimensions 2.4x2.4x2.4 mm^3, FOV=210x210mm^2, 88x88 matrix, AP phase-encoding, bandwidth 279 Hz/Px).The first five scans were excluded from further analyses, in order to reduce artifacts resulting from magnetic saturation effects. All MRI data were pre-processed using the statistical parametric mapping software for Matlab (SPM, version 12, Wellcome Department of Cognitive Neurology, London, UK). MRI data were temporally realigned and corrected for residual geometric distortion on the basis of the magnetic field map, spatially realigned and corrected for micromovements and normalized to a standard MNI (Montreal Neurological Institute, Quebec, Canada) EPI template. Following this step, an isotropic Gaussian kernel for group analysis (8 mm full width at half maximum) was applied to the images.

Statistical analyses

Analyses of the neuroimaging data were performed according to previous studies, by firstly modelling the experimental conditions (faces vs. shapes) of the fMRI task in a generalized linear model (GLM) using the statistical parametric mapping software for Matlab (SPM, version 12, Wellcome Department of Cognitive Neurology, London, UK) to compute the first-level contrast images that contrast brain activation during the task conditions against implicit baseline (contrast: faces – baseline, shapes – baseline) and against another (contrast: faces – shapes). According to the crossover design, a flexible factorial model with the factors treatment (OXY vs. PLC), period (OXY at first vs. OXY at second measurement) and subject was set up in SPM12 and first-level contrast images (see above) were included to test the treatment effects of OXY versus PLC on amygdala activation. Treatment effects on amygdala activation during the presentation of emotional face stimuli and shapes were assessed combined and separately, to confirm that the effects were specific to the emotion-processing condition. The carryover effect was evaluated by including the period-by-treatment interaction in the flexible factorial model (Lim & In, 2021). To control for sex effects, sex (male vs. female) was included as covariate. Due to the strong a priori hypothesis for OXY's effects on the amygdala, a region-of-interest (ROI) approach was used to determine the significance of OXY effects on the amygdala response to emotional stimuli. To this end, a standardized anatomical amygdala mask from the Wake Forest University PickAtlas was used to define the left and right amygdala ROI (WFU PickAtlas, version 3.0.2,

https://www.nitrc.org/projects/wfu_pickatlas). Significance was set to a local small-volume-corrected a family-wise error rate (FWE) corrected p value of $p_{FWE}<.05$. To complement the analyses of averaged brain activation, we also extracted block-wise activation values from the left and right Amygdala (betas) using the region of interest mask for the left Amygdala and the right Amygdala and the MarsBaR toolbox (version 0.45, https://marsbar-toolbox.github.io/index.html) for Matlab. According to Wellek and Blettner (2012), the hypothesis of negligible carryover effect was initially tested by performing a t-test for independent samples for the intraindividual sums of the betas of sequence group AB (OXY-PLC) and sequence group BA (PLC-OXY). Subsequently, the intraindividual differences of the betas from sequence group AB (OXY-PLC) and sequence group BA (PLC-OXY) for faces blocks and shapes blocks were compared separately using t-tests for independent samples to test the specificity of the treatment effect. The analysis was performed using IBM SPSS Statistics (version 27.0). Additional post-hoc power analyses for the t-tests for independent samples of the intraindividual differences of the betas in the left and right amygdala for faces blocks were performed using G*Power (version 3.1.9.7) (Faul et al., 2009). Intervention effects on alcohol craving (AUQ before and after fMRI) and performance data i.e., accuracy and reaction times during face matching task were analysed using mixed models with treatment (OXY vs. PLC), sequence (OXY at first vs. OXY at second measurement) and sex (male vs. female) as fixed effects, and subjects as random effect in IBM SPSS Statistics (version 27.0). The period-by-treatment interaction was included in the mixed model analysis to account for potential carryover effects. In addition, baseline characteristics were compared to control for sequence effects or any systematic differences between the sessions. Statistical significance level was set to $\alpha = 0.05$.

To investigate the hypothesized association between amygdala activation and alcohol craving, we extracted functional brain activation in the left and right amygdala ROI using the MarsBaR software package (version 0.45, https://marsbar-toolbox.github.io/index.html) and imported the values into IBM SPSS Statistics (version 27.0). Associations of functional brain activation in the left and right amygdala ROIs with craving (AUQ) were tested using Pearson correlation coefficient and robustness was confirmed using bootstrapping (i.e. the Bias corrected and accelerated [BCa] bootstrapping procedure).

2.2.4 Results

Sample characteristics

In total, 19 (9 males) individuals with AUD (mean age = 45.63 ± 11.69) were included in the primary analyses with no significant differences in baseline characteristics between the OXY and PLC assessment sessions (see **Tables 2.2** and **Table 2.3**).

The whole-brain analysis of the main effects of task conditions confirmed that face stimuli induced higher brain activation than shapes in a cluster of brain areas including the fusiform gyrus, the occipital gyrus, lingual gyrus and calcarine (p_{FWE}<.05 whole-brain cluster-level corrected) and in the left and right amygdala (p_{FWE}<.05 SVC-corrected). Higher amygdala activation was only observed for the face-matching condition, but not in the shapes condition, indicating the specificity of amygdala activation for face stimuli (see **Table 2.4**).

Table 2.2. Demographic data and substance use patterns.

	Individuals with AUD ***N* = 19**
Demographical variables	
Sex (female/male)	10/9
Age [years; mean (SD)]	45.63 (11.69)
Substance use characteristics	
Number of AUD criteria last 12 months	3.26 (2.21)
ADS [total score; mean (SD)]	7.47 (4.29)
Smoker (yes/no)	3/16
FTND[#] [total score; mean (SD)]	1.37 (2.77)

Note. AUD = Alcohol Use Disorder, ADS = Alcohol Dependence Scale, FTND = Fagerstrøm Test for Nicotine Dependence, [#] assessed in smokers only.

Table 2.3. Depiction of the baseline characteristics, alcohol craving and performance data for the sample of individuals with mild to severe alcohol use disorder for both treatment visits.

Individuals with AUD (*N* = 19) Baseline chracteristics	Mean (SD) OXY	Mean (SD) PLC	statistics	p-value
OCDS (sumscore)	9.47 (5.72)	9.16 (6.00)	$F(1,17) = 0.60$	$p = .451$
STAI (state sumscore)	38.05 (9.49)	38.00 (10.15)	$F(1,17) = 0.01$	$p = .906$
PSS (sumscore)	17.84 (6.22)	17.05 (5.36)	$F(1,17) = 0.53$	$p = .477$
BDI-II (sumscore)	9.79 (6.04)	9.21 (6.40)	$F(1,17) = 0.23$	$p = .640$
Alcohol craving	Mean (SD) OXY	Mean (SD) PLC	statistics	p-value
$AUQ_{baseline}$ (sumscore)	13.74 (5.85)	13.37 (5.79)	$F(1,17) = 1.80$	$p = .677$
AUQ_{preMRI} (sumscore)	14.42 (5.93)	16.21 (7.89)	$F(1,17) = 1.73$	$p = .206$
$AUQ_{postMRI}$ (sumscore)	16.42 (7.97)	16.74 (9.02)	$F(1,17) = 0.78$	$p = .784$
Performance data	Mean (SD) Oxytocin	Mean (SD) Placebo	statistics	p-value
Accuracy during **faces** conditions (%)	99.78 (0.96)	99.12 (2.23)	$F(1,17) = 1.14$	$p = .301$
Reaction time during **faces** task (ms)	1170.41 (226.95)	1210.48 (258.95)	$F(1,17) = 1.24$	$p = .280$
Accuracy during **shapes** condition (%)	96.93 (4.13)	96.05 (4.70)	$F(1,17) = 0.29$	$p = .597$
Reaction time during **shapes** task (ms)	1196.43 (276.04)	1198.83 (252.27)	$F(1,17) = 0.32$	$p = .582$

Note. AUD = Alcohol Use Disorder, OXY = Oxytocin, PLC = Placebo, OCDS-G = Obsessive Compulsive Drinking Scale, STAI = State and Trait Anxiety Inventory, PSS = Perceived Stress Scale, BDI-II = Beck Depression Inventory.

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Table 2.4. Brain regions that show significant condition- (faces vs. shapes)-dependent activation (p_{FWE} < 0.05 whole-brain cluster-level corrected).

Side	Brain regions	Cluster size	MNI Coordinates			t_{max}
Faces > Shapes						
R	Fusiform Gyrus, Inferior Occipital Gyrus, Lingual Gyrus, Middle Occipital Gyrus, Calcarine, Superior Occipital Gyrus, Cuneus	4123	-30	-86	-10	11.19
R	Amygdala[#]	79	20	-4	-16	5.64
L	Fusiform Gyrus, Middle Occipital Gyrus, Inferior Occipital Gyrus, Lingual Gyrus, Calcarine, Superior Occipital Gyrus	4123	-30	-86	-10	11.19
L	Amygdala[#]	86	-20	-6	-16	6.73
Faces < Shapes						
-						

Note. t_{max} = maximum t-value; p_{FWE} <.05; [#]= Region of interest (ROI) based small volume corrected (SVC) analysis.

