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Neuroimaging biomarkers in Alcohol Use Disorder: clinical relevance and relapse prediction.

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Preface

This work is a cumulative dissertation based on empirical studies, and part of the results have already been published as peer-reviewed publications. Therefore, certain sections, tables, or figures of this thesis will be identical to these publications. Please find the list of peer-reviewed publications below.

Publication 1: Tan, H., Hubertus, S., Thomas, S., Lee, A. M., Gerhardt, S., Gerchen, M. F., Sommer, W. H., Kiefer, F., Schad, L., & Vollstädt-Klein, S. (2023). Association between iron accumulation in the dorsal striatum and compulsive drinking in alcohol use disorder. Psychopharmacology, 240(2), 249-257. https://doi.org/10.1007/s00213-022-06301-7.

Publication 2: Tan, H., Gerchen, M. F., Bach, P., Lee, A. M., Hummel, O., Sommer, W., Kirsch, P., Kiefer, F., & Vollstädt-Klein, S. (2023). Decoding fMRI alcohol cue reactivity and its association with drinking behaviour. BMJ Mental Health, 26(1), e300639. https://doi.org/10.1136/bmjment-2022-300639.

For **Publication 1**, the corresponding chapter in the dissertation is **Empirical Studies 2.1 Study 1**. For **Publication 2**, the corresponding chapter in the dissertation is **Empirical Studies 2.2 Study 2**. The detailed description of the personal contribution to each of the publication are listed in the table below.

Work steps	**Publication 1**	**Publication 2**
Conception (%)	95	95
Literature research (%)	95	95
Ethics submission (%)	0	0
Animal experiment application (%)	n/a	n/a
Data collection (%)	0	0
Data evaluation (%)	100	100
Interpretation of results (%)	90	90
Writing the manuscript text (%)	95	95
Revision (%)	90	90
State which figures/tables are the result of this doctoral thesis.	All figures and tables	All figures and tables
Detail which data/figures/tables are based on research by others.	0	0

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ABBREVIATIONS

AAL	Automated Anatomical Labelling atlas
ADS	Alcohol Dependence Scale
AUD	Alcohol Use Disorder
AUQ	Alcohol Urge Questionnaire
BA	Brodmann Area
BBB	Blood-brain barrier
BOLD	Blood oxygenation level dependent
CSF	Cerebrospinal fluid
dCNN	Deep convolutional neural network
dlPFC	Dorsolateral prefrontal cortex
DRKS	Deutsches Register Klinischer Studien (German Clinical Trials Register)
DSM	Diagnostic and Statistical Manual of Mental Disorders
EEG	Electroencephalogram
FDR	False Discovery Rate
FEF	Frontal eye fields
fMRI	Functional magnetic resonance imaging
FWE	Family-wise error
GLM	General linear model
GRE	Gradient echo sequence
HC	Healthy controls
ICD	International Classification of Disease
iRISA	Impaired response inhibition and salience attribution
MEG	Magnetoencephalography
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NAcc	Nucleus accumbens
OCDS	Obsessive Compulsive Drinking Scale
PET	Positron emission tomography
PPI	Psychophysiological interaction
QSM	Quantitative Susceptibility Mapping
RA	Reward Appraisal

ROI	Region of interest
RSA	Representational similarity analysis
RDM	Representational distance matrix
SCID	Structured Clinical Interview for DSM
SPM	Statistical Parametric Mapping
SUD	Substance Use Disorder
SVM	Support Vector Machine
VAS	Visual analogue scale
VOR	Visual Object Recognition

1 INTRODUCTION

Alcohol Use Disorder (AUD) represents one of the world's most significant addiction problems and has a large impact on global public health with associations of morbidity and mortality (Rehm & Shield, 2019; World Health Organization, 2019, 2020). Individuals with AUD could have impaired control over their alcohol consumption and chronical and heavy pattern of alcohol use with a high risk to relapse after detoxification, despite serious detrimental costs to their overall health (American Psychiatric Association, 2013; Carvalho et al., 2019).

1.1 Alcohol Use Disorder: prevalence and diagnosis

The harmful use of alcohol is a causal factor in more than 200 disease and injury conditions, and results in 3 million deaths every year (5.3% of all deaths). AUD is one of the most prevalent addictive disorders (and also mental disorders) in the world, which is affecting 8·6% of men and 1·7% of women based on the report from WHO. Twelve-month prevalence of AUD among adults decreases in middle age, being greatest among individuals 18 to 29-years-old (16.2%) and lowest among individuals age 65 years and older (1.5%) (World Health Organization, 2019, 2020). AUD is associated with a high burden of disease, disability and high mortality through medical conditions such as liver cirrhosis or injury (American Psychiatric Association, 2013; Rehm & Shield, 2019; Samokhvalov et al., 2010). A study including 1158486 person-years from 1987 to 2006 in Denmark, Finland and Sweden showed that people hospitalized with AUD have an average life expectancy of 47–53 years (men) and 50–58 years (women) and die 24–28 years earlier than people in the general population. Moreover, the risk of AUD related mortality is associated with socioeconomic status, and an interaction between alcohol use and socioeconomic status was observed (Westman et al., 2015).

AUD are defined by the Diagnostic and Statistical Manual of Mental Disorders (DSM) (American Psychiatric Association, 2013) and the International Classification of Disease (ICD) (World Health Organization, 2004) and characterized by loss of control over alcohol intake, compulsive alcohol use, and a negative emotional state when not drinking. In the fifth edition of DSM (DSM-5) (American Psychiatric Association, 2013),

AUD is described by a cluster of behavioral and physical symptoms, including withdrawal, tolerance, and craving, which correspond to 11 criteria listed below:
A problematic pattern of alcohol use leading to clinically significant impairment or distress, as manifested by at least two of the following, occurring within a 12-month period:

1. Alcohol is often taken in larger amounts or over a longer period than was intended.
2. There is a persistent desire or unsuccessful efforts to cut down or control alcohol use.
3. A great deal of time is spent in activities necessary to obtain alcohol, use alcohol, or recover from its effects.
4. Craving, or a strong desire or urge to use alcohol.
5. Recurrent alcohol use resulting in a failure to fulfill major role obligations at work, school, or home.
6. Continued alcohol use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of alcohol.
7. Important social, occupational, or recreational activities are given up or reduced because of alcohol use.
8. Recurrent alcohol use in situations in which it is physically hazardous.
9. Alcohol use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by alcohol.
10. Tolerance, as defined by either of the following:
    a. A need for markedly increased amounts of alcohol to achieve intoxication or desired effect.
    b. A markedly diminished effect with continued use of the same amount of alcohol.
11. Withdrawal, as manifested by either of the following:
    a. The characteristic withdrawal syndrome for alcohol (refer to Criteria A and B of the criteria set for alcohol withdrawal, pp. 499-500).
    b. Alcohol (or a closely related substance, such as a benzodiazepine) is taken to relieve or avoid withdrawal symptoms.

Withdrawal symptoms usually develop after reduced intake following prolonged heavy drinking, and they can be unpleasant and intense. To relieve negative affect, AUD

individuals may continue to consume alcohol despite adverse consequences. Tolerance is defined as the same amount of alcohol not bring AUD individuals desired effect. Craving for alcohol is indicated by a strong desire to drink, and it could make AUD individuals difficult to think of anything else. These symptoms of AUD, like in other substance use disorders, have been conceptualized as elements of both impulsivity and compulsivity that yield a composite addiction cycle composed of three stages: 'binge/intoxication', 'withdrawal/negative affect', and 'preoccupation/anticipation' (Koob & Volkow, 2010, 2016).

1.2 Neuroimaging in Alcohol Use Disorder

With the developments of neuroimaging, researchers conducted studies in humans in the past decades to understand AUD (Voon et al., 2020). Neuroimaging can provide a critical window into underlying neural mechanisms of AUD, deliver clinical biomarkers of diagnosis and prognosis, and highlight possible treatment and therapy targets. Structural imaging studies have revealed that chronic alcohol use is accompanied by volume reductions of gray and white matter, as well as microstructural disruption of various white matter tracts (Bühler & Mann, 2011; Pando-Naude et al., 2021). For example, alcohol dependence was found associated with lower thickness more specifically in bilateral putamen, right thalamus, right globus pallidus and left Nucleus accumbens (NAcc) along with bilateral posterior cingulate and superior frontal cortex (Makris et al., 2008). Approaches of positron emission tomography (PET) and Proton Magnetic Resonance Spectroscopy (MRS) have revealed metabolic changes in the brain, from aspects of, e.g., dopaminergic, opioids and Gamma-aminobutyric acid mechanisms (Chen et al., 2021; Heinz et al., 2005). Moreover, using task-based functional magnetic resonance imaging (fMRI), studies focused on cognitive processes that underlie functional network impairments related to alcohol use. Many tasks were developed for investigating the cognitive processes, such as cue-reactivity, inhibitory control and decision making (Voon et al., 2020). In alcohol users, altered activation was observed using these neuropsychological paradigms, and based on previous task-based fMRI findings, Zilverstand et al. summarized the impaired response inhibition and salience attribution (iRISA) model for understanding the mechanism of substance use (including alcohol use), which demonstrated specific impairments within six large-scale brain networks (reward, habit, salience, executive, memory, and self-directed networks) (Zilverstand et al., 2018). Furthermore, some neuroimaging findings could

serve as biomarkers or targets for the treatment in AUD in clinical practice (Garrison & Potenza, 2014; Voon et al., 2020). Meta-Analyses of cue-reactivity fMRI studies report that altered activity in the regions of mesocorticolimbic circuit could be the common feature of AUD individuals (Jasinska et al., 2014), and have a translational value for AUD treatment development.

However, neuroimaging features of AUD still need further description, and the underlying neural mechanisms are not fully understood. This work aimed to identify novel neuroimaging biomarkers of AUD, from the aspects of brain iron accumulation and neural patterns decoding alcohol cues, as well as their clinical relevance.

1.3 Brain iron accumulation

Brain iron concentration has emerged as a potential contributing factor to psychiatric disorders. Previous research found brain iron levels to be associated with aging and neurodegeneration (Möller et al., 2019), but also with some psychiatric disorders, such as mood disorders and schizophrenia (Necus et al., 2019; Yao et al., 2017), whereby the role of concomitant alcohol use remains unclear in these studies. Regarding Substance Use Disorder (SUD), iron accumulation was observed in the globus pallidus of cocaine users, which strongly correlated with overall duration of cocaine use (Ersche et al., 2017). Similarly, iron accumulation in globus pallidus and substantia nigra was found in methamphetamine-exposed animals (Melega et al., 2007). These findings showed that the basal ganglia exhibited an increased iron concentration in SUD. In 2017 (Juhás et al., 2017), brain iron accumulation in the deep gray matter of AUD patients was ascertained from resting-state fMRI signal, by combining multi-channel complex phase signal in raw fMRI data using an adaptive method. Patients exhibited higher iron levels in basal ganglia regions, including caudate nucleus, putamen, globus pallidus and dentate nucleus compared to healthy subjects. Recently, a study based on UK Biobank also found moderate alcohol consumption was associated with higher iron in putamen, caudate and substantia nigra (Topiwala, Wang, Ebmeier, Burgess, Bell, Levey, Zhou, McCracken, Roca-Fernández, et al., 2022). However, these studies of AUD used predefined Regions of interest (ROIs) with a priori knowledge to examine iron accumulation, while some studies in neurodegeneration showed iron could also accumulate at cortical gray matter (Acosta-Cabronero et al., 2013; Ravanfar et al., 2021). Therefore, the current work aimed to compare whole-brain iron levels in individuals with AUD and healthy participants using gradient multi-echo imaging to find

regions with iron accumulation in the context of the whole brain, and further investigate its association with drinking patterns with the goal of developing a brain-iron-related biomarker of AUD.

1.4 Alcohol cue-reactivity

Another construct associated with habitual and compulsive alcohol consumption is cue-reactivity, the enhanced sensitivity to conditioned cues. In AUD, these conditioned cues can trigger conditioned emotional or motivational reactions, which provide the basis for experiencing craving, and comprise the anticipation of reward or the occurrence of withdrawal symptoms in the case of not consuming the substance (Carter & Tiffany, 1999; Koob & Volkow, 2010). Therefore, cue-reactivity may also be suitable to develop biomarkers from. In the literature, three models of cue-reactivity have been proposed (Drummond, 2000): 1) the conditioned withdrawal model, 2) the conditioned compensatory response model and 3) the conditioned appetitive-motivational model, which were recently unified in a framework of addiction (Koob & Volkow, 2016; Lüscher et al., 2020) where cue-reactivity was conceptualized as the motivational change associated with addiction. The neural activity triggered by cues was extensively reported and reviewed in previous publications. Existing neuroimaging evidence suggests that salient cues elicit increases in activity throughout the mesocorticolimbic and nigrostriatal systems (Jasinska et al., 2014). The mesocorticolimbic system reflects the representations of reward values of cues and the motivational processes of incentive salience, and the nigrostriatal system is critical to habit learning and automatic behavior. Moreover, sensory and motor functions were suggested to importantly contribute to the cue-reactivity in addiction. In both animals and humans, the activity of visual cortices could be modulated in viewing reward-mounted cues (Yalachkov et al., 2010). However, most previous studies compared brain activity between an alcohol-condition and a neutral condition with a-priori assumptions on brain response patterns, and reported cue-triggered brain activity as a contrast (Carter & Tiffany, 1999; Jasinska et al., 2014; Zeng et al., 2021). These studies usually only assessed the alcohol-cue-elicited activation (Jasinska et al., 2014; Voon et al., 2020), while the sub-processes leading to this change in the brain remain elusive. For example, how does the brain recognize the alcohol cues, and how are reward values represented in the brain? Until now, only a few studies considered the different reward values of alcohol cues in cue-reactivity tasks, which could help to

understand the process of reward appraisal in AUD individuals. Therefore, the second aim of the current work was to separately model processes of Visual Object Recognition (VOR) and Reward Appraisal (RA) in cue-reactivity, and examine if the altered patterns of neural activity of AUD were associated with clinical features in order to develop a potential biomarker. Moreover, the levels of enhancement of the neural patterns were then used for predicting relapse within six months to investigate their translational values.