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Effects of OXY on emotion processing in the amygdala

Our analyses of local activation in the amygdala showed that OXY significantly attenuated the response to emotional face stimuli (first-level contrast: faces - baseline) in the right amygdala ($t(33)= 3.32$, $p_{FWE\ SVC} = .035$, see **Figure 2.8**), while no significant OXY effect was found on activation in the left amygdala. No significant OXY effect was observed during the presentation of geometric figures (first-level contrast: shapes - baseline) in the left or right amygdala. In addition, post-hoc analyses of the extracted mean activation for the faces and shapes blocks showed negligibility of carryover effects and that OXY versus PLC attenuated activation in the right amygdala during face-matching blocks ($t_{faces(17)}=-2.447$, $p = .026$, Cohen's d = -1.137) but not during shape-matching blocks ($t_{shapes(17)}=-1.362$, $p = .191$, Cohen's d = -.633) blocks (see **Figure 2.8**), indicating that the effect of OXY was specific to the processing of emotional stimuli while the attenuated activation of OXY versus PLC during the face-matching blocks was not observed in the left amygdala ($t_{faces(17)}=-.444$, $p = .662$, Cohen's d = -.206). A post-hoc power analysis was conducted to evaluate the statistical power of the t-tests for independent samples of the faces-blocks in the left and right amygdala. Based on the effect size observed in our data and the alpha level of 0.05, the analysis indicated that the achieved power was 76% for detecting differences between OXY and PLC in the right amygdala and 11% for detecting differences between OXY and PLC in the left amygdala. Given the observed effect sizes, the power analysis suggests that the sample size was not sufficient to detect the effects of small size in the left amygdala. Additional whole-brain analysis revealed no significant treatment effects (OXY vs. PLC) in brain areas beyond the amygdala during the presentation of emotional face stimuli.

To confirm that the observed differences in amygdala responses were not due to effects of OXY versus PLC on task performance, we compared reaction times between both treatment conditions. Here, we observed no significant difference between OXY versus PLC sessions concerning reaction times ($F_{shapes(1,17)} = 0.315$, $p = .582$, $eta^2=.018$; $F_{faces(1,17)} = 1.243$, $p = .280$, $eta^2=.068$) or accuracy ($F_{shapes(1,17)} = 0.290$, $p = .597$, $eta^2=.017$; $F_{faces(1,17)} = 1.138$, $p = .301$, $eta^2=.063$) during the face- and shape-matching trials of the fMRI task (see **Table 2.3**).

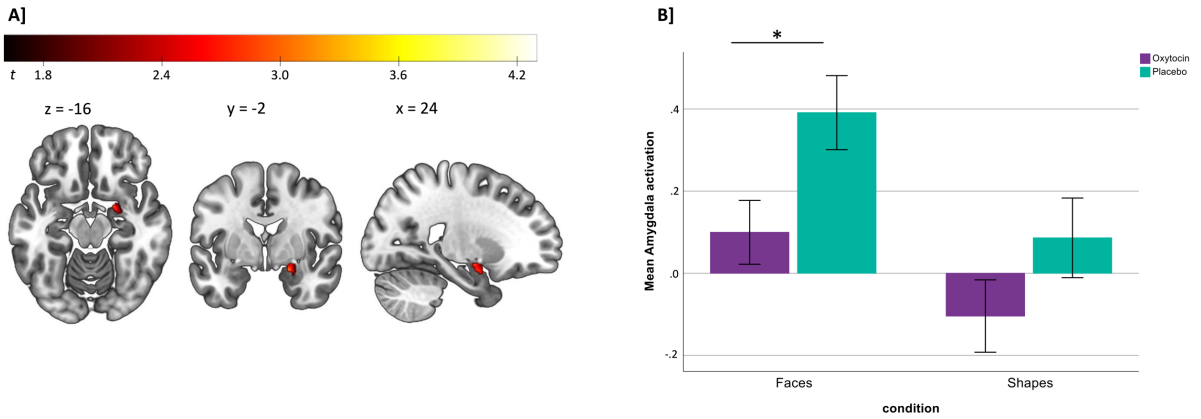

Figure 2.8. Depiction of the activation cluster in the right Amygdala, which showed a significant treatment effect with significantly lower activation after Oxytocin (OXY) compared to Placebo (PLC) administration (p_{FWE} < .05 small volume corrected for the Amygdala as pre-specified region of interest) and B] the difference in mean brain activation in the right Amygdala between OXY and PLC administration in the faces ($t_{faces(}18)$=-2.609, p = .018, Cohen's d = -.599) and shapes ($t_{shapes}(18)$=-1.574, p = .133, Cohen's d = -.361) blocks (as part of a post hoc test, mean activation for the faces and shapes blocks was extracted using the region of interest mask for the right Amygdala and the marsbar toolbox for Matlab; error bars represent +/- 1 standard error).

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Effects of OXY on alcohol craving

There was no significant effect of OXY versus PLC on situational alcohol craving at second ($F_{(1, 17)}$ = 1.730, p = .206, eta^2=.092) and third assessment ($F_{(1, 17)}$ = 0.078, p = .784, eta^2=.005) of the AUQ questionnaire after OXY or PLC administration during the experimental session.

Associations between Amygdala activation and behavioural data

Right amygdala activation during the presentation of emotional face stimuli was significantly associated with situational alcohol craving after treatment with OXY or PLC and after cue-exposure with the favorite alcoholic drink which was measured directly before fMRI (second AUQ assessment; r = .332, Bias corrected and accelerated 95% confidence interval [95% BCa CI]=-.044 to .624, p = .042; see **Figure 2.9** and **Supplementary Figure S2.4**), but the association with situational alcohol craving after fMRI measurement failed to yield significance (third AUQ assessment; r = .303, 95% BCa CI=-.015 to .556, p = .064).

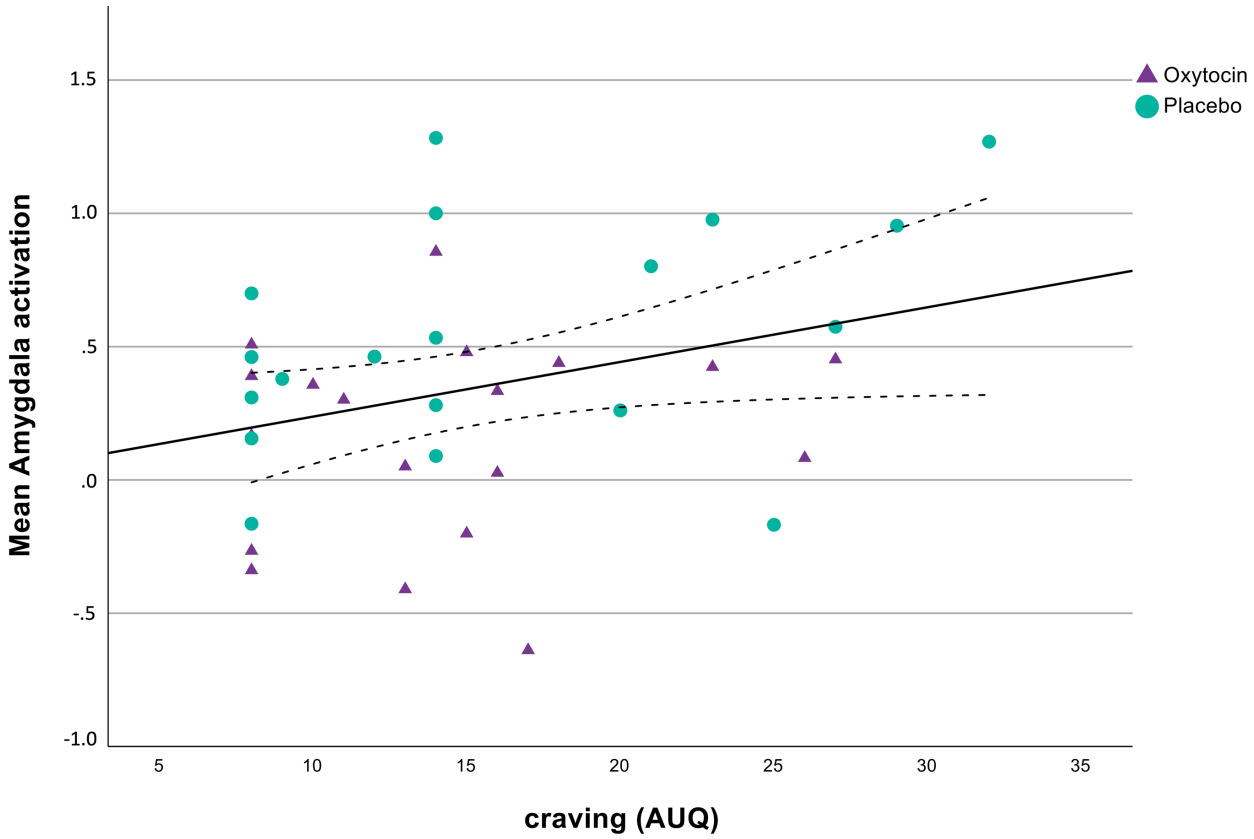

Figure 2.9. Depiction of the significant positive correlation between activation in the right amygdala and alcohol craving after treatment with oxytocin or placebo (second assessment of the Alcohol Urge Questionnaire (AUQ); r = .332, Bias corrected and accelerated 95% confidence interval [95% BCa CI]=-.044 to .624, p = .042).

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2.2.5 Discussion

The results of this randomized double-blind placebo-controlled crossover study show that a single dose of OXY significantly attenuates amygdala activation during emotion processing, which in turn positively correlated with situational alcohol craving after cue-exposure with the favorite alcoholic drink. However, no direct treatment effect of OXY on situational alcohol craving after cue-exposure with the favorite alcoholic drink was observed. These findings suggest that OXY has significant effects on emotion-processing neurocircuits in AUD, which were implicated in goal-directed drug choice during negative affective states (Giannone et al., 2024; Hogarth, 2020). However, the effect of OXY on craving might depend on the presence of negative affective states, indicating the context-dependence of OXY's effects on alcohol craving with stronger effects when experiencing negative affective states. In accordance to previous research, which demonstrated a close link between negative affect and alcohol craving (Bresin et al., 2018; Suzuki et al., 2020), we observed a significant association between the amygdala response to fearful and angry face expressions and situational alcohol craving after cue-exposure with the favorite alcoholic drink, suggesting a higher sensitivity of the amygdala to emotional stimuli in individuals reporting higher situational alcohol craving after alcohol cue-exposure in the Bar Lab setting. Previous findings of our research group in social drinkers and independent studies in healthy individuals mirror presented findings (Bach et al., 2020a; Domes et al., 2007; Kirsch et al., 2005), suggesting a robust effect of OXY an amygdala activation during the processing of emotional stimuli and also substantiating the association between amygdala activation and alcohol craving (Bach et al., 2020a). Further studies also established direct links between amygdala activation and alcohol craving, supporting the role of emotion-processing neurocircuits in AUD and craving (Childress et al., 1999; Garrison et al., 2023; Schacht et al., 2013). In the light of these findings, presented results indicate that our prior findings in male social drinkers generalize to males and females with AUD, supporting the potential of OXY for treating craving induced by negative affective states.
Previous studies indicated that OXY's effects might be different in males and females, even though findings are inconsistent (Hansson & Spanagel, 2020; Lieberz et al., 2020; Potretzke et al., 2023). The presented trial was not designed to compare effects between sexes, but instead intended to recruit a balanced number of female and male individuals and control for sex in the analysis to allow generalizability of the effects of

OXY effects in AUD for both biological sexes. In addition, this trial is designed as cross-over design, which allows to control for inter-individual characteristics.

According to previous work, which showed that OXY effects on alcohol craving are linked to negative affect or more specific attachment anxiety (Mitchell et al., 2016), one could speculate that OXY might be especially effective in individuals experiencing negative affective states and anxiety through attenuation of dysbalanced amygdala activation. Further, the observed OXY effects on the BOLD response are unlikely to be performance effects, rather, they are more likely associated with the emotional component as OXY affects emotional processing without altering task performance during fMRI. However, OXY does affect the negative emotional states resulting from emotion processing, which in turn are related to craving. This might also explain why OXY is especially effective in individuals experiencing anxiety and negative affect. Even though presented results replicate previous findings and thus substantiate the robustness of OXY's effects in AUD, future larger studies are needed to confirm OXY's effects and determine whether effects attenuate after repeated OXY exposure.

Limitations

The here presented study included a well-characterized sample of male and female individuals with mostly mild to moderate AUD. Thus, the results might not generalize to individuals with severe AUD, who typically suffer from higher craving states (Hansson et al., 2018). The success of the blinding was not systematically assessed, however, due to the matching of the OXY and PLC nasal spray and the blinding procedure no differences were reported by the study personnel or participants. Future studies should consider to assess the success of blinding systematically, which could further strengthen the validity of the results. In this trial, a balanced number of female and male individuals was enrolled and we controlled for sex in the analysis to allow generalizability of the effects of OXY in AUD for both biological sexes. Fluctuations of progesterone levels throughout the menstrual cycle might be associated with altered amygdala response and altered sensitivity for fearful faces in females (Derntl et al., 2008; Domes et al., 2010). However, the menstrual cycle or contraception was not assessed and therefore, not specifically considered in the analyses. The analyses did not reveal sex-effects, but it cannot be ruled out that the menstrual cycle in females may have affected the data. Additionally, due to delays of the study start caused by the COVID-19 pandemic, optimization of the MRI device, and regulatory requirements, which led to an adaptation of the study design and recruitment procedure where only

participants from a well-characterized cohort were recruited, resulting in a reduction of the pool of eligible individuals, of whom some were no longer interested in participating in another study, the sample size differed from the originally planned sample size. The final sample size has limited the capacity to detect smaller effects, in particular in the left amygdala. Therefore, we have performed a post-hoc power analysis to evaluate the power of the effect of OXY versus PLC during faces-blocks in the left and right amygdala, which revealed that the sample size was not sufficient to detect effects of small size in the left amygdala. This suggests that potential effects in the left amygdala might have remained undetected in the presented study, due to the small sample size. While some studies observed effects on facial emotions only in the left amygdala, other studies found effects only in the right amygdala or in the bilateral amygdalae (Tully et al., 2018). There is some evidence for lateralization of effects, and the effects of sex and implicit/explicit paradigms have been discussed. However, more research is needed to further investigate lateralization. Our data do not directly support the lateralization hypothesis, as the effect in the left and right amygdala had at least descriptively the same direction, although the effect was not significant in the left amygdala. Still, the present study is the largest neuroimaging trial to investigate OXY's effects in AUD so far, and power was increased through the crossover design.
Further studies are needed to confirm that the effects of OXY on neural circuits translate to clinical endpoints, such as craving and relapse over longer treatment periods. To this end, it is still unclear which schedule and dose of OXY is optimal for repeated administration over longer periods.

2.2.6 Conclusion

The presented results provide evidence that a single-dose of OXY selectively and significantly attenuates amygdala activation during processing of negative emotional stimuli in individuals with AUD, supporting the effects of OXY on emotion-processing circuits underlying negative affective states, craving and relapse in AUD. The significant association between amygdala response to emotional stimuli and situational alcohol craving indicate the potential of OXY to relief craving-related pathology in AUD during negative affective states.

2.2.7 Supplements

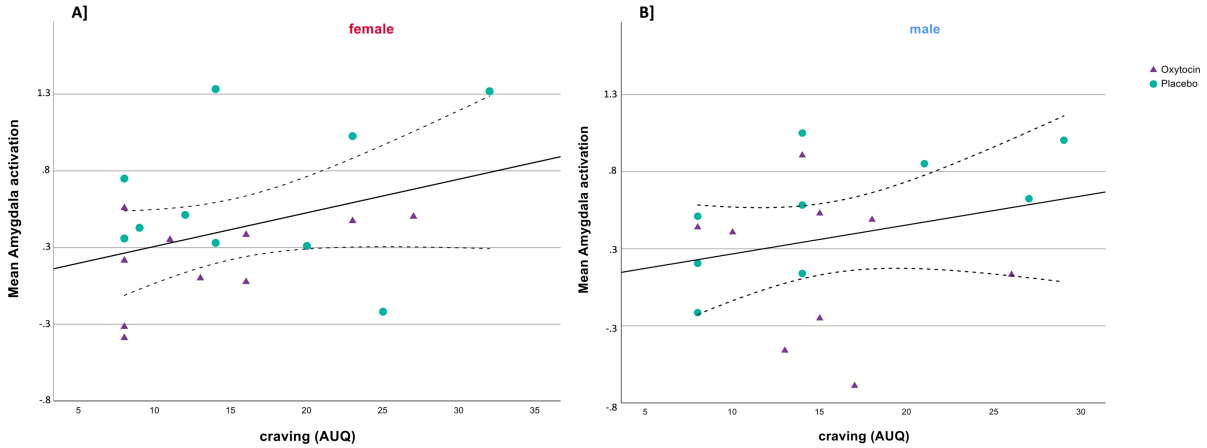
Supplementary Tables

Supplementary Table S 2.4. Demographic data and substance use patterns of all randomized individuals (*N* = 23).

	Individuals with AUD ***N* = 23**
Demographical variables	
Sex (female/male)	12/11
Age [years; mean (SD)]	45.87 (11.88)
Substance use characteristics	
Number of AUD criteria last 12 months	3.52 (2.13)
ADS [total score; mean (SD)]	7.04 (4.73)
Smoker (yes/no)	4/19
FTND [total score; mean (SD)]	1.53 (2.83)

Note. AUD = Alcohol Use Disorder, ADS = Alcohol Dependence Scale, FTND = Fagerstrøm Test for Nicotine Dependence

Supplementary Figures



Supplementary Figure S 2.4. Depiction of the positive correlation between activation in the right amygdala and alcohol craving measured by the Alcohol Urge Questionnaire (AUQ) in A] female compared to B] male individuals with Alcohol Use Disorder.

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3 DISCUSSION

The results of the two here presented empirical studies indicate an effect of both 800mg CBD and 24 I.U. OXY in non-treatment-seeking individuals with AUD. The results are discussed in the following sections 3.1 and 3.2. Both studies have limitations, which are discussed in section 3.3. Finally, implications are derived in section 3.4, and future directions are outlined in section 3.5.

3.1 Cannabidiol

The results of the first empirical study, a randomized, double-blind, placebo-controlled trial in 28 individuals with AUD, indicated that, compared to placebo, the acute administration of a single dose of 800mg CBD reduced alcohol cue-induced bilateral NAc activity and alcohol craving following a combined stress and alcohol cue exposure session, as well as craving during an fMRI-based alcohol cue-reactivity task. Additionally, CBD plasma levels were measured, which were higher in the CBD group and negatively correlated with alcohol craving during the cue-exposure experiment and craving during fMRI, as well as NAc activation. The increase in alcohol craving (AUQ scores) from baseline to after the combined stress- and alcohol cue-exposure session is consistent with previous findings indicating that exposure to alcohol stimuli enhances alcohol craving (Sinha, 2012; Sinha et al., 2011). The increase in alcohol craving from baseline to after the combined stress- and alcohol cue-exposure session, as well as alcohol craving during the fMRI-based cue-reactivity, was higher in individuals who received PLC compared to individuals who received CBD. This is in line with preclinical findings suggesting that CBD attenuates context- and stress-induced reinstatement behavior in rats (Gonzalez-Cuevas et al., 2018) and reduces reinforcement and motivational effects of ethanol in mice, thereby preventing relapse to ethanol (Viudez-Martínez et al., 2018b).