1.5 Basis of the current work

To measure brain iron, the current work used an emerging magnetic resonance imaging (MRI) technique, Quantitative Susceptibility Mapping (QSM), with gradient multi-echo images, which were collected by and compiled from several previously conducted studies. QSM calculates the tissue-frequency shift using phase information at different echo times from gradient echo images, and then reconstructs the susceptibility maps (Haacke et al., 2015; Kurz et al., 2021; Möller et al., 2019; Wang & Liu, 2015). Studies have shown that in gray matter structures, there is a strong linear correlation between chemically determined iron concentration and bulk magnetic susceptibility (Langkammer et al., 2012). This method has been extensively validated to be able to identify altered deep gray matter iron in normal aging as well as in many neurological disorders (Deistung et al., 2017; Haacke et al., 2015; Wang & Liu, 2015). FMRI is a method developed over the past few decades to allow mapping of the functioning human brain, which provides researchers an opportunity to acquire in-vivo brain images, and it is noninvasive, low-risk, with no radiation involved (Ulmer, 2013). The most widely used fMRI approach is blood oxygenation level dependent (BOLD) contrast, which results from magnetic susceptibility effects due to deoxyhemoglobin. Red blood cells are relatively oxygenated in the active state, since blood supply greatly exceeds oxygen demand, resulting in only a small perturbation of the main magnetic field. In the resting state, they are relatively deoxygenated, and signal from nearby protons is spoiled (Detre & Wang, 2002). As the physiological basis, a complex interaction between changes in blood flow, blood volume, and oxygenation consumption accompanying neural activity leads to the change of BOLD signal, and BOLD fMRI allows an image spatial resolution that is of the order of a few millimeters with a temporal resolution of a few seconds (Matthews & Jezzard, 2004).

By using BOLD fMRI together with psychological paradigms, researchers are able to indirectly detect the increase in neuronal activity at the moment that a person inside the scanner performs a particular task, compared to another moment when that task is not executed. The signal intensity of each voxel within the image could be compared to the expected BOLD responding to the task, to detect regional signal changes in the brain correlated with the behavior in the paradigm (Matthews & Jezzard, 2004; Smith, 2004).

In the current work, fMRI data were modeled with representational similarity analysis (RSA), a multivariate technique to examine neural patterns (Kriegeskorte et al., 2006; Kriegeskorte et al., 2008). This novel approach aims to measure brain-activity patterns with computational models, which reflect hypothesized brain information processes. Using a representational distance matrix (RDM), it characterizes response patterns elicited by a set of stimuli. RDMs could reflect the distinctions between stimuli in both neural activity and computational models, and the distance between neural RDM and model RDM could indicate the regional involvements of brain networks during information processing. Based on RSA, searchlight analysis provides a method of continuously mapping pattern information throughout the entire measured volume (Kriegeskorte et al., 2006). The searchlight RSA of fMRI data is carried out with spherical clusters of voxels centered at each voxel, which are used to calculate neural RDMs. Then an RDM-correlation map for each model RDM can be obtained to reveal the brain regions representing the hypothesized models.

This work was based on secondary analyses of a large dataset from previous studies (Bach et al., 2021; Bach et al., 2019; De Santis et al., 2019; Gerchen et al., 2021; Hansson et al., 2018; Karl et al., 2021; Vollstädt-Klein et al., 2020; Vollstädt-Klein et al., 2011), which included gradient multi-echo images for measuring brain iron and task-based fMRI of alcohol cue-reactivity. For constructing models of reward appraisal, a standalone dataset of attribute-rating task (outside the MRI scanner) for the stimuli of the alcohol cue-reactivity task were used. Besides, clinical datasets including assessments of drinking patterns, severity of AUD and six months relapse of a subsample were used to examine the relationship between information processing patterns and clinical features, which is important for the clinical evaluation of the findings to develop neuroimaging biomarkers.

1.6 Aims of this work

This work aimed to find biomarkers from the aspects of brain iron accumulation and neural patterns decoding alcohol-related cues, as well as the clinical relevance of these biomarkers. It is hypothesized that,

H1: Brain iron was accumulated in AUD individuals, especially in the basal ganglia.

H2: Brain iron accumulation was associated with amount of previous drinking, with AUD severity and with previous obsessive-compulsive drinking patterns.

H3: AUD individuals showed different neural patterns decoding the processes of visual object recognition and reward appraisal of alcohol cues compared to healthy participants.

H4: The involvements of neural patterns were correlated with craving, with obsessive-compulsive drinking patterns and with AUD severity.

H5: Decoding involvement of enhanced neural patterns of cue-reactivity predicted relapse within six months.

Please note that the results of this thesis have already been published. The results examining hypotheses 1 and 2 were published in Study 1 (Tan, Hubertus, et al., 2023) and the results examining hypotheses 3, 4 and 5 were published in Study 2 (Tan, Gerchen, et al., 2023) by the doctoral candidate as a first author. Therefore, certain sections, tables, or figures of this thesis will be identical to these publications.

2 EMPIRICAL STUDIES

2.1 Study1: Association between iron accumulation in the dorsal striatum and compulsive drinking in Alcohol Use Disorder[1]

2.1.1 Abstract

Rationale: Brain-iron accumulation has been observed in neuropsychiatric disorders, and shown to be related to neurodegeneration.
Objectives: In this study, we used Quantitative Susceptibility Mapping (QSM), an emerging MRI technique developed for quantifying tissue magnetic susceptibility, to examine brain-iron accumulation in individuals with Alcohol Use Disorder (AUD) and its relation to compulsive drinking.
Methods: Based on our previous projects, QSM was performed as a secondary analysis with gradient echo sequences images, in 186 individuals with AUD and 274 healthy participants. Whole-brain susceptibility values were calculated with morphology-enabled dipole inversion and referenced to cerebrospinal fluid. Then the susceptibility maps were compared between AUD individuals and healthy participants. The relationship between drinking patterns and susceptibility was explored.
Results: Whole-brain analyses showed that the susceptibility in dorsal striatum (putamen and caudate) among AUD individuals was higher than healthy participants, and was positively related to the Obsessive Compulsive Drinking Scale (OCDS) scores and the amount of drinking in the past three months.
Conclusions: Increased susceptibility suggests higher iron accumulation in dorsal striatum in AUD. This surrogate for the brain-iron level was linearly associated with the compulsive drinking pattern and the recent amount of drinking, which provides us a new clinical perspective in relation to brain iron accumulation, and also might indicate an association of AUD with neuroinflammation as a consequence of brain iron accumulation. The iron accumulation in striatum is further relevant for functional imaging studies in AUD by potentially producing signal dropout and artefacts in fMRI images.

[1] **Published as:** Tan, H., Hubertus, S., Thomas, S., Lee, A. M., Gerhardt, S., Gerchen, M. F., Sommer, W. H., Kiefer, F., Schad, L., & Vollstädt-Klein, S. (2023). Association between iron accumulation in the dorsal striatum and compulsive drinking in alcohol use disorder. Psychopharmacology, 240(2), 249-257. https://doi.org/10.1007/s00213-022-06301-7.

2.1.2 Introduction

As a chronic relapsing disease, Alcohol Use Disorder (AUD) represents one of the world's most significant addiction problems and has a large impact on global public health. It is characterized by recurrent compulsive alcohol-use despite significant alcohol-related behavioral, cognitive, physiological, and social problems. The Diagnostic and Statistical Manual of Mental Disorders fifth version (DSM-5) criteria of substance use disorder also emphasizes the compulsive quality as a central aspect of addiction (American Psychiatric Association, 2013).

The underlying neurobiological mechanism of compulsive consumption is currently still not fully understood. Converging evidence suggests the dorsal striatum to be critical in compulsive drug-seeking. In animal studies, a large increase in dopamine levels was observed in the dorsal striatum in long-term cocaine-use (Ito et al., 2002), and when inactivating the dorsolateral striatum, the habitual behavior was reduced (Vanderschuren et al., 2005). In addition to the deep gray matter of the striatum, a circuit involving the frontal cortex is suggested to be important for the development of compulsivity. In human imaging studies, our previous results from cue-reactivity tasks indicated that the cue-induced activation of the ventral striatum in social drinkers is higher than in heavy drinkers, while in heavy drinkers it was higher in dorsal striatum (Vollstädt‐Klein et al., 2010). This suggested that dorsal striatum became the dominant region in compulsive alcohol use. In 2013, Sjoerds and colleagues also found a dysfunction of the anterior putamen in alcohol-dependent patients using an instrumental learning task, which was related to habit control (Sjoerds et al., 2013). From an anatomical perspective, studies using structural MRI have indicated that basal ganglia were affected in alcohol-users, including the caudate, putamen and nucleus accumbens (Fritz et al., 2022).

Brain iron concentration has emerged as a potentially contributing factor to psychiatric disorders. In 2017 (Juhás et al., 2017), brain iron accumulation in the deep gray matter of AUD patients was ascertained from resting-state functional MRI (fMRI) signal, by combining multi-channel complex phase signal in raw fMRI data using an adaptive method. Patients exhibited higher iron levels in basal ganglia regions including caudate nucleus, putamen, globus pallidus and dentate nucleus compared to healthy subjects. Recently, a study based on UK Biobank also found moderate alcohol consumption was associated with higher iron in putamen, caudate and substantia nigra (Topiwala, Wang, Ebmeier, Burgess, Bell, Levey, Zhou, McCracken, Roca-Fernández, et al., 2022).

Previous research found brain iron levels to be associated with aging and neurodegeneration (Möller et al., 2019), but also with some psychiatric disorders, such as mood disorders and schizophrenia (Necus et al., 2019; Yao et al., 2017), whereby the role of concomitant alcohol use remains unclear in these studies. With regards to Substance Use Disorder (SUD), iron accumulation was observed in globus pallidus of cocaine users, which strongly correlated with overall duration of cocaine use (Ersche et al., 2017). Similarly, accumulation of iron in globus pallidus and substantia nigra was found in methamphetamine-exposed animals (Melega et al., 2007). These findings showed that in SUD the basal ganglia exhibited an increased iron concentration. The mechanism of this restricted pattern of iron accumulation in the brain are not well understood. The profound effect of alcohol on systemic iron storage is well established (Duane et al., 1992; Whitfield et al., 2001), and animal studies suggest an involvement of dopamine signaling in brain iron metabolism (Ben-Shachar et al., 1993; Ben-Shachar & Youdim, 1990). Interestingly, the basal ganglia, especially the ventral and dorsal striatum, as mentioned above, are also at the core of the shift from hedonic to compulsive consumption.

Quantitative Susceptibility Mapping (QSM) is an emerging MRI technique. It calculates the tissue-frequency shift using phase information at different echo times from gradient echo images, and then reconstructs the susceptibility maps (Haacke et al., 2015; Kurz et al., 2021; Möller et al., 2019; Wang & Liu, 2015). Studies have shown that in gray matter structures there is a strong linear correlation between chemically determined iron concentration and bulk magnetic susceptibility (Langkammer et al., 2012). This method has been extensively validated to be able to identify altered deep gray matter iron in normal aging as well as in many neurological disorders (Deistung et al., 2017; Haacke et al., 2015; Wang & Liu, 2015).

Here, we applied QSM with gradient multi-echo imaging collected by and compiled from several previously conducted fMRI studies to compare brain iron levels in individuals with AUD and healthy participants. We hypothesized that AUD individuals show increased accumulation of brain iron especially in the basal ganglia, and that the concentration of brain iron relates to compulsive drinking.

2.1.3 Methods

Participants

This study was based on previous projects (Bach et al., 2021; Bach et al., 2019; De Santis et al., 2019; Gerchen et al., 2021; Hansson et al., 2018; Karl et al., 2021; Vollstädt-Klein et al., 2020) in AUD conducted in our lab (Supplementary Table 2.1-1), which were all designed with similar inclusion criteria and used the same gradient echo sequence (GRE). Data were collected for 186 AUD individuals (DSM-IV or DSM-5 criteria, see supplementary material) and 274 healthy participants recruited between 2011 and 2019 at the Central Institute of Mental Health, Mannheim, Germany. The demographic and clinical overview of the participants is summarized in Table 2.1-1. AUD individuals did not use other substances except nicotine, which was verified by a urine drug screen (nal von minden GmbH Drug-Screen® Diptest, Version 1.0). The healthy participants had no history of alcohol or drug addiction or any current psychiatric disorder. Participants in both groups were excluded if they had any history of serious medical (including psychiatric or neurological) complications, brain injury, use of psychotropic medications (other than during the detoxification process), or did not meet magnetic resonance safety criteria for our imaging facility, for example because of metal-implants or pregnancy.