While the here presented results are the first results of a randomized-controlled trial in individuals with AUD, previous randomized controlled trials in abstinent individuals with heroin use disorder have already demonstrated that the first administration of 400 or 800mg CBD significantly reduced cue-induced heroin craving and anxiety symptoms compared to PLC (Hurd et al., 2019) and, similarly, administration of 400 or 800mg CBD led to a reduction in cannabis use in individuals with cannabis use disorder (Freeman et al., 2020). In contrast, the administration of 800mg CBD did not reduce

drug cue-induced craving in cocaine use disorder compared to PLC (Mongeau-Pérusse et al., 2021). Compared to neutral stimuli, alcohol cues induced higher brain activation in clusters including the occipital, temporal, parietal and frontal cortices, in the orbitofrontal cortex, as well as in the NAc, cuneus, middle and posterior cingulum, precuneus, hippocampus, and the cerebellum. This reflects for the most parts the brain activation clusters reported in the original work on the development of the *ALCUE task*, except that we did not observe brain activation in the thalamus, dorsal striatum and amygdala (Vollstadt-Klein et al., 2012) and is in line with the results of a meta-analysis indicating that alcohol cues induce activation of the ventral striatum and ventromedial prefrontal cortex in individuals with AUD (Schacht et al., 2013).

Contrary to the meta-analysis, we did not observe activation in the anterior cingulate cortex (Schacht et al., 2013), but in the middle and posterior cingulate. Similar to Bach et al. (2024), the fMRI was performed after a combined stress and alcohol cue exposure session. However, we did not find increased alcohol cue-induced activation in the left anterior insula. Our results showed that individuals who received CBD showed less brain activation in the left and right NAc, while these effects were specific to alcohol stimuli and no effects of CBD were observed in other brain areas. In line with preclinical studies indicating that observed effects of CBD on ethanol use were associated with alterations in gene-expression in both cannabinoid receptors CB1 and CB2, GPR55 and OPRM1 receptors in the NAc, the NAc was predefined as region of interest for fMRI analyses. The present results are also consistent with the results of a meta-analysis showing that the anterior cingulate cortex, the right pallidum and the ventral striatum are linked to drug cue-reactivity and self-reported craving in alcohol dependent individuals and other SUDs (Kühn & Gallinat, 2011). Several studies have demonstrated that the reduction of cue-induced brain activation in the ventral striatum is a key target for pharmacological treatments and thus an important predictor for the effectiveness of AUD treatment (Bach et al., 2020b; Courtney et al., 2016; Sangchooli et al., 2024; Schacht et al., 2017).

The effects of CBD on cue-induced NAc activation, which were specific to the presentation of alcohol stimuli and were not observed with neutral stimuli, provide supporting evidence for the potential of CBD to influence neurobiological disease mechanisms of AUD. CBD's effects on the brain have also been observed in previous randomized-controlled trials following a single dose of 600mg CBD during a verbal memory task (Bhattacharyya et al., 2018) and an emotion processing task (Davies et al., 2020)

during fMRI in individuals with high risk for psychosis. In addition, other neuroimaging studies found that CBD inhibited brain activation in the left temporal cortex and the insula in a go/no go task during fMRI (Borgwardt et al., 2008) and another emotion processing task during fMRI revealed a reduced connectivity between the anterior cingulate cortex and the amygdala during the processing of fearful face stimuli (Fusar-Poli et al., 2010).

In addition, CBD plasma levels were determined, showing that CBD was rapidly absorbed even after a single administration of 800mg CBD. CBD plasma levels correlated negatively with cue-induced NAc activity and alcohol craving, suggesting dose-dependent effects of CBD, consistent with findings from preclinical studies on alcohol use (Nona et al., 2019). The determination of CBD plasma levels may, on the one hand, help to define the therapeutically effective range of CBD (Morel et al., 2021), particularly in studies with repeated CBD administration as it has been shown that the CBD blood concentration increases over 12 weeks, indicating higher bioavailability of CBD with longer treatment duration (Morissette et al., 2021). On the other hand, substantial inter-participant variability in CBD plasma levels was observed in our sample, which was not explained by sex, age, BMI, smoking status or recent THC/CBD use. This suggests inter-individual differences likely resulting from the complex metabolism and the high lipophilicity of CBD (Calapai et al., 2020; Millar et al., 2018), highlighting the importance of monitoring CBD plasma levels in future studies.

3.2 Oxytocin

The results of the second empirical study, a randomized double-blind placebo-controlled crossover study in 24 non-treatment seeking individuals with AUD, showed that a single dose of 24 I.U. OXY significantly attenuates amygdala activation during emotion processing, which in turn positively correlated with situational alcohol craving after cue-exposure with the favorite alcoholic beverage. Contrary to our hypothesis, no direct treatment effect of OXY on situational alcohol craving after cue-exposure with the favorite alcoholic beverage in a laboratory bar setting was observed compared to PLC. The results of this study suggest that OXY has a significant effect on emotion-processing neurocircuits in AUD associated with goal-directed drug choice during negative affective states (Giannone et al., 2024; Hogarth, 2020).

Previous studies have already shown that stress and alcohol exposure in individuals with alcohol dependence is associated with the induction of negative affect and alcohol

craving (Fox et al., 2007) and that the experimental induction of negative affect leads to an increase in alcohol craving and alcohol consumption (Bresin et al., 2018). In addition, Suzuki et al. (2020) showed in an fMRI study in individuals with AUD that the regulation of alcohol craving and negative affect involve the same as well as different neuronal regulatory systems and that, in particular, reduced neuronal activation in the amygdala was associated with the regulation of negative affect, but not with the regulation of alcohol craving. The positive correlation between amygdala activation and situational alcohol craving induced by alcohol exposure observed in the present study supports the previously observed relationship between negative affect and alcohol craving (Bresin et al., 2018; Fox et al., 2007) as well as the role of the amygdala in regulating negative affect (Suzuki et al., 2020). Other studies also support the link between amygdala activation and craving in individuals with AUD and thus imply the role of emotion-processing neurocircuits in AUD and craving (Childress et al., 1999; Garrison et al., 2023; Schacht et al., 2013). The presented results of the second study mirror previous findings of our own research group in heavy social drinkers (Bach et al., 2020a) as well as the findings of independent studies in healthy individuals (Domes et al., 2007; Kirsch et al., 2005), which demonstrated an association between intranasal OXY administration and the amygdala response during the processing of emotional stimuli. Moreover, Bach et al. (2020a) also identified a link between amygdala response and alcohol craving. The results of the second empirical study suggest that, in both male and female individuals with AUD, the effect of OXY on craving depends on the presence of negative affective states. This highlights the context-dependence of OXY's effects with stronger effects observed when experiencing negative affective states. These findings indicate that the context, such as negative affective states, and stable inter-individual differences such as personality variables can play an important role (Bartz et al., 2011). For instance, it has been shown that the effects of OXY on alcohol craving vary inter-individually depending on attachment anxiety, with OXY reducing alcohol craving in more anxiously attached individuals, while an increase in alcohol craving was observed in less anxiously attached individuals (Mitchell et al., 2016). In contrast to several previous studies (Bach et al., 2020a; Bach et al., 2019; Flanagan et al., 2019; Hansson et al., 2018; Stauffer et al., 2019), the second study examined the effects of OXY in both female and male individuals with AUD. This allowed to replicate the observed effects of Bach et al. (2020a) in a mixed-sex sample, therefore verifying the generalizability of the effects across sexes, although this study

was initially not specifically designed with the aim of comparing effects between sexes, but instead intended to recruit a balanced sample consisting of female and male individuals to control for sex in the analyses. Previous studies indicated that OXY may have different effects in male and female individuals (Bethlehem et al., 2013; Hansson & Spanagel, 2020; Lieberz et al., 2020; Potretzke et al., 2023; Wigton et al., 2015). In a preclinical model Potretzke et al. (2023) postulated that intranasal OXY selectively reduced alcohol consumption in male but not in female prairie voles. Some studies observed differences in the brain response between males and females. Lieberz et al. (2020) showed that intranasal administration of OXY enhances the salience of social cues in females by increasing the neural responsiveness to social cues in the amygdala and the striatum, while OXY attenuates the amygdala response in males and thus tended to elicit anxiolytic effects. In line with that, other systematic reviews also reported opposite brain activation patterns after OXY administration in female and male individuals in the amygdala and the temporal lobes (Bethlehem et al., 2013; Wigton et al., 2015). Peris et al. (2020) also reported differences in OXTRs in the NAc between the sexes and due to the estrus cycle, which could explain differences in the inhibitory effects of OXY on alcohol stimuli in male and female individuals. Hansson and Spanagel (2020) have also stated that discrepant findings may result from sex hormone levels as well as the estrus cycle and that female rodents and alcohol dependent individuals do not show changes in the OXY system, which is why they recommended to investigate OXY interventions for AUD in males only. In contrast, other studies recommend controlling for sex effects in the analyses (Bethlehem et al., 2013) or specifically investigating both sexes (Quintana et al., 2021). Interestingly, there are not only sex effects in OXY-administration, but also in negative affect between the sexes, which further emphasizes the importance of considering sex effects by conducting sex-specific analyses. Guinle and Sinha (2020) found that females report more negative affect compared to males and that males are more likely to use alcohol to increase positive affect while females are more likely to use alcohol in response to negative affect which has been linked to increasing rates of AUD among females. In line with the recommendations of Quintana et al. (2021) and Bethlehem et al. (2013) we have included a balanced sample of male and female individuals with AUD in the second empirical study and have controlled for sex-effects. No sex-effect was found in any of our analyses, male and female individuals did not differ in brain activation patterns in the amygdala, in the intervention effects on alcohol craving or in task performance.

In the past, researchers have found a reduction in alcohol consumption, withdrawal symptom severity and relapse risk following the administration of OXY in rodent models (Bowen & Neumann, 2017) and initial preliminary studies were able to replicate the positive effects of OXY on alcohol withdrawal symptoms (Pedersen et al., 2013) and alcohol craving (Mitchell et al., 2016; Pedersen et al., 2013) and observed a reduction in neuronal cue-reactivity to alcohol stimuli (Hansson et al., 2018) as well as an attenuation of NAc in response to the exposure with alcohol stimuli during fMRI which was associated with alcohol craving (Bach et al., 2019), after administration of OXY compared to PLC. However, in the present study no direct treatment effect of OXY on situational alcohol craving following cue-exposure with the favorite alcoholic beverage in a laboratory bar setting was observed. Although this is in contrast with our initial hypothesis, several other studies were also not able to find an effect of OXY on alcohol craving (Flanagan et al., 2019; Flanagan et al., 2022; Melby et al., 2021; Stauffer et al., 2019). It is important to note, that the studies differ considerably in terms of samples, study design, dose and timing of OXY administration and the outcomes examined. With regard to the effects of OXY on alcohol-related outcomes some studies investigated non-treatment seeking social drinkers (Bach et al., 2020a; Bach et al., 2019; Hansson et al., 2018; Mitchell et al., 2016), others investigated alcohol-dependent patients admitted for medical detoxification (Melby et al., 2019; Melby et al., 2021; Melby et al., 2022; Pedersen et al., 2013), individuals with AUD and comorbid posttraumatic stress disorder (Flanagan et al., 2019; Stauffer et al., 2019) or individuals with AUD and co-occurring intimate partner aggression (Flanagan et al., 2022). In our study individuals with predominantly mild to moderate AUD according to DSM-5 were investigated. Some studies have included only male individuals (Bach et al., 2020a; Bach et al., 2019; Flanagan et al., 2019; Hansson et al., 2018; Stauffer et al., 2019), while others have included both male and female individuals (Flanagan et al., 2022; Melby et al., 2019; Melby et al., 2021; Melby et al., 2022; Mitchell et al., 2016; Pedersen et al., 2013), as we did in our second empirical study.

Several studies have employed between-subjects-designs (Flanagan et al., 2022; Melby et al., 2019; Melby et al., 2021; Melby et al., 2022; Pedersen et al., 2013) with sample sizes between 11 (Pedersen et al., 2013) and 40 alcohol dependent patients (Melby et al., 2019) und 100 couples with AUD co-occurring intimate partner aggression (Flanagan et al., 2022). In contrast, other studies used crossover or within-subjects designs (Bach et al., 2020a; Bach et al., 2019; Hansson et al., 2018; Mitchell et

al., 2016; Stauffer et al., 2019), with samples varying between 12 (Hansson et al., 2018) and 32 non-treatment seeking social drinkers (Mitchell et al., 2016) and 47 patients with alcohol dependence (Stauffer et al., 2019). Thus, with 24 male and female individuals with AUD, our crossover study is one of the largest randomized controlled trials that did not exclusively study social drinkers.

The studies also differed in methodology with regard to the dose and timing of the OXY administration. Some studies investigated the acute effects using a single dose ranging from 24 IU (Bach et al., 2020a; Bach et al., 2019; Hansson et al., 2018) to 40 IU (Flanagan et al., 2019; Flanagan et al., 2022; Mitchell et al., 2016), other studies applied repeated OXY administrations, such as 24 IU twice daily for 3 days (Melby et al., 2019; Melby et al., 2022; Pedersen et al., 2013), 8 IU OXY or PLC up to three times daily (reaching a maximum of 24 IU) over a four-week period (Melby et al., 2021) or 20 IU, 40 IU and PLC each administered once and one week apart (Stauffer et al., 2019). In addition, the studies differed in the time span in which the effects were examined after intranasal OXY administration. While Mitchell et al. (2016) examined the effects 30 minutes and Melby et al. (2022) 40 minutes after intranasal application, behavioral and neuronal effects were examined after 45 minutes in most of the studies (Bach et al., 2019; Flanagan et al., 2019; Flanagan et al., 2022; Hansson et al., 2018). However, other studies investigated behavioral and neuronal effects 60 minutes (Bach et al., 2020a) and 65 minutes (Stauffer et al., 2019) after intranasal application. Apart from the studies investigating effects of OXY on alcohol-related outcomes, most of the other studies applied dosages between 20 to 48 IU, with the most common dose being 24 IU, and behavioral effects and neuronal response were mainly investigated between 20 and 90 minutes after intranasal administration due to the short half-life (Quintana et al., 2021). Accordingly, in our empirical study, we administered a single dose of 24 IU OXY to investigate the acute effects on the amygdala response approximately 45 minutes after intranasal application and thus followed the most commonly used dose and timing.

Another key difference between previous studies and our study is that the studies each examined different outcomes after OXY administration. The effect of OXY on alcohol craving (Bach et al., 2020a; Melby et al., 2021; Pedersen et al., 2013; Stauffer et al., 2019) or cue-induced alcohol craving (Bach et al., 2019; Flanagan et al., 2019; Flanagan et al., 2022; Mitchell et al., 2016) was examined most frequently, but also the effects on alcohol use and days until relapse (Bach et al., 2020a; Melby et al.,

2021). In addition, some studies specifically investigated the effect of OXY on withdrawal symptoms and on the use of lorazepam or oxazepam during detoxification (Melby et al., 2019; Pedersen et al., 2013). Several studies have also investigated the effect of intranasal OXY application on sleep (Melby et al., 2019; Melby et al., 2021), anxiety and tension (Pedersen et al., 2013), aggression (Flanagan et al., 2022), heart rate (Stauffer et al., 2019), stress response (Flanagan et al., 2019; Flanagan et al., 2022) and performance during emotion processing (Bach et al., 2020a; Melby et al., 2022). Additionally, our own research group has also conducted several neuroimaging studies on the effects of intranasal OXY on neuronal cue-reactivity to alcohol stimuli (Hansson et al., 2018), NAc connectivity during processing of alcohol stimuli (Bach et al., 2019) and amygdala response during processing of negatively valenced facial expressions (Bach et al., 2020a).

In the here presented empirical study, we primarily replicated the effect of intranasal OXY on the amygdala response during emotion processing in a larger sample of individuals of both sexes with AUD. In addition to the effect of acute OXY administration on the neuronal response, the effects on situational alcohol craving and task performance were investigated. Consistent with Bach et al. (2020a), it was shown that 24 IU OXY reduced the response of the amygdala and that this attenuating effect was specific for negatively valenced face expressions of an emotion processing task, indicating a robust effect of OXY on the amygdala response.

In contrast to Bach et al. (2020a), however, the presented empirical study did not find an effect in the bilateral amygdala, but only in the right amygdala. In a systematic review, effects of OXY on the processing of facial expressions were found in the left amygdala in some studies and in the right amygdala or bilateral amygdala in other studies (Tully et al., 2018). There is inconsistent evidence regarding the lateralization of OXY effects in the amygdala. However, differences in task type and sex may contribute to these variations. Studies including explicit paradigms more often report effects in the left amygdala whereas studies including implicit paradigms tend to report effects in the right and bilateral amygdala and a few studies in female participants found that OXY increased amygdala activation during explicit tasks (Tully et al., 2018). The results of our empirical study do not directly support the lateralization hypothesis, as the effects in the left and right amygdala showed at least descriptively the same direction. Although the effect in the left amygdala did not reach significance, potentially due to limited statistical power, which is discussed in section 3.3, this pattern still

suggests a bilateral reduction of amygdala activation, consistent with the findings reported by Bach et al. (2020a).

3.3 Limitations

Both empirical studies have limitations, which are discussed below. In both empirical studies, the acute effects of a single dose of CBD and OXY were investigated. While this allows to assess acute effects of CBD and OXY, no conclusions about the long-term effects of the respective substance can be drawn from single-dose studies without follow-up measures. Intranasally administered OXY already reaches its peak after 30 minutes (Daughters et al., 2015) and the concentration drops significantly approximately 105 minutes after intranasal administration (Daughters et al., 2015; Spengler et al., 2017), indicating that no effects on the amygdala response are to be expected beyond this period. Orally administered CBD reaches its peak within 3-4 hours (Hurd et al., 2019; Paulus et al., 2022) and has a half-life of between 18 and 32 hours (Hurd et al., 2019). While there is evidence in other SUDs suggesting that the effects of CBD administration may persist after discontinuation (Freeman et al., 2020; Gonzalez-Cuevas et al., 2018; Hurd et al., 2019; Nona et al., 2019), there is no evidence that a single administration of CBD could produce stable, lasting effects in AUD. No conclusions can be drawn from both empirical studies about the effect of long-term administration of OXY or CBD, thus further studies with repeated administration are required to investigate the effect of repeated administration (Britch et al., 2021; Lee et al., 2016; Mitchell et al., 2016; Paulus et al., 2022). Both CBD and OXY have very limited bioavailability when administered orally (CBD) and intranasally (OXY), particularly due to the lipophilic properties of CBD (Millar et al., 2018; Navarrete et al., 2021; Paulus et al., 2022; Yau et al., 2023) as well as the very small amount of OXY that crosses the blood-brain barrier through nasal absorption (Quintana et al., 2021) and may also vary due to anatomical differences in the nasal cavity (Quintana et al., 2017) or differences due to individual stress levels (Martinetz & Neumann, 2016). Nevertheless, oral and intranasal administration represent a non-invasive and feasible application method, which is why those application methods are preferred despite these limitations (Bethlehem et al., 2013; Quintana et al., 2021; Yau et al., 2023). However, it seems important to assess plasma levels to determine how much medication was actually absorbed based on inter-individual differences if the effects of the medication are to be evaluated. In the first empirical study, CBD plasma levels were determined, showing

substantial inter-participant variability that was not explained by sex, age, BMI, smoking status or recent THC/CBD use, but presumably by other inter-individual differences such as the metabolism and the high lipophilicity of CBD (Calapai et al., 2020; Millar et al., 2018). In the second empirical study, however, OXY plasma levels were not measured, so it remains unclear how much OXY was actually absorbed. Differences in the density of OXTRs and differences in endogenous OXY caused by variations of the OXY system activity may also influence the effects of OXY (Martinetz & Neumann, 2016), which cannot be assessed without determining OXY plasma levels.