Before taking part in the scanning procedure, participants completed the following questionnaires: Form90 (Scheurich et al., 2005), the Alcohol Dependence Scale (ADS, (Kivlahan et al., 1989)), the Alcohol Urge Questionnaire (AUQ, (Bohn et al., 1995)) and the Obsessive Compulsive Drinking Scale (OCDS, (Anton et al., 1995; Mann & Ackermann, 2000)) (For details, Tabular Appendix). With the Form90 retrospectively recorded the amount of alcohol drunk everyday, and calculated the cumulative amount in the past 90 days. With the Form90 the amount of daily alcohol consumption was assessed, and the cumulative amount in the past 90 days was calculated. All participants provided informed written consent according to the declaration of Helsinki, and all projects in this study were approved by the ethics committee of the University of Heidelberg.

Table 2.1-1 Group characteristics of all participants (N=460).

	AUD Individuals	Healthy Controls	Statistics (T/χ2-value)	df	p-value
N	186	274			
Age (years)	48.3 ± 10.8	37.5 ± 15.3	8.337	458	<0.001

Sex (female)	34, 18.3%	66, 24.2%	1.924	1	0.165
Duration of drinking (years)	19.8±12.9	-	-	-	-
Cumulative amount of alcohol (gram in the last 90 days) [a]	15332.2 ± 13752.9	1092.1 ± 3967.3	9.227	206	<0.001
Current smoke (yes)	115, 67.6%	30, 13.8%	116.206	1	<0.001
ADS score	11.8 ± 8.0	2.5 ± 3.5	9.271	130	<0.001
AUQ score	13.5±6.5	10.3±3.6	4.503	218	<0.001
OCDS global [b]	14.8 ± 7.5	2.8 ± 3.9	15.619	251	<0.001

Abbreviations: Alcohol Dependence Scale (ADS), Alcohol Urge Questionnaire (AUQ) and the Obsessive Compulsive Drinking Scale (OCDS).

a. Based on FORM90.

b. Calculation rules of OCDS based on Mann et al. (Mann & Ackermann, 2000).

MRI acquisition

Neuroimaging data was acquired using a Siemens 3 Tesla whole-body-tomograph (MAGNETOM Trio, TIM technology, Siemens, Erlangen, Germany) with a 12-channels head coil. A multislice 2D-GRE was used for the QSM analysis: TR = 358 ms; TE1 = 5.19 ms and TE2 = 7.65 ms; matrix size = 64 × 64 × 42; voxel size = 3 × 3 × 3 mm3; flip angle = 60°. This sequence was originally implemented as a sequence for fieldmap correction of fMRI data to control for distortions of the functional images in the previous projects.

Quantitative susceptibility mapping (QSM)

The GRE raw data were reconstructed manually by using a sum-of-squares approach for the magnitude and exponential addition for the phase after referencing the phase of each channel to the first echo. QSM reconstruction was done with the MEDI toolbox from Cornell MRI Research Lab (de Rochefort et al., 2010; Liu et al., 2011), which included procedures of fitting the complex MRI data, phase unwrapping with a region- growth approach, brain mask generation with morphological operators and 5 mm erosion of the boundary, background field removal by solving the Laplacian boundary value (Sun & Wilman, 2014; Zhou et al., 2014), Furthermore, field inversion with MEDI used a weighting factor of 1000, which was based on the parameter optimization (from 10^-3 to 10^6) with 10%

random sub-sampling (for detailed methodological description see (Hubertus et al., 2019a, 2019b)), and the susceptibility maps were also referenced to the averaged susceptibility in the cerebrospinal fluid (CSF). The CSF-referenced susceptibility values were relative values without units.

Data analysis

The CSF-referenced susceptibility maps were then normalized using SPM12 (Wellcome Centre for Human Neuroimaging, London, UK, https://www.fil.ion.ucl.ac.uk/spm/) to SPM12 TPM MNI template for statistical comparison. Whole-brain susceptibility values for each subject were included in a one-tailed t-test to find brain regions with differences in QSM intensity between the groups of AUD and healthy participants. Age and current smoke status were added as covariates of no interest. Although we had specific hypotheses for the basal ganglia, we conducted whole brain analyses to also exploratory look at other brain regions. A voxel-wise-threshold of $P< 0.001$ in combination with a cluster-extent-threshold determined with random field theory in SPM12 was used for a corresponding cluster-level family-wise error (FWE) significance threshold of $P< 0.05$. We then generated a region of interest (ROI) using the significant voxels of the group-comparison, and averaged the susceptibility values in this ROI. A linear partial correlation - controlling for age and smoke-status was conducted between the mean susceptibility within the ROI and psychometric variables using SPSS (Statistical Package of the Social Sciences, version 25; SPSS Inc., Chicago, IL, USA). Correlation analyses were done in all participants, because in group of healthy participants there were also light to moderate drinkers, which could bring more information on linear relations between susceptibility and psychometrics. Psychometric data included the sum of ADS score, AUQ score, and the OCDS global score, according to the calculation-rules from a previous study (Mann & Ackermann, 2000).

2.1.4 Results

Whole-brain susceptibility in AUD and healthy participants

To examine the brain-iron level, we voxel-wise compared the whole-brain susceptibility in AUD individuals with the healthy participants. AUD individuals showed increased CSF-referenced susceptibility in the bilateral putamen and caudate (Table 2.1-2 and

Figure 2.1-1). This revealed higher iron accumulation in the dorsal striatum of AUD individuals.

Table 2.1-2 Brain areas with increased susceptibility in AUD individuals compared to healthy controls.

(n=460 subjects, combined voxel-wise- [p<0.001] and FWEc=29 voxels, corresponding to cluster-pFWE<0.05).

Side	Brain regions	Percentages in cluster	Cluster size	MNI coordinates (x y z)	tmax
Left	Caudate	86.4% [a]	44	-20 -20 22	5.1025
Right	Caudate	83.6% [b]	67	18 2 20	5.0614
Right	Putamen	100%	37	28 6 6	4.6831
Left	Putamen	100%	29	-28 -2 6	4.0510

a and b. the rest voxels of these two clusters were unlabeled in AAL-Atlas.

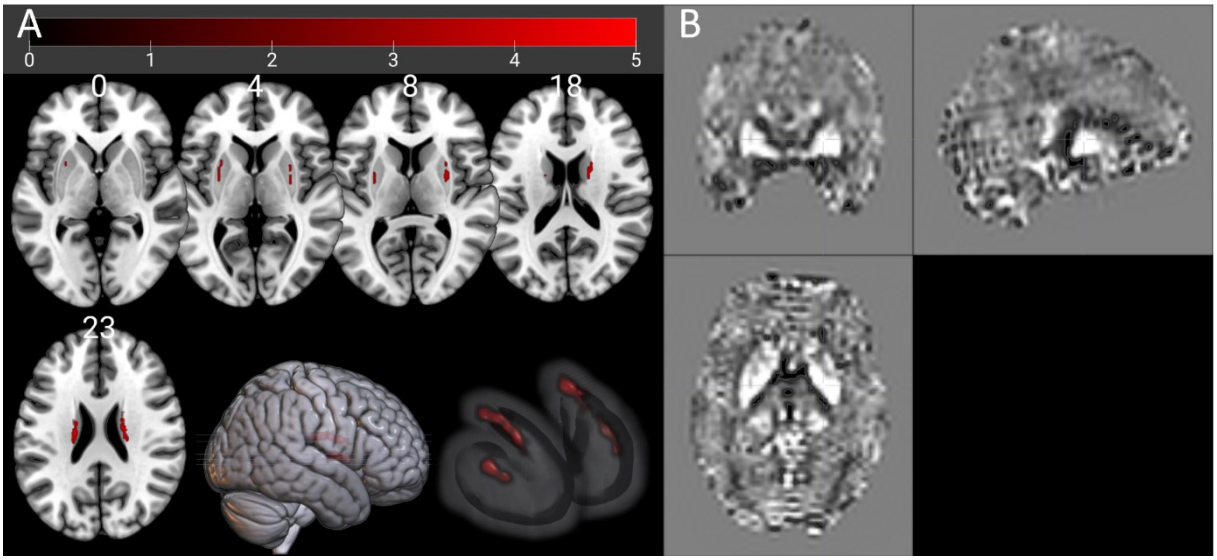


Figure 2.1-1 Brain regions with iron accumulation.

A) whole-brain two-sample t-test of susceptibility between AUD individuals and healthy participants (combined voxel-wise- [p<0.001] and extent threshold FWEc=29 voxels, corresponding to cluster-pFWE<0.05); B) an exemplary susceptibility map from an AUD individual. This figure is from published work (Tan, Hubertus, et al., 2023).

Correlation of susceptibility and psychometrics

To explore whether the increased susceptibility in dorsal striatum is related to the pattern of alcohol-consumption, further correlation analyses were conducted. The mean susceptibility in the ROI of all 177 voxels in four clusters based on the results of whole brain analysis was positively linearly correlated to the cumulative amount of alcohol consumption in the past three months, controlling for age and smoke-status (Table 2.1-3 and Figure 2.1-2). Furthermore, the ROI-susceptibility was also significantly correlated to OCDS global scores (Table 2.1-3 and Figure 2.1-3). There was no significant correlation with ADS and AUQ observed (Supplementary Figure 2.1-1 and Supplementary Figure 2.1-2), and the linear correlations were not significant within the AUD group.

Table 2.1-3 Correlation of mean ROI-susceptibility and psychometric variables in all participants.

controlled age and smoke-status	All participants			AUD group		
	coefficient	df	P	coefficient	df	P
Cumulative amount of drinking (gram in the last 90 days)	0.201	185	0.006	0.068	110	0.478
Global score of OCDS [a]	0.146	225	0.028	-0.072	118	0.434
Sum of ADS	0.139	120	0.127	0.190	40	0.228
Sum of AUQ	0.118	201	0.093	0.097	104	0.320

Abbreviations: Alcohol Dependence Scale (ADS), Alcohol Urge Questionnaire (AUQ) and the Obsessive Compulsive Drinking Scale (OCDS).

a. Calculation rules of OCDS based on Mann et al. (Mann & Ackermann, 2000).

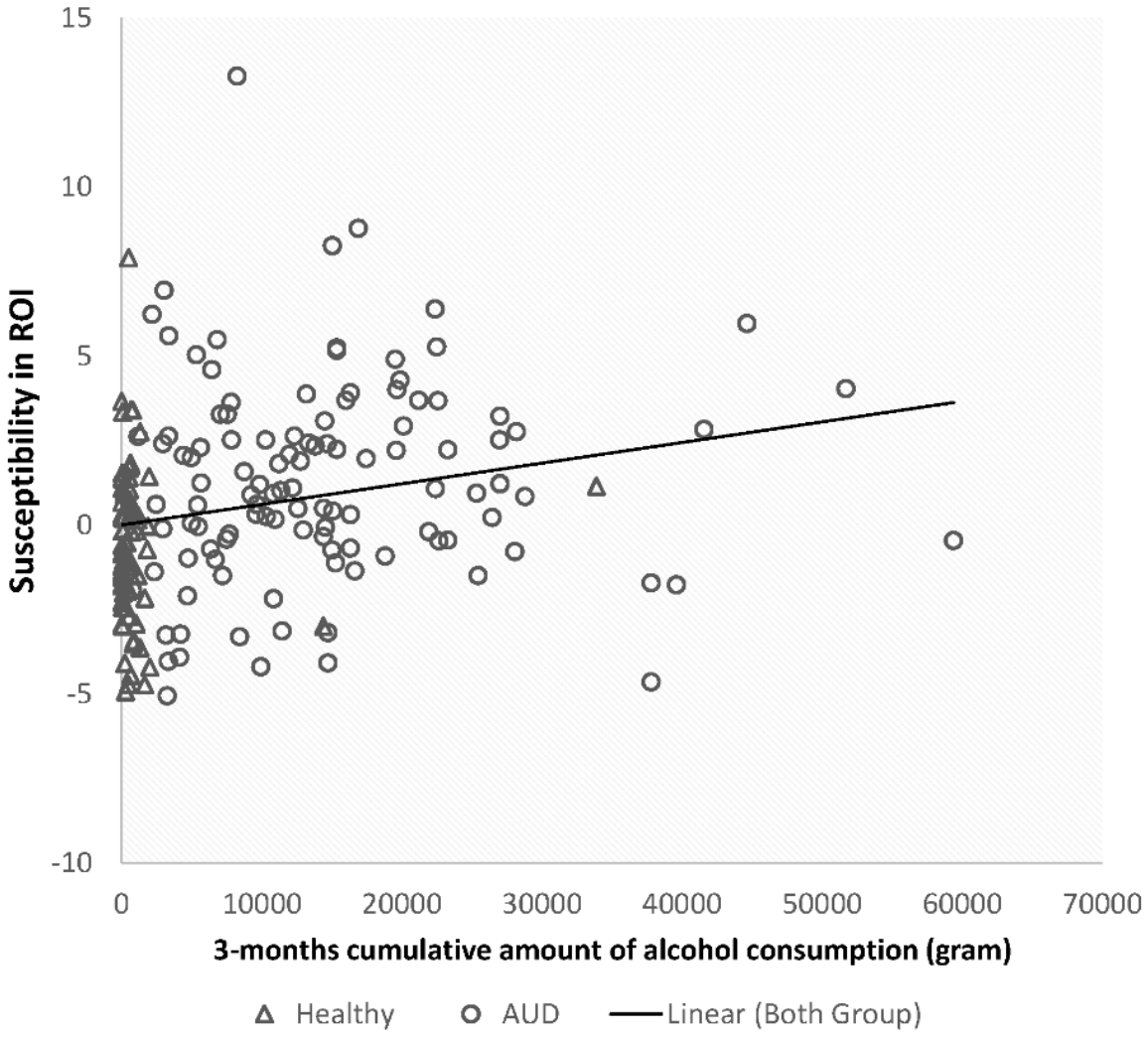


Figure 2.1-2 Correlation of the cumulative amount of drinking (alcohol in gram, in the last 90 days period) and susceptibility, controlling for age and smoking-status.