Even other interindividual differences such as age, body weight, sex may have an impact on the effects of OXY or CBD (Bis-Humbert et al., 2020; Huffmeijer et al., 2013; Matheson et al., 2022; Quintana et al., 2021). Hence, in the first empirical study, age, sex, BMI, smoking status and recent CBD use were considered in the analyses and no effects of these interindividual factors were observed in our sample. In the analyses of the second empirical study, we controlled for sex, but not for age or body weight. While no effects of sex were observed in any of the analyses in our sample, no conclusions can be drawn about the extent to which body weight or age may have influenced the effects of OXY. Both studies included both sexes, which represents a strength of the studies and allows for generalization of the effects of OXY and CBD to both sexes. The analyses of both studies also controlled for sex, however, menstrual cycle was not considered. Preclinical studies have shown that the estrus cycle may lead to differences in CBD effects (Fabris et al., 2022; Kim et al., 2022). Similarly, in studies in female individuals it was observed that fluctuations of progesterone levels throughout the menstrual cycle might be associated with altered amygdala response and altered sensitivity for fearful faces in females after OXY application (Derntl et al., 2008; Domes et al., 2010). However, in both studies the menstrual cycle or contraception was not assessed and therefore, not specifically considered in the analyses. Both analyses considered sex effects and no sex-effects were observed in our samples, but it cannot be ruled out that the menstrual cycle in females may have affected the data of both analyses. A further limitation concerns the measurement of alcohol craving, which in both empirical studies is based exclusively on self-reported measures, which may lead to bias (Hurd et al., 2019; Mongeau-Pérusse et al., 2021) it is therefore recommended to combine self-reports with biomarkers (Mellentin et al., 2023). While alcohol craving was examined exclusively as a self-report and not additionally by biomarkers, at least the neuronal response during fMRI represents a further objective

parameter in addition to self-reported craving. In both empirical studies, alcohol craving was induced by cue exposure prior to the fMRI examination. Hence, in the first empirical study a combined stress- and alcohol cue-exposure session, consisting of a combination of the TSST (Kirschbaum et al., 1993) and an alcohol cue-exposure task as previously established (Bach et al., 2024; Kwako et al., 2015) was conducted and in the second empirical study an alcohol cue-exposure session with the preferred alcoholic beverage in a laboratory bar setting according to an established procedure (Kwako et al., 2015; Monti et al., 1987). It is likely that the combined stress- and cue-exposure in the first study as well as the alcohol cue-exposure in the second study prior to the fMRI influenced the outcomes measured during fMRI. Therefore, the results of both studies cannot be interpreted independently of the effects of these prior exposures. As a result, the effects of CBD on alcohol cue-reactivity cannot be generalized to cue-reactivity paradigms in general without prior stress- and cue-exposure as well as the effects of OXY cannot be generalized to the amygdala response to negatively valenced face stimuli without prior alcohol cue-exposure. Nevertheless, as the exposure was the same in both study arms of the first study, and due to the crossover design in both assessment days of the second study, the outcomes between PLC and CBD and PLC and OXY are still comparable.

Further, it is important to note that the sample of both empirical studies consisted of non-treatment seeking individuals with AUD. Therefore, the results cannot be generalized to treatment-seeking individuals with usually more severe AUD. Notably, there is preclinical and clinical evidence that OXY could be more effective in more severe AUD or individuals that consume higher quantities of alcohol (Melby et al., 2022; Tunstall et al., 2019) and that the effect of CBD on craving in cannabis withdrawal symptoms is limited in particular to the acute phase (Bonaccorso et al., 2019). However, there are other findings that have demonstrated that treatment-seeking and non-treatment-seeking individuals with mild to moderate AUD do not differ in cue-reactivity, thus the cue-reactivity of non-treatment-seeking individuals may be informative for the investigation of pharmacologic treatments (Venegas & Ray, 2020).

Lastly, the sample sizes of both empirical studies including 28 individuals with AUD in the first empirical study and 24 individuals with AUD in the second empirical study are limited. Small sample sizes and low statistical power are still a common issue in OXY (Bethlehem et al., 2013; Bowen & Neumann, 2017; Quintana et al., 2021) and CBD research (Kirkland et al., 2022). However, in the first empirical study, an a priori power

analysis was calculated and the sample size of the parallel group design was chosen to detect medium or larger effects of CBD on the primary outcomes with a minimum power of 80%. In the second study, no a priori power analysis was calculated, but the sample size was estimated based on the sample sizes of previous studies and the power was increased by implementing a crossover design. In addition, a post hoc power analysis was conducted, to evaluate the statistical power to detect the effect of OXY versus PLC during faces-blocks in the left and right amygdala. This analysis aimed to determine, whether small effects, in particular in the left amygdala, may have been undetected due to the small sample size. The results indicated that the sample size was insufficient to reliably detect small effects in the left amygdala. This suggests that potential effects in the left amygdala may have remained undetected in the second empirical study due to the limited sample size resulting in inadequate statistical power. Although both studies included relatively small sample sizes, which may have led to small effects outside the NAc in the first study and outside the right amygdala in the second study being not detected, these two empirical studies still represent important contributions to the research on OXY and CBD.

3.4 Implications

Despite these limitations, the presented results suggest several implications, which are discussed below with reference to the research question. With reference to **RQ 1**, i.e., *Does the administration of 800mg CBD reduce cue-reactivity to alcohol stimuli and alcohol craving in individuals with AUD and what are the implications of the effects of CBD for the treatment of AUD?,* specific implications can be derived from the results of this thesis and the empirical study presented in section 2.1. The results indicate that a single administration of 800mg CBD led to a reduction in bilateral activation of the NAc, as well as a reduction in alcohol craving. The first empirical study thus provides initial evidence that CBD can positively influence neurobiological disease mechanisms and core symptoms of AUD. With regard to the theoretical framework of Koob (2015, 2024), CBD's effects could be particularly promising during the *binge/intoxication stage*. The observed reduction of cue-induced reactivity in the NAc and alcohol craving by CBD could attenuate incentive salience and thus possibly hinder the progression of the development of habitual and compulsive patterns of alcohol use.

Previous research has demonstrated a significant correlation between NAc activation, alcohol craving (Kühn & Gallinat, 2011; Schacht et al., 2013) and relapse risk in AUD (Bach et al., 2015). Moreover, cue-induced response of the NAc has been identified as a predictor of treatment response and clinical efficacy of pharmacotherapeutic approaches in AUD (Bach et al., 2020b; Pfisterer et al., 2025; Schacht et al., 2017). Furthermore, higher cue-reactivity is associated with more alcohol use (Kirsch et al., 2024). Therefore, the reduction of cue-reactivity in the NAc could imply that CBD may have significant effects on alcohol use and relapse risk, potentially contributing to better treatment outcomes in AUD. Compared to PLC, CBD administration did not only lead to a reduction in cue-induced craving during the presentation of alcohol stimuli in the fMRI, but also resulted in a lower increase in alcohol craving following the combined stress and alcohol exposure with the preferred alcoholic beverage. The findings suggest that CBD may not only reduce alcohol craving, but may also exert a stress-dampening effect in individuals with AUD as already shown in a preclinical study in rats (Gonzalez-Cuevas et al., 2018). However, this contrasts with findings in cocaine use disorder, where CBD did not result in a reduction of cortisol levels (Mongeau-Pérusse et al., 2022). While CBD's effects on stress cannot be validated using objective markers such as cortisol levels in the first study, the increase in CBD plasma levels was investigated as an objective marker for the absorption of CBD. The analysis of CBD plasma levels showed a rapid absorption even after a single administration of 800mg CBD and a negative correlation with cue-induced NAc activity and alcohol craving, suggesting dose-dependent effects of CBD as already observed in preclinical studies on alcohol use (Nona et al., 2019). In the here presented empirical study, the administered dose of 800mg CBD was well tolerated and no adverse events or severe adverse events were observed. CBD was also well tolerated in previous studies and rated as overall safe (World Health Organization, 2019; Wright et al., 2020). Consistent with previous evidence demonstrating the potential of CBD to reduce craving and anxiety in individuals with heroin use disorder (Hurd et al., 2019) and to reduce cannabis use in cannabis use disorder (Freeman et al., 2020), the present findings in individuals with AUD also support the potential of CBD as a treatment approach for AUD. CBD may complement existing pharmacotherapeutic strategies.