This figure is from published work (Tan, Hubertus, et al., 2023).

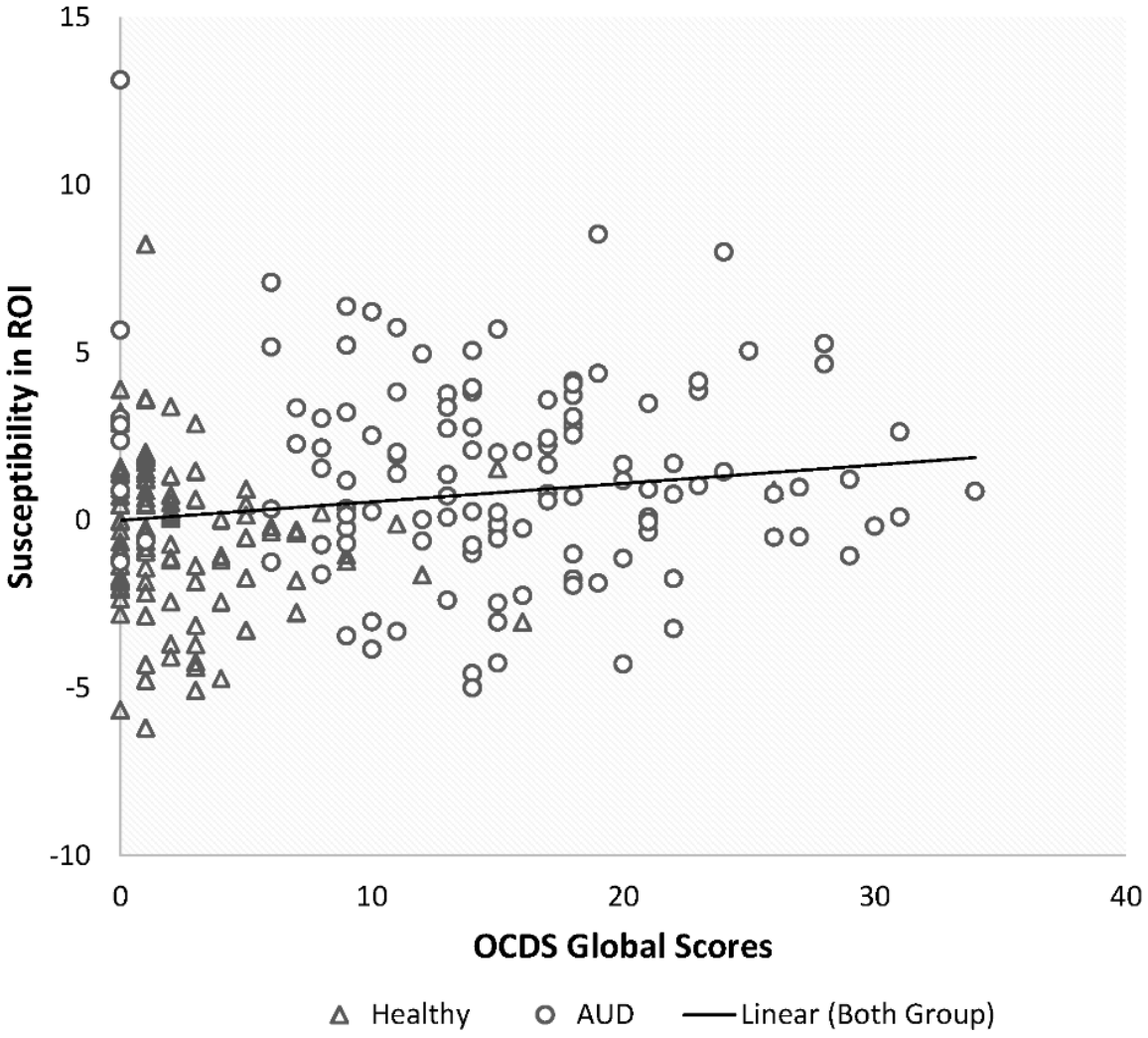


Figure 2.1-3 Correlation of OCDS scores and susceptibility, controlling for age and smoking-status.

This figure is from published work (Tan, Hubertus, et al., 2023).

2.1.5 Discussion

The most salient message of the current study is that AUD patients show increased iron accumulation in the dorsal striatum, and that iron levels are associated with the measure of drinking pattern. Specifically, AUD subjects had bilaterally increased magnetic susceptibility in the dorsal striatum when compared to healthy participants. Importantly, this iron accumulation was strongly and positively correlated to the alcohol exposure in the last three months and with OCDS score. This finding suggests that the behavioral pattern of compulsive drinking is related to the concentration of brain iron in the dorsal striatum, a brain region involved in habituation and automated behaviors.

The specificity of this finding is supported by the lack of correlation of momentary alcohol urges and severity of alcohol dependence with the striatal iron levels.

Accumulation of brain iron in AUD

As hypothesized, increased accumulation of brain iron was observed in AUD participants. Alcohol use has a significant and wide-ranging impact on multi-systems/organs and might be associated with systemic iron accumulation in the body. Alcohol use may increase intestinal iron absorption and be related to abnormal hepcidin signaling (Duane et al., 1992; Juhás et al., 2017; Kohgo et al., 2008). The liver, as the major storage site for iron, as well as the principal targets for alcohol injury, suffers from iron overload (Ioannou et al., 2004; Tavill & Qadri, 2004). Further, it is reported that alcohol use disrupts the blood-brain barrier (BBB) integrity (Haorah et al., 2005; Pimentel et al., 2020), which could have impact on iron transport and contributes to brain iron accumulation (Olmedo-Díaz et al., 2017). What is more, the pre-clinical experimental literature reports increased brain iron after acute and chronic alcohol exposure in animals (Crews & Nixon, 2009; Rouach et al., 1997; Rouach et al., 1990), which was hypothesized to be related to free radicals and oxidative stress, and consequently in neuroinflammation. Studies in humans linked long-term alcohol use and AUD to signs of increased immune signaling in the central nervous system (Coller & Hutchinson, 2012), pro-inflammatory state in the brain (Rubio‐Araiz et al., 2017) and microglia activation (Kempuraj et al., 2016; Petrakis et al., 2019). However, the mechanism underlying increased brain iron in AUD and its relation to neuroinflammation and neurodegeneration is still not fully understood.

Specific brain iron accumulation in the dorsal striatum

Our findings indicate a specific brain iron accumulation in the dorsal striatum of AUD participants. Whole-brain analysis showed significantly higher susceptibility in the dorsal striatum of AUD participants compared to healthy controls. A potential reason why striatal regions might be particularly sensitive to iron accumulation is its high energetic demands resulting from dopaminergic activity. In dopamine synthesis, iron is a co-factor of tyrosine hydroxylase, which converts tyrosine to dopamine. Tissue culture experiments in peripheral blood cells have shown that dopamine alters cellular iron homeostasis by increasing iron incorporation (Dichtl et al., 2018). The dorsal striatum is particular vulnerable to alterations of the iron homeostasis because it holds the highest density in dopaminergic terminals, and dopamine turnover and metabolism are energetically extremely demanding with iron and dopamine forming a potent redox

couple (Hare & Double, 2016; Scheurich et al., 2005), which might also underlie the higher sensitivity of the dorsal vs ventral striatal regions to neurodegeneration in Parkinson's disease. Following the dopamine synthesis, molecules from oxidation in the dopamine degradation could be neurotoxic to catecholaminergic cells (Muñoz et al., 2012), and iron was found as a mediator of the neurotoxicity in Parkinson's disease via Fe−dopamine complex (Paris et al., 2005).

Thus, regions with high dopaminergic activity appear to be vulnerable to iron accumulation, This in turn might lead to cognitive and behavioral impairment (Rodrigue et al., 2020; Schröder et al., 2013; Spence et al., 2020; Tonekaboni & Mollamohammadi, 2014). In fact, evidence from human PET and postmortem studies and corresponding animal experiments demonstrated profound alterations in the dopamine system in AUD (Hansson et al., 2019; Hirth et al., 2016). Ventral and dorsal striatum play different dopamine-mediated roles in addiction, and the dorsal striatum is more related to compulsive use. (Ito et al., 2000; Lüscher et al., 2020; Uhl et al., 2019; Vollstädt‐Klein et al., 2010). In the present study, AUD participants had been drinking for 19.8 years, on average, and were therefore likely in the stage of compulsive use, to varying degrees as assessed by the OCDS. Correspondingly, the dopaminergic activity in the dorsal striatum might have become dominant in their alcohol use behavior, which led to increased iron accumulation in this region.

Connections between brain iron accumulation and compulsive drinking in AUD

The current study found a positive correlation between dorsal striatal susceptibility, i.e. iron load, and compulsive drinking behavior as measured by the OCDS (Vollstädt‐Klein et al., 2010). This correlation further strengthens the hypothesis that the dorsal striatum is specifically involved with mediating compulsive drinking behavior and that a potential underlying neural mechanism contributing to this might be iron overload (Tonekaboni & Mollamohammadi, 2014). An interesting question these findings raise is whether brain iron accumulation in the dorsal striatum is a predisposing factor for compulsive behavior and the development of AUD or whether it is the result of long-term alcohol consumption. In order to explore this question it would be useful to follow individuals over trajectory of addiction development, to make a direct intra-individual comparison of iron levels over time. While the present study is limited by its cross-sectional design and found a positive correlation between brain iron accumulation and the drinking amount, some recent studies have attempted to elucidate the relationship between brain iron, cognitive function, and age in non-AUD populations. Interestingly,

in healthy individuals greater iron load was predictive of deficits in a working memory task, especially in younger and middle-aged participants, when compared to older ones (Rodrigue et al., 2020). However, a different study (Larsen et al., 2020) in which the longitudinal trajectories of striatal iron load were examined came to the conclusion that greater cognitive ability is increasingly associated with greater iron concentration through late adolescence and young-adulthood. Meanwhile, we did not find significant correlations between dorsal striatal susceptibility and AUQ or ADS scores. We did not find this result surprising given that the AUQ assesses 'state' as opposed to 'trait', which reflects a temporary condition and would be unlikely to correlate with a cumulative, chronic indicator like iron-load. The ADS, on the other hand, does in fact measure trait (severity of alcohol dependence), but one which consists of several domains beyond compulsivity, including negative emotion, preoccupation and salience. Therefore, it seems likely that the ADS may associate with neural activity that goes beyond the dorsal striatum. The OCDS is a tool that is specific to the assessment of trait compulsive drinking and its positive correlation with dorsal striatal susceptibility makes a compelling case that increased iron load in the dorsal striatum is directly related to increased compulsive drinking patterns.

Clinical perspective

These results provided us with a new perspective on clinical assessment and treatment. Brain iron concentration from imaging examinations could function as a potential biological marker in AUD diagnosis, providing an objective measure associated with recent alcohol exposure and compulsive drinking, which might be helpful for individualized treatment of AUD.

Importantly, it must be noted that increased brain iron accumulation leads to signal loss and hence systematic artefacts when acquiring fMRI images because of the static field inhomogeneities. This represents a specific challenge for clinical addiction researchers using fMRI, because it is exactly these regions – putamen, pallidus, insula, caudate – that have been hypothesized to have special relevance for the development and maintenance of addiction. Meanwhile, these regions are disproportionately affected by iron accumulation when compared to healthy individuals (Puckett et al., 2018; Song, 2001). This likely has significant implications when analyzing fMRI data, and should be regarded as a potentially impacting factor in studies of AUD.

Limitation

Our re-analyses following this innovative method included existing datasets from previous projects using the same inclusion criteria and scanning parameters. This resulted in limitations regarding data resolution. Second, although our GRE sequence appears suitable for standard QSM methods (Haacke et al., 2015), its spatial resolution is relatively low, which may have limited our ability to detect iron increases in smaller brain regions of the mid and hind brain as previously reported (Juhás et al., 2017; Topiwala, Wang, Ebmeier, Burgess, Bell, Levey, Zhou, McCracken, Roca-Fernandez, et al., 2022) and prevented the exploration of striatal subregions. However, this work performed whole-brain analysis and warrants further investigation using QSM of adequate spatial resolution. Moreover, our analyses only found a significant correlation in both groups, but not within the AUD group alone. This might be because of the classification based on DSM criteria, which results in different distributions in the AUD and healthy individuals. Third, we had no access to blood markers (e.g., iron levels, ferritin and transferrin saturation), and were unable to study the relationship between iron metabolism and brain iron accumulation. Thus, future studies need to address these issues by using state-of-the-art sequences, including biomarkers of peripheral iron metabolism, and most importantly by positing an a priori and pre-registered hypothesis on the effect of iron accumulation on behavioral and other clinical outcomes.

2.1.6 Conclusion

This is the first study exploring whole-brain iron accumulation in AUD using GRE sequences with a large clinical sample. It is also the first time that compulsive behavioral patterns in AUD have been related to brain iron accumulation. In summary, treating compulsive patterns of alcohol use is one of the main aims in clinical practice with regards to AUD. The neural mechanisms underlying habituation and compulsivity are still not fully understood. This study using QSM susceptibility measures finds increased iron accumulation in the dorsal striatum to be associated with the behavior of compulsive drinking, which might bring a new perspective to clinical practice. Further, neuroinflammation might be a consequence of brain iron accumulation which might relate AUD to neuroinflammation mechanisms. Lastly, our results also had implications for fMRI methods used in addiction research, because iron accumulation results in signal dropout when echo planar imaging images are acquired. This means that regions of basal ganglia, specifically of interest in general SUD research, have a

potentially systematically disturbed signal, which may affect the quality of the analysis. The method used in the current study is easy to implement and offers the possibility to examine brain iron accumulation with images using short GRE sequences, which might already have been acquired in previous studies as images for fieldmap correction.

2.1.7 Supplementary Material

Supplementary Table 2.1-1 Information of included studies.

Study name	Inclusion criterion *	Sample size		ClinicalTrials/ German Clinical Trials Register (DRKS)
		AUD	HC	
Transalc [22]	1) Alcohol Use Disorder according to DSM-5; 2) for heavy-drinker, at least 84g pure alcohol in the previous 90 day; 3) treatment-seeking and abstinent.	33	37	DRKS: DRKS00003357
SFB_Folgestudie [23]	1) alcohol dependence according to the DSM-IV and ICD 10; 3) treatment-seeking and abstinent.	82	53	DRKS: DRKS00003388
ADHS [24]	1) Alcohol Use Disorder according to the DSM-5; 2) treatment-seeking and abstinent.	21	20	DRKS: DRKS00004929
eMEDs [25]	1) alcohol dependence according to the DSM-IV and ICD 10; 2) treatment-seeking and abstinent.	30	58	DRKS: DRKS00003341
NALCUE [26]	1) Alcohol Use Disorder according to the DSM-5; 2) at least 60g for men and 40g for women pure alcohol, at least 5 days/week; 3) non-treatment-seeking.	20	1	ClinicalTrials: NCT02372318

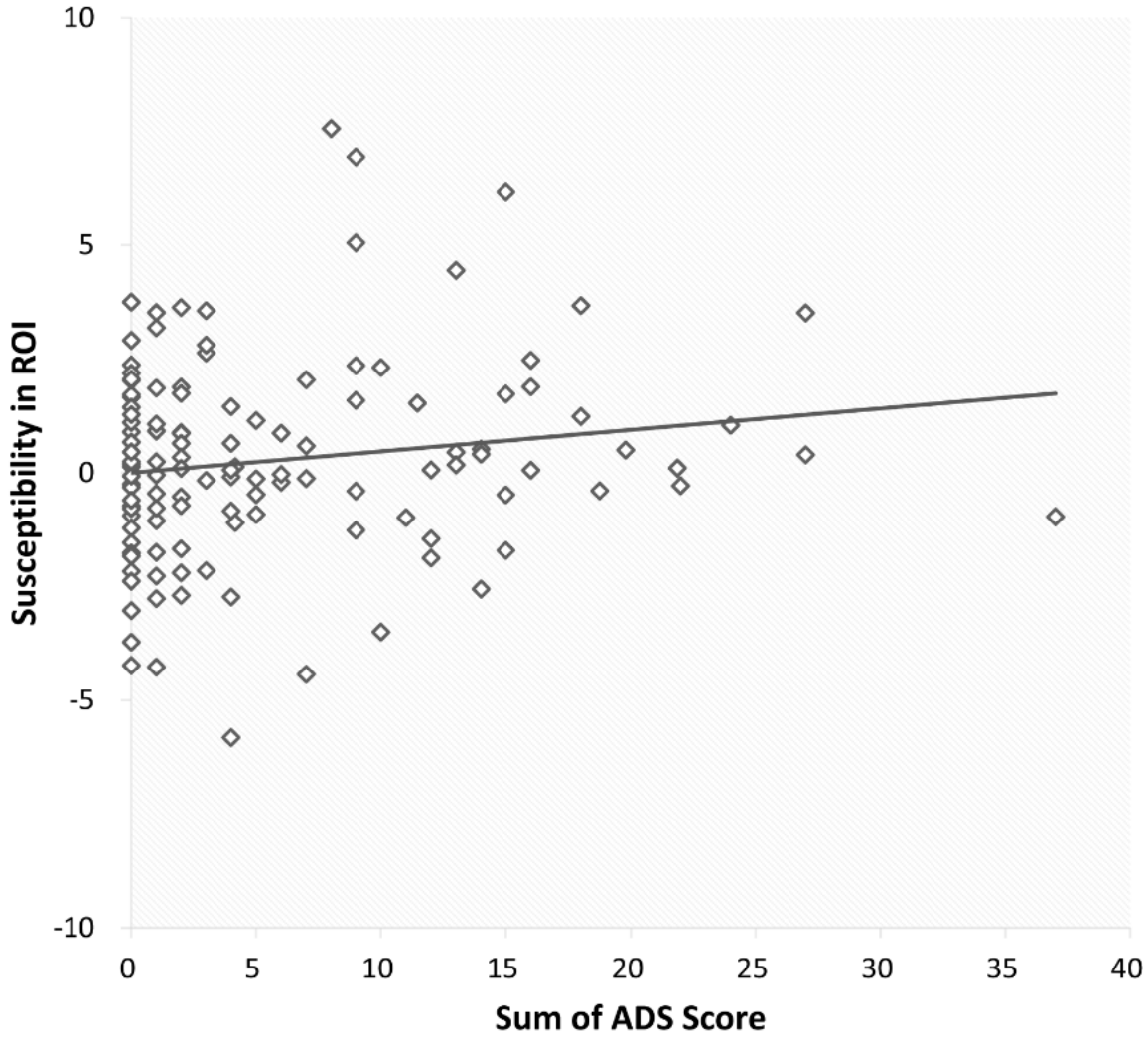
Oxytocin [27]	healthy participants **	0	13	DRKS: DRKS00009253
Avatar [28]	healthy participants	0	49	DRKS: DRKS00009439
CBD-IS (not yet published)	healthy participants	0	43	ClinicalTrials: NCT02051387

Abbreviations: diagnostic and statistical manual of mental disorders (DSM), international classification of diseases (ICD), Alcohol Use Disorder (AUD), healthy control (HC).

* General criterion of inclusion and exclusion:
These studies included AUD individuals who 1) between 18 and 75 years, 2) right-handed;
and excluded AUD individuals who 1) comorbid axis-I disorders (other than nicotine dependence) in the last year, 2) treatment with psychotropic or anticonvulsive medications in the last three months, 3) severe neurological or physiological disease (i.e. liver cirrhosis), 4) positive drug screening, 5) ineligibility for MRI scanning (e.g. metal implants), 6) history of severe head trauma.

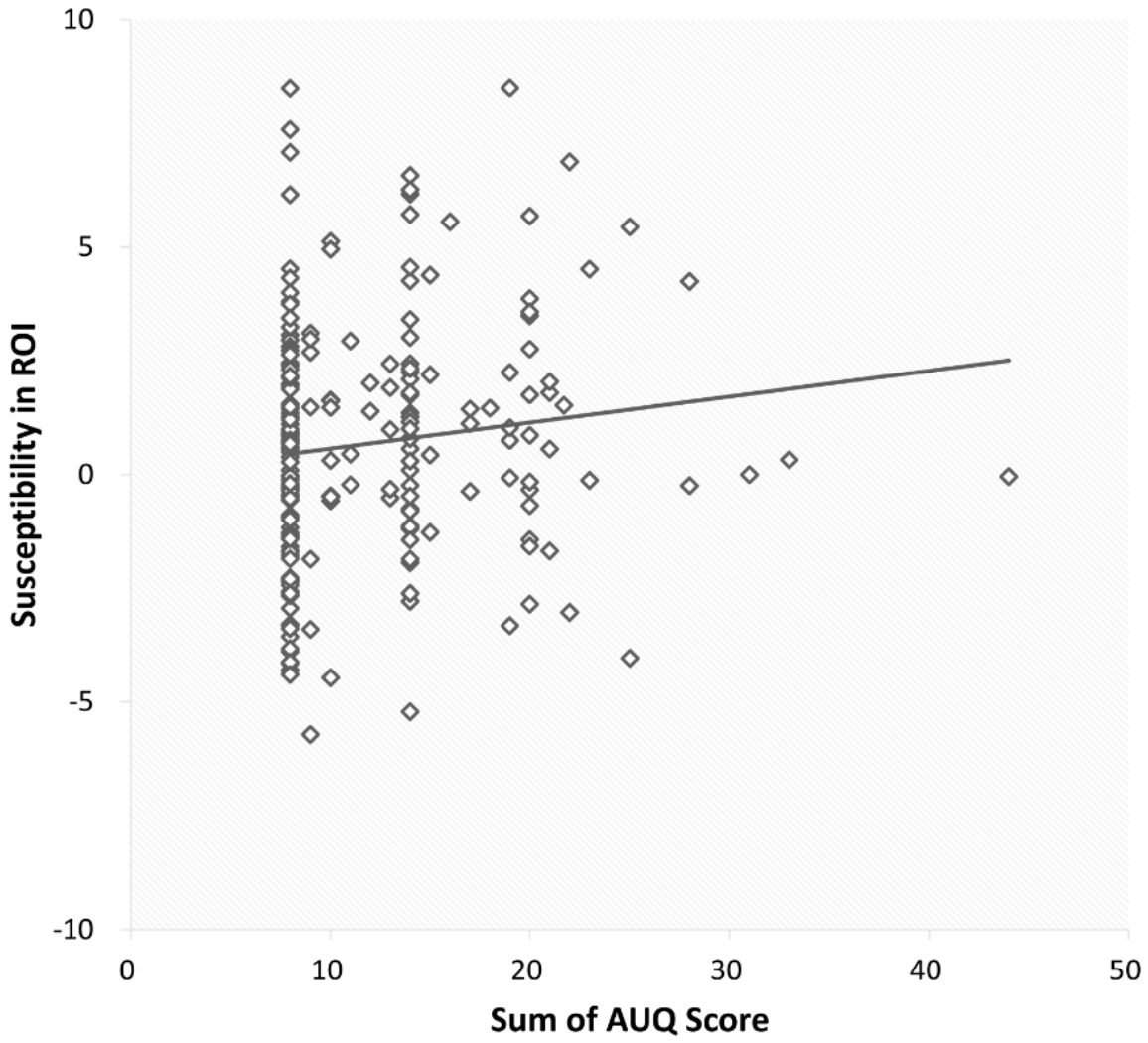
These studies included healthy participants/light to moderate drinkers who 1) were aged between 18 and 75 years, 2) right-handed, 3) had an average alcohol consumption below 14g pure alcohol;
and excluded healthy participants who 1) comorbid axis-I disorders (other than nicotine dependence) in the last year, 2) treatment with psychotropic or anticonvulsive medications in the last three months, 3) severe neurological or physiological disease (i.e. liver cirrhosis), 4) positive drug screening, 5) ineligibility for MRI scanning (e.g. metal implants), 6) history of severe head trauma.[27]

** light to moderate drinkers were classified as healthy participants.



Supplementary Figure 2.1-1 Correlation of sum of ADS scores and susceptibility, controlling age and smoke-status.

This figure is from published work (Tan, Hubertus, et al., 2023).



Supplementary Figure 2.1-2 Correlation of sum of AUQ scores and susceptibility, controlling age and smoke-status.

This figure is from published work (Tan, Hubertus, et al., 2023).

2.2 Study 2: Decoding fMRI alcohol cue-reactivity and its association with drinking behaviour[2]

2.2.1 Abstract

Background: Cue-reactivity, the enhanced sensitivity to conditioned cues, is associated with habitual and compulsive alcohol consumption. However, most previous studies in Alcohol Use Disorder (AUD) compared brain activity between alcohol and neutral conditions, solely as cue-triggered neural reactivity.

Objective: This study aims to find the neural sub-processes during processing of visual alcohol cues in AUD individuals, and how these neural patterns are predictive for relapse.

Methods: Using cue-reactivity and rating tasks, we separately modeled the patterns decoding the processes of visual object recognition and reward appraisal of alcohol cues with Representational Similarity Analysis, and compared the decoding involvements (i.e. distance between neural responses and hypothesized decoding models) between AUD and healthy individuals. We further explored connectivity between the identified neural systems and the whole brain, and predicted relapse within six months using decoding involvements of the neural patterns.