With reference to **RQ 2**, i.e., *Does the administration of 24 IU OXY reduce the neural response of the amygdala during processing of negative emotional stimuli and alcohol craving in individuals with AUD and what are the implications of the effects of OXY for*

the treatment of AUD?, specific implications can be derived from the results of this thesis and the empirical study presented in section 2.2. The results indicate that a single intranasal administration of 24 IU OXY led to a reduced amygdala response to negatively valenced facial expressions during emotion processing. This reduction was positively correlated with situational alcohol craving following cue-exposure with the preferred alcoholic beverage. These findings suggest that OXY acts on emotion-processing neurocircuits in AUD, particularly those involved in goal-directed alcohol-seeking behavior during negative affective states (Giannone et al., 2024; Hogarth, 2020). With regard to the theoretical framework of Koob (2015, 2024), OXY's effects could be particularly promising during the *withdrawal/negative affect stage*, where the function of the reward system in the ventral striatum is reduced while the stress system in the extended amygdala is activated, creating stress-like states that increase motivation for alcohol use. By modulating this circuit, OXY may reduce stress-induced negative affect and alcohol craving. A treatment approach that specifically targets emotion-processing neurocircuits in AUD could be a valuable complement to currently approved anti-craving medications, particularly given that previous studies have established a close link between negative affect and alcohol-related outcomes. Firstly, research has shown that exposure to stress and alcohol cues elicits both negative affect and alcohol craving (Fox et al., 2007). Secondly, the experimental induction of negative affect has been associated with higher alcohol craving and alcohol use (Bresin et al., 2018). Thirdly, both craving and negative affect can be effectively regulated using cognitive-behavioral treatment strategies and, notably, the regulation of negative affect was, similar to the effects observed following OXY administration, associated with decreased amygdala activity (Suzuki et al., 2020). Lastly, negative affect is also hypothesized to be one of the risk factors for relapse in AUD (e.g., (Guinle & Sinha, 2020; Koob, 2009; Sliedrecht et al., 2019). A pharmacotherapeutic approach that can regulate negative affect could therefore be a promising strategy to reduce the burden of disease in individuals with AUD who experience negative affect or craving in response to negative affective states. The findings of the second study, that the amygdala response is correlated with situational craving following cue exposure, although no direct treatment effect of OXY on alcohol craving was observed, suggest that OXY may not be effective as a conventional anti-craving medication. Rather, it may have potential as a short-term intervention for individuals experiencing acute negative affective states which may lead to craving. The favorable safety profile, the absence of psychoactive effects and, thus, a low

abuse potential, as well as the rapid onset and short half-life, support the potential of OXY as a candidate for acute as-needed interventions in AUD. In addition to the observed effects, previous research suggests that OXY may also exert a positive effect on the HPA axis and may have anxiolytic properties (Lee & Weerts, 2016; Martinetz & Neumann, 2016; Takayanagi & Onaka, 2021), which could be particularly beneficial during negative affective states.

Taken together, the results of both empirical studies are in line with the theoretical framework of AUD postulated by Koob (2015, 2024) and suggest that both, CBD and OXY, may target different stages of the addiction cycle. CBD's effects on NAc cue-reactivity and craving suggest modulating effects during the *binge/intoxication stage*. In contrast, OXY's effects on the amygdala activation during emotion processing, that were associated with situational alcohol craving after cue-exposure suggest modulating effects on the stress-related mechanisms during the *withdrawal/negative affect stage*. Therefore, OXY and CBD may represent complementary pharmacological approaches for AUD treatment that may supplement existing approved medications, such as naltrexone and acamprosate, that focus exclusively on alcohol craving.

3.5 Recommendations for Future Work

With regard to future research, there are some recommendations to extend and replicate the current findings. Firstly, larger randomized placebo-controlled trials are needed to investigate the effects of CBD and OXY in a larger sample of male and female individuals with AUD. To date, both CBD and OXY studies have suffered from small sample sizes and limited effect sizes (Bethlehem et al., 2013; Bowen & Neumann, 2017; Kirkland et al., 2022; Quintana et al., 2021). Secondly, studies are needed that apply repeated administration of CBD and OXY (Britch et al., 2021; Lee et al., 2016; Martinetz & Neumann, 2016; Mitchell et al., 2016; Paulus et al., 2022) and investigate alcohol-related outcomes over a longer period of time in order to evaluate long-term effects, including possible side-effects with long-term administration and thus suitability as a potential novel pharmacotherapy. The use of the medication as an "as needed" medication (Melby et al., 2021) and the combination of OXY and CBD with established, already approved medications (Viudez-Martínez et al., 2018a; Zimmermann et al., 2022) may also be evaluated in future studies. In order to be able to provide evidence on clinical efficacy, the samples in future studies should be expanded to include treatment-seeking individuals with more severe AUD or at least

treatment-seeking status should be recorded as a variable (Melby et al., 2022; Stauffer et al., 2019; Tunstall et al., 2019). In addition, individual factors such as age, sex, body weight and comorbidity should be systematically recorded and included in the analyses in future studies (Bis-Humbert et al., 2020; Fischler et al., 2022; Huffmeijer et al., 2013; Matheson et al., 2022; Mellentin et al., 2023; Quintana et al., 2021; Ryabinin & Zhang, 2022). The amount of alcohol previously consumed should also be considered as a variable, as there are findings, at least for OXY, that the effects can be modulated by this (Melby et al., 2022). If female individuals are included in the sample, which is recommended in order to evaluate the treatment effects for the population in which it could be used, the menstrual cycle and the use of hormonal contraceptives should be recorded and controlled for in the analyses, as these can influence the effects of CBD and OXY (Derntl et al., 2008; Domes et al., 2010; Fabris et al., 2022; Hansson & Spanagel, 2020; Kim et al., 2022). While many previous studies analyze self-reported outcomes, as we did in part with regard to cue-induced craving, this may lead to bias (Hurd et al., 2019; Mongeau-Pérusse et al., 2021). Therefore, it is recommended that future studies ensure that validated psychometric measures are used (Mellentin et al., 2023) and that these are ideally supplemented with objective measures or biomarkers. While the dosages used in both empirical studies follow the dose regimens established in other studies, the optimal dose still remains unclear (Bowen & Neumann, 2017; Mongeau-Pérusse et al., 2021; Nona et al., 2019; Quintana et al., 2021). Thus, dose-response effects should be investigated in future studies. As in the first empirical study, it is recommended to measure plasma levels (Calapai et al., 2020; Millar et al., 2018; Quintana et al., 2021) to ensure that the medication has been sufficiently absorbed and to account for interindividual differences in absorption. This would also allow for the differentiation between responders and non-responders in the analysis. In addition, pharmacodynamic effects following administration should be investigated, as previous evidence suggests, that the effects may persist beyond the administration period (Freeman et al., 2020; Gonzalez-Cuevas et al., 2018; Hurd et al., 2019; Lee & Weerts, 2016; Nona et al., 2019). Finally, as some reviews and meta-analyses identified an overall risk of bias, at least for the effects of OXY in many trials (Mellentin et al., 2023) and some studies were unable to replicate previous effects (Mellentin et al., 2023; Ryabinin & Zhang, 2022), which - as discussed in detail in section 3.2 - could be due to methodological differences or short-half-life (Fischler et al., 2022; Mellentin et al., 2023; Ryabinin & Zhang, 2022), methods to reduce bias are strongly recommended.

This includes pre-registration of studies including hypotheses, outcome measures, power calculations and statistical analysis plan before data acquisition, as well as conducting replication studies, reporting of possible null results and increasing power by applying within-subjects study designs or pre-registration of one-sided p-value thresholds (Quintana et al., 2021).

3.6 Conclusion

In conclusion, the results of the presented work provide evidence that both CBD and OXY may represent potential pharmacological approaches for the treatment of AUD. CBD appears to be particularly promising modulator of cue-reactivity in the bilateral NAc and alcohol craving. CBD's effects were specific to the processing of alcohol stimuli, suggesting an effect of CBD on reward processing, which is associated with other alcohol-related outcomes such as relapse risk. Together with the observed reduction of alcohol craving, this points to the potential of CBD to alleviate core symptoms of AUD. Furthermore, the observed correlation between CBD plasma levels, NAc activation and alcohol craving supports the hypothesis of a dose-dependent effect, emphasizing the pharmacological relevance of CBD. In contrast, OXY showed a dampening effect on the amygdala response during processing of negatively valenced facial expressions. This neural response was positively correlated with situational alcohol craving following alcohol cue-exposure with the preferred alcoholic beverage. These findings suggest that OXY may be particularly effective in the context of negative affective states that are associated with increased alcohol craving or heightened risk of relapse. As no direct effect of OXY on alcohol craving was observed, OXY should not be considered as a conventional anti-craving medication. Instead, OXY may warrant further investigation as a potential candidate for acute intervention in high-risk states characterized by negative affect. The distinct neurobiological targets of CBD and OXY suggest either a context-specific application depending on individual high-risk states (i.e., characterized by cue-reactivity and craving or negative affect that contributes to craving or relapse risk) or, due to the complementary efficacy profiles, even a combined strategy with other anti-craving medications (Viudez-Martínez et al., 2018a; Zimmermann et al., 2022), for example in the form of an "as needed medication" (Melby et al., 2021) for anticipated cue-exposure or as an acute intervention for negative affective states when currently approved medications are not sufficiently effective. However, neither CBD nor OXY has yet been approved for the treatment of AUD.

Therefore, further randomized, placebo-controlled studies with larger samples including male and female individuals with AUD are needed. Future research should aim to replicate the encouraging findings, to evaluate the clinical relevance in more severely affected individuals with AUD and to investigate the long-term efficacy through repeated administrations and follow-up periods in order to evaluate the therapeutic potential of these substances.

4 SUMMARY

Over 400 million people worldwide meet the criteria for an alcohol use disorder, which is associated with health impairments, increased mortality and negative social consequences. A key symptom is alcohol craving, which can be triggered by exposure to alcohol stimuli or stressors. Alcohol craving, cue-reactivity and negative affect are considered risk factors for alcohol relapse. Although naltrexone and acamprosate are approved to reduce alcohol craving, their clinical efficacy is limited, despite the urgent need for effective treatments. Therefore, novel effective and well-tolerated treatment approaches are warranted to close the existing treatment gap. Cannabidiol and Oxytocin are considered promising candidates to complement existing treatment approaches due to their potential effects on central mechanisms of alcohol use disorder. Preclinical and preliminary clinical studies indicate positive effects of Cannabidiol on substance use and craving, relapse, impulsivity and alcohol-related damage to the liver and brain. Oxytocin has been shown to have positive effects on alcohol craving, alcohol withdrawal symptoms, stress regulation and the reduction of negative affect. However, previous studies involved in most cases small, often exclusively male samples. Therefore, the aim of this dissertation was to investigate the effects of Cannabidiol on the reduction of alcohol cue-reactivity in the nucleus accumbens and alcohol craving as well as the effects of Oxytocin on the reduction of amygdala activation during emotion processing and alcohol craving. In the first randomized double-blind placebo-controlled study, 28 male and female individuals with alcohol use disorder received either 800mg Cannabidiol or a placebo capsules. Afterwards a combined stress- and alcohol-exposure as well as an examination of neuronal alcohol cue- reactivity using functional magnetic resonance imaging and repeated measurements of subjective alcohol craving were conducted. In the second randomized double-blind placebo-controlled cross-over study, 24 male and female individuals with alcohol use disorder each received 24 IU oxytocin intranasally and a placebo once. This was followed by alcohol exposure with the preferred alcoholic beverage and an examination of the neuronal activation of the amygdala during emotion processing using functional magnetic resonance imaging and repeated measurements of subjective alcohol craving. Cannabidiol reduced bilateral activation of the nucleus accumbens as well as alcohol craving while being presented with alcohol stimuli during functional magnetic resonance imaging. Compared to placebo, a smaller increase in alcohol craving after the combined stress and alcohol

exposure was observed. Cannabidiol blood plasma-levels were significantly higher after Cannabidiol administration and correlated negatively with the cue-reactivity of the nucleus accumbens, indicating a dose-dependent effect of cannabidiol. Oxytocin attenuated the activation of the right amygdala during the processing of negative emotional stimuli, which correlated with situational alcohol craving after the alcohol exposure. However, no direct effect of Oxytocin on alcohol craving was observed. Both substances were well tolerated, can be administered non-invasively and have a low abuse potential. While Cannabidiol effectively reduces cue-reactivity and craving, Oxytocin does not appear to be effective as pharmacological treatment for alcohol craving. Oxytocin, on the other hand, could be effective as an intervention for negative affective states when alcohol craving occurs as a result, which in turn may be associated with an increased risk of relapse. Oxytocin and Cannabidiol target distinct neurobiological mechanisms underlying alcohol use disorder, suggesting either a context-specific application based on the risk factors (cue-reactivity or negative affect) or a combination with existing pharmacotherapies, for example as an as-needed medication in the case of alcohol exposure or acute negative affective states, when currently approved medications are insufficient. Since neither Oxytocin nor Cannabidiol are currently approved for the treatment of alcohol use disorder and both empirical studies only investigated acute effects in individuals with mild to moderate alcohol use disorder, larger randomized placebo-controlled trials including males and females with more severe alcohol use disorder and multiple applications are needed to replicate the results and evaluate the clinical relevance over a longer period of time.

5 ZUSAMMENFASSUNG

Weltweit erfüllen über 400 Millionen Menschen die Kriterien einer Alkoholkonsumstörung, die mit gesundheitlichen Beeinträchtigungen, erhöhter Sterblichkeit und negativen sozialen Folgen einhergeht. Ein zentrales Symptom ist das Alkoholverlangen, das durch die Exposition mit Alkoholreizen oder Stressoren ausgelöst werden kann. Alkoholverlangen, Reizreaktivität und negativer Affekt gelten als Risikofaktoren für Alkoholrückfälle. Obwohl der Bedarf einer wirksamen Behandlung hoch ist und bisher zugelassene Pharmakotherapien wie Naltrexon und Acamprosat auf die Reduktion von Alkoholverlangen abzielen, zeigen diese nur eine begrenzte Effektivität. Daher sind neue wirksame und gut verträgliche Behandlungsansätze erforderlich, um die bestehende Versorgungslücke zu schließen. Cannabidiol und Oxytocin gelten aufgrund der potentiellen Effekte auf zentrale Mechanismen der Alkoholkonsumstörung als vielversprechende Kandidaten zur Ergänzung bisheriger medikamentöser Behandlungsansätze. Präklinische und erste klinische Studien deuten auf positive Effekte von Cannabidiol auf Substanzkonsum und -verlangen, Rückfall, Impulsivität und alkoholbezogene Schädigungen der Leber und des Gehirns hin. Für Oxytocin wurden positive Effekte auf Alkoholverlangen, Alkoholentzugssymptome, Stressregulation und die Reduktion von negativem Affekt gezeigt. Frühere Studien basierten jedoch überwiegend auf kleinen, oft ausschließlich männlichen Stichproben. Ziel dieser Dissertation war es daher die Effekte von Cannabidiol auf die Reduktion der Alkohol-Reizreaktivität im Nucleus Accumbens und das Alkoholverlangen sowie die Effekte von Oxytocin auf die Reduktion der Amygdala-Aktivierung während der Emotionsverarbeitung und das Alkoholverlangen zu untersuchen. In der ersten randomisierten doppelblinden Placebo-kontrollierten Studie erhielten 28 Männer und Frauen mit Alkoholkonsumstörung entweder 800mg Cannabidiol oder ein Placebo in Kapselform. Anschließend erfolgte eine kombinierte Stress- und Alkoholexposition sowie eine Untersuchung der neuronalen Alkohol-Reizreaktivität mittels funktioneller Magnetresonanztomographie und wiederholte Messungen des subjektiven Alkoholverlangens. In der zweiten randomisierten doppelblinden Placebo-kontrollierten Crossover Studie erhielten 24 Männer und Frauen mit Alkoholkonsumstörung intranasal jeweils einmal 24 IE Oxytocin und einmal ein Placebo. Anschließend wurde eine Alkoholexposition mit dem bevorzugten alkoholischen Getränk sowie eine Untersuchung der neuronalen Aktivierung der Amygdala während der Emotionsverarbeitung mittels funktioneller Magnetresonanztomographie und

wiederholte Messungen des subjektiven Alkoholverlangens durchgeführt. Cannabidiol reduzierte die bilaterale Aktivierung des Nucleus Accumbens sowie das Alkoholverlangen bei der Betrachtung von Alkoholreizen im funktionellen Magnetresonanztomographen. Verglichen mit dem Placebo zeigte sich ein geringerer Anstieg des Alkoholverlangens nach der kombinierten Stress- und Alkoholexposition. Die Cannabidiolspiegel im Blutplasma waren signifikant höher nach der Gabe von Cannabidiol und korrelierten negativ mit der Reizreaktivität des Nucleus Accumbens, was auf eine dosisabhängige Wirkung von Cannabidiol hindeutet. Oxytocin reduzierte die Aktivierung der rechten Amygdala während der Verarbeitung negativer emotionaler Stimuli, was mit situativem Alkoholverlangen nach der Alkoholexposition korrelierte. Ein direkter Effekt von Oxytocin auf das Alkoholverlangen konnte nicht beobachtet werden. Beide Substanzen wurden gut vertragen, sind nicht-invasiv applizierbar und weisen ein geringes Missbrauchspotential auf. Während Cannabidiol insbesondere auf die Reduktion von Reizreaktivität und Alkoholverlangen wirkt, scheint Oxytocin nicht als Medikation gegen Alkoholverlangen geeignet zu sein. Oxytocin könnte dagegen als Intervention bei negativem Affekt wirksam sein, wenn infolge von negativem Affekt Alkoholverlangen auftritt, das mit einem erhöhten Rückfallrisiko einhergehen kann. Oxytocin und Cannabidiol zielen auf unterschiedliche neurobiologische Mechanismen der Alkoholkonsumstörung ab was entweder für eine kontextspezifische Anwendung je nach Risikosituation (Alkohol-Reizreaktivität oder negativer Affekt) oder für eine Kombination mit bisher zugelassenen Medikamenten, zum Beispiel als Bedarfsmedikation bei bevorstehender Alkoholexposition oder akutem negativen Affekt, wenn bisher zugelassene Medikamente nicht ausreichend wirksam sind, spricht. Da bisher weder Oxytocin noch Cannabidiol zur Behandlung der Alkoholkonsumstörung zugelassen sind und in beiden empirischen Studien ausschließlich akute Effekte bei Individuen mit leichter bis mittelgradiger Alkoholkonsumstörung untersucht wurden, sind größere, randomisierte Placebo-kontrollierte Studien mit männlichen und weiblichen Personen mit stärker ausgeprägter Alkoholkonsumstörung und mehrfacher Applikation erforderlich, um die Ergebnisse zu replizieren und die klinische Relevanz über einen größeren Zeitraum zu evaluieren.

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8 PUBLICATION LIST

Included Publications

Zimmermann, S., Teetzmann, A., Baeßler, J. et al. Acute cannabidiol administration reduces alcohol craving and cue-induced nucleus accumbens activation in individuals with alcohol use disorder: the double-blind randomized controlled ICONIC trial (2024). *Molecular Psychiatry*, 1-8, https://doi.org/10.1038/s41380-024-02869-y.

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Other Publications / Co-Authorship

Bach, P., Zaiser, J., **Zimmermann, S**., Gessner, T., Hoffmann, S., Gerhardt, S., ... & Kiefer, F. (2024). Stress-induced sensitization of insula activation predicts alcohol craving and alcohol use in alcohol use disorder. *Biological Psychiatry, 95*(3), 245-255.

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