Findings: AUD individuals, compared to healthy individuals, showed higher involvement of motor-related brain regions in decoding visual features, and their reward, habit and executive networks were more engaged in appraising reward values. Connectivity analyses showed the involved neural systems were widely connected with higher cognitive networks during alcohol cue processing in AUD individuals, and decoding involvements of frontal eye fields and dorsolateral prefrontal cortex could contribute to relapse prediction.

Conclusions: These findings provide insight into how AUD individuals differently decode alcohol cues compared to healthy participants, from the componential processes of visual object recognition and reward appraisal.

Clinical implications: The identified patterns are suggested as biomarkers and potential therapeutic targets in AUD.

[2] **Published as:** Tan, H., Gerchen, M. F., Bach, P., Lee, A. M., Hummel, O., Sommer, W., Kirsch, P., Kiefer, F., & Vollstädt-Klein, S. (2023). Decoding fMRI alcohol cue reactivity and its association with drinking behaviour. BMJ Mental Health, 26(1), e300639. https://doi.org/10.1136/bmjment-2022-300639.

2.2.2 Background

Alcohol Use Disorder (AUD) is a major international public health issue with high associated morbidity and mortality. It can be characterized as a disorder of neurocircuitry interacting with environmental and social factors (World Health Organization, 2020).

Cue-reactivity is the enhanced sensitivity to conditioned cues. In AUD, these conditioned cues can trigger conditioned emotional or motivational reactions (i.e., cue-reactivity), which provide the basis for experiencing craving, comprise the anticipation of reward or the occurrence of withdrawal symptoms in the case of not consuming the substance (Carter & Tiffany, 1999; Koob & Volkow, 2010). In the literature, three models of cue-reactivity have been proposed (Drummond, 2000): the conditioned withdrawal model, the conditioned compensatory response model and the conditioned appetitive-motivational model, which were recently unified in a framework of addiction (Koob & Volkow, 2016; Lüscher et al., 2020) where cue-reactivity was conceptualized as the motivational change associated with addiction.

The neural activity triggered by cues was extensively reported and reviewed in previous publications. Existing neuroimaging evidence suggests that salient cues elicit increases in activity throughout the mesocorticolimbic system and nigrostriatal system (Jasinska et al., 2014). Activity in the mesocorticolimbic system, including the ventral tegmental area, ventral striatum, amygdala, anterior cingulate, prefrontal cortex, insula, and hippocampus, as well as in sensory and motor cortices, reflected the neural representations of reward values of cues and the motivational processes of incentive salience that guide drug-seeking behavior. On the other hand, the nigrostriatal system is critical to habit learning and a transition from controlled to automatic behavior, which consists primarily of dopamine projections from the substantia nigra to caudate and putamen (also referred to as the dorsal striatum) and globus pallidus. When increases in dorsal striatum cue-reactivity were observed, the dorsal striatum circuits were also involved in planning and execution of motor responses (Jasinska et al., 2014; Yalachkov et al., 2012). Moreover, researchers found that sensory and motor function could also importantly contribute to the cue-reactivity in addiction (Smolka et al., 2006; Yalachkov et al., 2013; Yalachkov et al., 2010), and in both animals and humans, activity of visual cortices could be modulated in viewing reward-mounted cues (Yalachkov et al., 2010). In a study using an animal model of chronic alcohol drinking, a functional dedifferentiation in visual and sensorimotor networks was observed

(Pérez-Ramírez et al., 2022). Recently, a paper reviewed previous work in addition research and demonstrated six large-scale brain networks (reward, habit, salience, executive, memory, and self-directed networks) for understanding the dysfunctions in addictive disorders (Zilverstand et al., 2018).

Most of the previous studies compared brain activity between an alcohol-condition and a neutral condition, and reported cue-triggered brain activity as a contrast (Carter & Tiffany, 1999; Jasinska et al., 2014; Zeng et al., 2021). However, comparing alcohol versus neutral cues usually only assesses the alcohol-cue-elicited activation, while the sub-processes leading to this change in the brain remain elusive. For example, how does the brain recognize the alcohol cues, and how are reward values represented in the brain? Until now, only a few studies considered the different reward values of alcohol cues in cue-reactivity tasks, which could help to understand the process of reward appraisal in AUD individuals.

2.2.3 Objective

This study was designed to separately model processes of Visual Object Recognition (VOR) and Reward Appraisal (RA) in cue-reactivity, and examine altered patterns of neural activity in AUD. Functional magnetic resonance imaging (fMRI) data were examined with representational similarity analysis (RSA), a multivariate technique to model neural patterns (Kriegeskorte et al., 2006; Kriegeskorte et al., 2008). We hypothesized that, compared to healthy participants, AUD individuals show specific enhanced patterns in visual object recognition and reward appraisal of the alcohol cues. Moreover, the level of enhancement of the neural patterns should be related to the clinical characteristics of AUD.

2.2.4 Methods

Participants

This study was based on a combined analysis of previous projects in AUD, which were designed with similar inclusion criteria and implemented with the same EPI sequence (Bach et al., 2019; De Santis et al., 2019; Gerchen et al., 2021; Hansson et al., 2018; Karl et al., 2021; Vollstädt-Klein et al., 2011) (For details, see Supplementary Table 2.2-1). It comprised datasets from 238 (53 female) alcohol dependent patients (DSM-IV and DSM-5 criteria) and 229 (50 female) healthy participants recruited at the Central Institute of Mental Health, Mannheim, Germany between 2008 and 2016. The average

ages of AUD individuals and healthy participants were 47.0 ± 10.8 and 45.7 ± 12.7 years, respectively. Psychometric assessments included the Alcohol Dependence Scale (ADS, (Kivlahan et al., 1989)), the Alcohol Urge Questionnaire (AUQ, (Bohn et al., 1995)), the Obsessive Compulsive Drinking Scale (OCDS, (Anton et al., 1995; Mann & Ackermann, 2000)) and FORM 90 (Scheurich et al., 2005) (For details, see Supplementary Methods and Tabular Appendix). The demographic and clinical overview of the participants is summarized in Supplementary Table 2.2-2. All participants provided informed written consent according to the declaration of Helsinki, and all projects in this study were approved by the local ethics committee of the University of Heidelberg (Ethics approval numbers: 2007-095F-MA, 2009-215N-MA, 2010-348N-MA, 2011-303N-MA, 2009-215N-MA, 2015-540N-MA).

Stimuli and Experimental Tasks

During the imaging session, visual alcohol and neutral stimuli were presented in blocks that were pseudo-randomized (Lang et al., 1997; Vollstädt-Klein et al., 2011) (tasks were described in Supplementary Methods). The alcohol picture series was previously evaluated in a pilot study 11 by AUD and healthy individuals with an attribute-rating task (outside the MRI scanner). In the task, the participants indicated their feeling towards these pictures from aspects of craving, valence and arousal (with three questions of “when seeing this picture, how strong is your craving; how pleasant/unpleasant do you feel; how excited do you feel?”).

Imaging Acquisition and Preprocessing

Scanning was performed using a 3-T whole-body tomography scanner (MAGNETOM Trio with TIM technology; Siemens, Erlangen, Germany). T2*-weighted, echo planar images covering the entire brain were acquired. Imaging parameters were set to repetition time = 2.41 s, echo time = 25 ms, flip angle = 80°, number of slices = 42, slice thickness = 2 mm, voxel-gap = 1 mm, voxel dimensions = 3 × 3 × 3 mm3, field of view = 192 × 192 mm2, in-plane resolution = 64 × 64. Visual stimuli were presented using Presentation software (Neurobehavioral Systems Inc.). fMRI data were processed and analyzed using SPM12 (Wellcome Centre for Human Neuroimaging, University College London, UK). The first five scans were excluded from imaging analyses to avoid any artefacts caused by the effects of magnetic saturation. All images were realigned spatially, normalized to the SPM12 TPM MNI template, and unsmoothed images were used in the following analyses in order to preserve the fine spatial details in the fMRI signal.

Representational similarity analysis (RSA)

To find altered neural patterns in AUD individuals during the cue-reactivity task, we used RSA with two separate models of Visual Object Recognition (VOR) and Reward Appraisal (RA) as shown in Figure 2.2-1. Patterns of neural activity (neural representational dissimilarity matrices (RDMs)) were compared to model RDMs (VOR and RA) with a searchlight approach (Nili et al., 2014) according to the hypotheses.

For the VOR, we used a pre-trained deep convolutional neural network (dCNN) for model construction (Eickenberg et al., 2017; Seeliger et al., 2018; Simonyan & Zisserman, 2014; Xie et al., 2020; Zhou et al., 2018) (for details, see Supplementary Methods). We extracted the last three fully connected layers (fc8, fc7 and fc6) of the neural network for each picture (Simonyan & Zisserman, 2014; Xie et al., 2020), and constructed the model RDMs of VOR. For the RDM of RA, we used ratings for each presented picture stimulus from a pilot study (Vollstädt‐Klein et al., 2010). The correlation distance (1 - Pearson's r) was used in distance-calculation for model RDMs (Figure 2.2-1).

With preprocessed images (unsmoothed), we extracted voxel-wise fMRI responses (beta values) for all pictures using the general linear model (GLM) in SPM12 including movement parameters. A previous study found Block-designed fMRI-tasks could also be modelled as event-related and might even better explain neural responses (Mechelli et al., 2003). Therefore, though our cue-reactivity tasks in this study were block-designed, event-modelling was used in analyses to investigate the neural patterns with the hypothesized models. We then created individual RDMs based on the correlation distance for each pair of all the pictures.

Searchlight Analysis

To quantify how well the different models were related to the neural patterns in the cue-reactivity task and assess the alerted involvement of brain regions in decoding information in AUD individuals, we correlated (Spearman's rank correlation) each model RDM with individual neural RDM with a searchlight analysis (radius = 10 mm). In this analysis, individual whole-brain maps of 'decoding involvement' (Fisher-transformed correlation coefficients) were obtained, which showed the similarity between the models and the brain response, and were compared between AUD and healthy individuals.

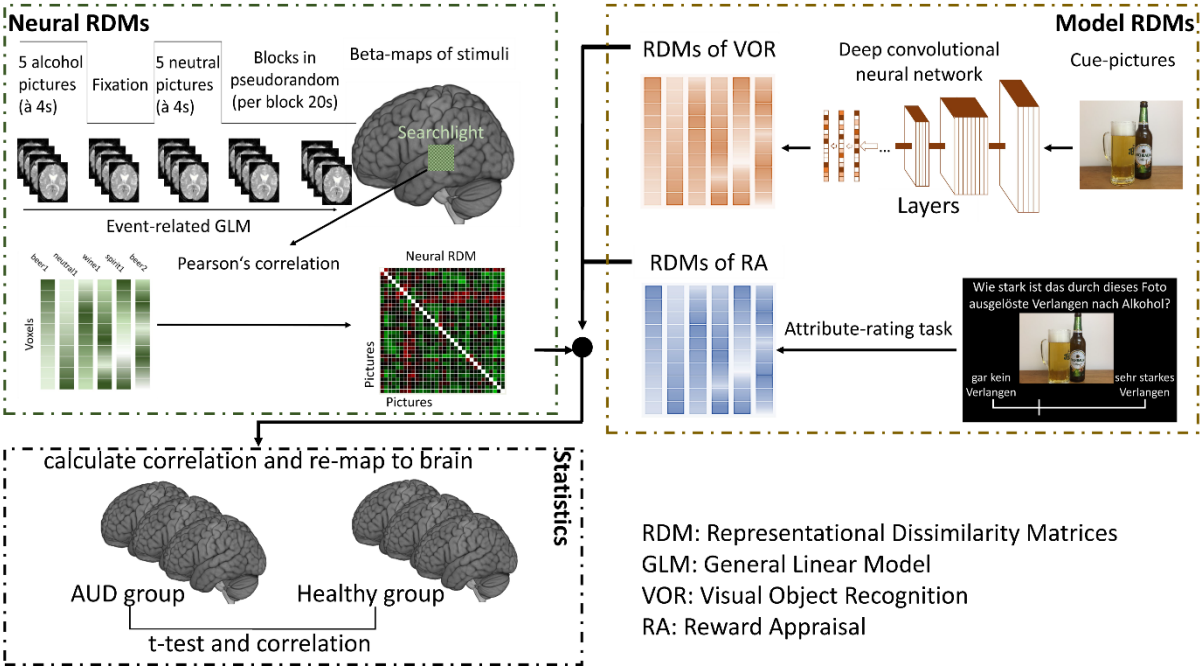


Figure 2.2-1 Procedure of representational similarity analysis with computational models.

The functional MRI data was used to calculate the neural representational dissimilarity matrices (RDMs), while RDMS of Visual Object Recognition (VOR) and Reward Appraisal (RA) were calculated with layers in deep convolutional neural network (dCNN) and ratings of pictures, respectively. Then patterns of neural activity (neural RDMs) were compared to model RDMs (VOR and RA) with a searchlight approach and followed with statistical tests. This figure is from published work (Tan, Gerchen, et al., 2023).

Whole-Brain Connectivity to neural patterns of cue-reactivity

To investigate the communication between the identified regions of neural representations of cue-reactivity and other brain areas in AUD individuals, we performed psychophysiological interaction (PPI) analyses (Friston et al., 1997; O'Reilly et al., 2012) (for details, see Supplementary Methods). The clusters with different neural representations identified by the group comparison of RSA were defined as seed regions (voxels in the clusters). The contrast "Alcohol vs. Neutral" was used in computing seed-to-voxel PPI in the whole brain.

Statistical analyses

The whole-brain decoding involvement maps of two groups were compared using SPM12 with a one-tailed two-sample t-test to find the enhanced neural pattern of AUD individuals. In following analyses, a two-tailed one-sample t-test was used to examine

the significant connectivity. To control for multiple comparisons, a voxel-wise-threshold of P< 0.0005 in combination with a cluster-extend-threshold determined with random field theory in SPM12 was used for the cluster-corrected-threshold of P< 0.05. Based on the results of the between-group comparison, significant regions from both VOR and RA were defined as two neural patterns, and correlation analyses were conducted between the mean decoding involvements of the patterns and psychometric assessments in SPSS 25.

Relapse Prediction with neural patterns

Finally, we conducted a prediction of relapse with the cue-reactivity neural patterns in a subsample of 59 AUD individuals, in whom follow-up data after cue-exposure based extinction training and Treatment As Usual was available (Vollstädt-Klein et al., 2011). The aim of prediction was relapse during a six months follow-up period. We extracted the mean 'involvement value' from all significant ROIs (Table 2.2-1) in the VOR and RA patterns separately as features, and used a Support Vector Machine (SVM) (Chang & Lin, 2011) and lasso penalized logistic regression to predict the relapse (relapse = 1 and abstinence = -1). The SVM model used a linear kernel, and was trained with Leave-one-out-cross-validation. The weights of support vectors were used for investigating the most informative ROIs. The lasso penalized logistic regression model was trained with Leave-one-out-cross-validation, with nested 10-fold cross-validation for removing redundant predictors. The ROIs with large positive weights in SVM and left after lasso regularization (with low cross-validation error) were considered as risk factors. These positive weighted ROIs were linked with AUD individuals who relapsed within six months, and ROIs with large negative weights were protective factors contributing to abstinence.

2.2.5 Findings

Different Neural Representation between AUD and healthy individuals

In VOR (RDM-fc8), motor-related brain areas (precentral and supplementary motor cortex) and postcentral cortex of AUD individuals showed higher involvement than in healthy individuals (Figure 2.2-2-A, Table 2.2-1 and Supplementary Table 2.2-3). Modelling with RDM-fc7 showed similar significant regions as RDM-fc8 (see Supplementary Table 2.2-4), while RDM-fc6 did not show significant difference between two groups.

With reward-appraisal modelling, AUD individuals showed different neural patterns in a large network compared to healthy individuals. Engaged areas involved the basal ganglia (caudate, putamen), the frontal cortex (inferior, middle and superior), pre/postcentral gyrus and also regions in occipital and temporal cortex. (Figure 2.2-2-B, Table 2.2-1 and Supplementary Table 2.2-3).

Table 2.2-1 Different Neural Representation of VOR and RA between AUD and healthy individuals.

Cluster Names	**AAL Labels**	**Brodmann Area (BA) Labels**
VOR1	Supp_Motor_Area_R	BA6: premotor cortex and supplementary motor cortex
VOR2	Precentral_R, Postcentral_R	BA4: primary motor cortex, BA3: primary somatosensory cortex
RA1	Occipital_Sup_L, Cuneus_L, Occipital_Mid_L	BA18: secondary visual cortex_V2, BA19: associative visual cortex (V3, V4 & V5)
RA2	Temporal_Mid_R, Temporal_Sup_R, Temporal_Pole_Mid_R	BA21: middle temporal gyrus
RA3	Frontal_Inf_Tri_L, Frontal_Inf_Orb_L, Frontal_Mid_Orb_L, Frontal_Mid_L	BA47: pars orbitalis, part of the inferior frontal gyrus, BA11: orbitofrontal area, BA10: anterior prefrontal cortex
RA4	Putamen_R, Caudate_R, Pallidum_R, Hippocampus_L, Olfactory_R, Amygdala_L	BA25: subgenual area
RA5	Precentral_R, Frontal_Inf_Oper_R, Rolandic_Oper_R, Insula_R, Frontal_Mid_R	BA6: premotor cortex and supplementary motor cortex, BA9: dorsolateral prefrontal cortex, BA13: insular cortex, BA44: part of Broca area

RA6	Frontal_Mid_L, Precentral_L, Frontal_Sup_L, Frontal_Inf_Oper_L, Frontal_Inf_Tri_L, Frontal_Sup_Medial_L, Supp_Motor_Area_L	BA8: frontal eye fields, BA9: dorsolateral prefrontal cortex, BA6: premotor cortex and supplementary motor cortex
RA7	Temporal_Mid_L, Temporal_Sup_L	BA21: middle temporal gyrus
RA8	Calcarine_L, Lingual_L	BA21: middle temporal gyrus, BA30: cingulate cortex
RA9	Lingual_R, Calcarine_R, Vermis_4_5	BA30: cingulate cortex, BA18: secondary visual cortex_V2
RA10	Occipital_Mid_R, Occipital_Sup_R	BA19: associative visual cortex_V3, V4 & V5
RA11	Lingual_R	BA30: cingulate cortex, BA27: piriform cortex
RA12	Frontal_Inf_Tri_R	BA45: part of Broca area (45)

Two-sample t-tests were used between Alcohol Use Disorder (N = 238) and healthy (N = 229) individuals, combining voxel-wise-p<0.0005 and FWEc=108 voxels for Visual Object Recognition (VOR) modelling and 88 voxels for Reward Appraisal (RA) modelling, corresponding to cluster-pFWE<0.05. See supplementary Table 2.2-3 for the full table with cluster sizes, MNI coordinates and peak T-values of clusters, as well as labels under five voxels.

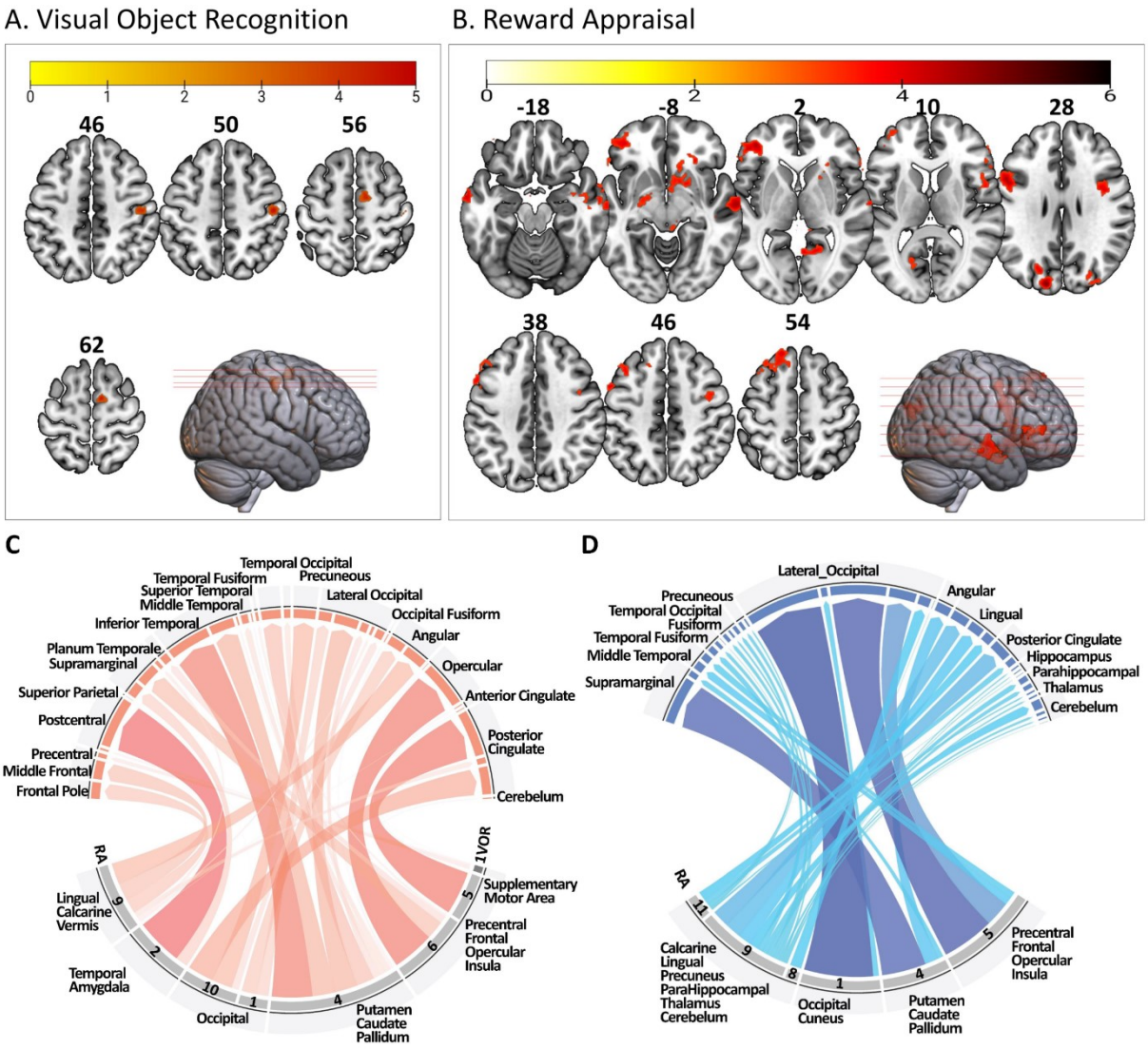


Figure 2.2-2 Increased decoding neural patterns and their connections to the whole brain.

(A) the increased decoding neural pattern in Visual Object Recognition (VOR). (B) the increased decoding neural pattern in Reward Appraisal (RA). (C) Positive connectivity in ROI-to-Voxel performed psychophysiological interaction (PPI) analyses: the lower half-circle displays the seed regions (RA1 to 12 for the ROIs from Reward Appraisal model and VOR1 and 2 for the Visual Object Recognition model), and the upper half displays the significant regions in the PPI analyses. The width of links corresponds to the size of seeds and significant regions. (D) Negative connectivity in ROI-to-Voxel PPI analyses. For (A) and (B), two-sample t-tests were used between Alcohol Use Disorder (AUD, N = 238) and healthy (N = 229) individuals, combining voxel-wise-$p<0.0005$ and FWEc=108 voxels for A and 88 voxels for B, corresponding to cluster-$pFWE<0.05$. For (C) and (D), one-sample t-tests were used within AUD group with a same multi-

comparison-correction approach for cluster-pFWE<0.05. This figure is from published work (Tan, Gerchen, et al., 2023).

Connectivity from the neural patterns of VOR and RA

The seed-to-voxel PPI analyses were based on the enhanced decoding regions of AUD individuals comparing to healthy participants, which were identified with VOR (RDM-fc8) and RA models. Supplementary Motor Area was positively connected to Pre/Postcentral and Opercular cortex during visual cue recognition in AUD individuals. During appraising reward value, a large network, which related to habit/reward function and executive function, as well as visual-sensory-motor process, was involved. (Figure 2.2-2-C and D, and Supplementary Table 2.2-5).

Correlation to Psychometrics and Relapse Prediction

The involvement of neural patterns in visual recognition was positively correlated to compulsive drinking, severity of AUD and also the AUQ score (Table 2.2-2). The enhanced neural pattern in reward appraisal was correlated to the scores of AUD and AUQ. With SVM, the relapse was predicted by decoding involvements in the 14 ROIs with a balanced accuracy of 0.6220 (sensitivity = 0.9583, specificity = 0.2857, and parameter C = 1). With lasso regularization, four predictors (RA6, RA8, RA9, RA10) entered the logistic regression model in most of the folds, and the balanced accuracy was 0.5104 (sensitivity = 0.7027 and specificity = 0.3182. See Supplementary Figure 2.2-6 for the Receiver operating characteristic (ROC) curves). In the SVM model, the most informative ROIs (whose weights were over the mean weight) were RA6, RA 10 and RA 11, mainly including frontal eye fields (FEF), dorsolateral prefrontal cortex (dlPFC), associative visual cortex and lingual gyrus (see Supplementary Table 2.2-6 for weights of all ROIs).

Table 2.2-2 Correlation of averaged t-value in the ROIs and the clinical variables in all participants

	Visual Object Recognition		**Reward Appraisal**	
	Coefficient	**P-value**	**Coefficient**	**P-value**
Global score of OCDS (n=281)[a]	0.174	0.003*	0.058	0.329

Sum of ADS (n=173)	0.167	0.028*	0.172	0.024*
Sum of AUQ (n=330)	0.133	0.016*	0.111	0.044*
Amount of drink (n=262)[b]	0.080	0.199	0.092	0.137

Abbreviations: Alcohol Dependence Scale (ADS), Alcohol Urge Questionnaire (AUQ) and the Obsessive Compulsive Drinking Scale (OCDS)

* P < 0.05 after False Discovery Rate (FDR) correction in the four psychometrics respectively.

[a]The calculation rules of OCDS was based on the study from Mann et al. (Mann & Ackermann, 2000) (see Supplementary Methods).

[b]The amount of drink was based on FORM90.

2.2.6 Discussion

Most studies of cue-reactivity in the past decades have not disentangled the processing of cues into components. For the first time, the current study comparing AUD individuals and healthy participants, found specific enhanced patterns in Visual Object Recognition (VOR) and Reward Appraisal (RA) of alcohol cues, as well as their relevance for clinical characteristics and outcome. Furthermore, we found the neural patterns connected to large-scale functional networks, and the decoding involvements of enhanced neural patterns could contribute to predicting relapse within six months.

The role of sensory and motor regions

Comparing the neural representation between AUD and healthy individuals, we found the sensory and motor system of AUD individuals had enhanced information decoding in both visual VOR and RA processes. Some recent neuroimaging studies found sensory and motor function could also be relevant in the development of addiction (Smolka et al., 2006; Yalachkov et al., 2013; Yalachkov et al., 2010). An animal study in 2022 reported that occipital cortical areas lost their specific interaction with sensory-insular cortex, striatal, and sensorimotor networks after chronic alcohol consumption, because of a regional increase in neuronal activity and overall correlation (Pérez-Ramírez et al., 2022).

The visual cortex is the first gate for visual cues in the cortex. Studies from animals and humans demonstrated that both primary and higher visual cortices exhibited value-based modulations of their activity responding to reward-mounted cues (Yalachkov et al., 2010). In our RA model of RSA, we observed the enhanced neural pattern located at higher visual cortices (BA18, 19, and 21), which means that the visual cortex of AUD individuals might represent the reward value of Alcohol cues better than in healthy individuals. Interestingly, it was observed in the VOR processing that the somatosensory cortex, which is mainly responsible for low-level tactile information, was also involved in the specific neural pattern in AUD individuals. A possible explanation might be that alcohol had powerful impact on the somatosensory circuits and the exposure to visual cues may meanwhile activate sensory representations in the haptic modality. In PPI analysis, fusiform gyrus and associative visual cortex (V3, V4 & V5) were connected to striatum (Putamen and Caudate), and this could be related to value modulation on visual recognition (top-down influences) (Gilbert & Li, 2013).

In both VOR and RA process, AUD individuals showed special neural representation in motor and premotor brain areas. One interpretation could be that the motor brain regions play a role in the formation of automatized drinking behavior, which is also known as habitual and compulsive drinking (Lüscher et al., 2020).

The positive correlation to OCDS scores could also support this interpretation. Especially VOR-related patterns were associated with compulsive drinking, whereas RA was only associated with severity of dependence and with craving. Habitual drinking has been explained with a concept of incentive habits (Belin et al., 2013), which is mainly related to the dorsolateral striatum. However, our findings in motor areas (also some regions in cerebellum) might be related to automatized action schemata (Du et al., 2022; Shiffrin & Schneider, 1977; Tiffany, 1990; Yalachkov et al., 2010), as a complement to incentive habits, which would match with previous findings that heavier substance users showed a more automatized consumption (Baxter & Hinson, 2001). In many cue-reactivity studies, motor related areas also have been reported that activated differently towards substance- related stimuli (Vollstädt‐Klein et al., 2010; Yalachkov et al., 2009). Here we would like to specifically note that the motor brain not only represents the reward values from visual analogue scale (VAS) scores, but also the cue features from computer-vision (deep convolutional neural network (dCNN) model), which was not reward-mounted in our hypothesis. This might imply that abnormal neural representation might be embedded deeply in the action

schemata of AUD, and could be crucial for therapy. Previous studies targeting automatic action tendencies showed improvements of treatment outcome in AUD (Rinck et al., 2018; Wiers et al., 2011).

From sensory to distributed processing

In addition to enhanced neural patterns in sensory cortex, AUD individuals also showed representation in the middle temporal gyrus. The middle temporal gyrus, as a part of associate visual cortex, plays a role as transit hub of visual attention pathway, whose activity might reflect the sensory analysis of the cue (Corbetta & Shulman, 2002). Meanwhile, our PPI analysis found a connectivity from middle temporal gyrus to the dorsal frontoparietal network (superior parietal lobule), which is involved in top-down control of visual attention.

When modelling the representation of the reward value, a large network showed high involvements in AUD individuals comparing to healthy participants, especially the orbitofrontal, anterior prefrontal, dorsolateral prefrontal cortex and striatum. These areas could be summarized as three large-scale networks: reward, habit and executive, which reviewed by Zilverstand et al. in 2018 based on the impaired response inhibition and salience attribution (iRISA) model in addiction research (Zilverstand et al., 2018). Previous studies of substance use disorder demonstrated that the hyperactivation of these three networks was involved in the appraisal of the subjective value of the salient cues, automatization of the reaction, and cognitive control toward processing the cues (Jasinska et al., 2014; Zeng et al., 2021). Moreover, when drug incentives elicited stronger activation in these three networks in individuals with substance use disorder compared to controls, the aberrant salience attribution to drug-related stimuli was found to interact with impaired response inhibition in drug addiction (Jasinska et al., 2014; Zeng et al., 2021; Zhukovsky et al., 2020; Zilverstand et al., 2018). Our recent work suggested that the interaction of these three key networks may be rebalanced by an opioid receptor antagonist (Grundinger et al., 2022).

It is indicated that even the passive-viewing cue exposure could involve key addiction networks beyond value appraisal, habit learning, and response inhibition (Everitt & Robbins, 2016) to higher cognitive processes (Goldstein & Volkow, 2011). Using PPI we found that AUD individuals showed increased connectivity from higher visual cortex and attention system to higher cognitive functioning related area, including regions of dorsolateral prefrontal cortex, supramarginal, angular gyrus and anterior prefrontal cortex, as well as the memory system (Hippocampus and Parahippocampal gyrus).

The supramarginal and angular gyrus were found as the crucial regions for active maintenance of information in a working memory task (Cohen et al., 1997). Besides, the connectivity from visual and attention system to anterior and dorsolateral prefrontal cortex could also reflect the information transfer towards value circuit executive system, which support goals-directed behavior, inhibitory control and also self-regulatory processes (Lucantonio et al., 2014; Moeller & Goldstein, 2014).

Limitation

We were not able to investigate the temporal characteristics of the neural pattern, because of the design of paradigm and the temporal resolution. To answer questions such as whether the VOR pattern responds to cues before RA pattern or synchronously, a long-event fMRI paradigm design could be used in the future, combining with finite impulse response modelling, or maybe MEG/EEG could also be used to characterize the temporal characters of the neural pattern. The prediction models, both SVM and lasso penalized logistic regression did not reach a higher accuracy compared to a previous study, which predicted relapse with cue-reactivity fMRI and structural MRI (Seo et al., 2015). In this study, we focused on neural cue processing, which might be not sufficient as the only predictor. Although this study had a relative large sample size, the sample size for participants with relapse data was small. Another limitation of using the lasso penalized logistic regression might be the relatively weak linear association between neural patterns and relapse.

2.2.7 Clinical Implications

With linear SVM, our results showed that the decoding involvement of enhanced neural patterns of cue-reactivity could contribute to predicting relapse within six months. Although the accuracy of prediction was moderate, it might still imply a potential neuroimaging marker for clinical practice. The high-weighted features in the SVM model, the decoding involvements of FEF, dlPFC, had large negative weights towards relapse, which could be considered as protective factors. The FEF and dlPFC could be related to the attention and executive function (dorsal frontoparietal network), and targeting the dorsal frontoparietal network, attentional bias modification therapy has been studied in substance use disorder (Cox et al., 2014). Moreover, transcranial magnetic stimulation with the target area of dlPFC was reported modulating neural activity in brain circuits that mediate cognitive processes relevant to addiction (Gorelick

et al., 2014), and the findings in our current study could suggest further potential target regions.

2.2.8 Conclusion

In this study, we found enhanced neural representation of alcohol cues in specific brain regions of AUD individuals in the context of visual object recognition and reward appraisal. Furthermore, we found small to moderate associations between neural patterns and clinical measures and relapse. The identification of these dysfunctional processes of cue-reactivity in AUD individuals might bring a deeper understanding of the neural and psychological mechanisms underlying AUD, and could be an important step toward the goal of precision medicine approaches in AUD.

2.2.9 Supplementary Materials

Supplementary Methods

Participants

The patients did not use other substances except nicotine, which was verified by a urine drug screening (nal von minden GmbH Drug-Screen® Diptest, Version 1.0). The healthy participants had no history of alcohol or drug addiction or any current psychiatric disorder, assessed by applying the Structured Clinical Interview for DSM-IV and -5 (SCID). Participants in both groups were excluded if they had any history of serious medical (including psychiatric or neurological) complications, brain injury, use of psychotropic medications (other than during the detoxification process), or did not meet magnetic resonance safety criteria for our imaging facility.

Before taking part in the scanning procedure, participants completed the following questionnaires: the Alcohol Dependence Scale (ADS, (Kivlahan et al., 1989)), the Alcohol Urge Questionnaire (AUQ, (Bohn et al., 1995)), the Obsessive Compulsive Drinking Scale (OCDS, (Anton et al., 1995; Mann & Ackermann, 2000)), FORM 90 (Scheurich et al., 2005) and visual analogue scale (VAS) ranging from 0 (no craving) to 100 (extremely extensive craving). The calculation rules of OCDS was based on the study from Mann et. al (Mann & Ackermann, 2000).

Details of cue-reactivity tasks and RDMs

The alcohol cue-reactivity (ALCUE) task was block-designed, in which one block consisted of five stimuli, each presented for 4 seconds, resulting in a total duration of 20 sec per block. Alcohol stimuli were taken from an own alcohol picture series (Vollstädt‐Klein et al., 2010), while neutral cues were taken from the International Affective Picture Series (Lang et al., 1997). After each block participants were asked to indicate their subjective craving for alcohol on a visual analogue scale ranging from 0 (‘no craving at all’) to 100 (’severe craving’). Participants had to rate their subjective craving in a maximum period of 10 sec. Thereafter a black fixation cross was presented on a white background for a minimum of 10 sec. The data in this study were from previous projects, and there were three versions of cue-reactivity tasks, including ALCUE, ALCUE-short, and ALCUEPV. The version of ALCUE tasks had different numbers of blocks: ALCUE had 30 blocks (18 alcohol + 12 neutral), ALCUE-short had 20 blocks (12 alcohol + 8 neutral), and ALCUEPV had 24 blocks (12 alcohol + 12 neutral). All these versions had same picture presenting blocks, which consisted of five

pictures, each presented for 4 seconds. The ALCUEPV version had no have craving rating parts, which was replaced with 20 seconds fixations. The percentages of ALCUE and ALCUEPV in AUD and Healthy groups were not significantly different.
For Visual Object Recognition, the dimensions of RDMs were 150x150, 100x100 and 120x120 for the three versions of the task, and the Reward Appraisal model only included the alcoholic pictures, the dimensions of RDMs were 90x90, 60x60 and 60x60. The RDMs of neural activity were in same dimensions respectively with the Visual Object Recognition and Reward Appraisal models.

Visual Object Recognition (VOR) model construction

For the VOR model, we used a pre-trained deep convolutional neural network (dCNN) to obtain the visual decoding information of the stimuli of the cue-reactivity task (Simonyan & Zisserman, 2014; Xie et al., 2020). Previous studies demonstrated high layers of visual dCNN containing high-dimensional representations of objects and complex features in visual recognition (Eickenberg et al., 2017; Xie et al., 2020). In this study, the dCNN model based on the ImageNet dataset (Deng et al.), VGG19 (Simonyan & Zisserman, 2014), was used to recognize the stimulus pictures with the MatConvNet toolbox (https://www.vlfeat.org/matconvnet/), and connected layers of the neural network were used to construct the model RDMs.

Connectivity analyses

Based on the identified regions of neural representations of alcohol cue-reactivity, seed-to-voxel psychophysiological interaction (PPI) analyses (Friston et al., 1997) were performed with the CONN toolbox v20.b (https://web.conn-toolbox.org/) (Whitfield-Gabrieli & Nieto-Castanon, 2012).

Supplementary Table 2.2-1 Study2: Information of included studies.

Study name	Inclusion criterion *	Sample size		ClinicalTrials/ German Clinical Trials Register (DRKS)
		AUD	HC	

Transalc (De Santis et al., 2019)	1) alcohol use disorder according to DSM-5; 2) for heavy-drinker, at least 84g pure alcohol in the previous 90 day; 3) treatment-seeking and abstinent.	30	36	DRKS: DRKS00003357
SFB_Haupt/ NGFN13 (Vollstädt-Klein et al., 2011)	1) alcohol dependence according to the DSM-IV and ICD 10; 3) treatment-seeking and abstinent.	79	71	ClinicalTrials: NCT00926900
SFB_Folgestudie (Gerchen et al., 2021)	1) alcohol dependence according to the DSM-IV and ICD 10; 3) treatment-seeking and abstinent.	80	52	DRKS: DRKS00003388
eMEDs (Bach et al., 2019)	1) alcohol dependence according to the DSM-IV and ICD 10; 2) treatment-seeking and abstinent.	28	57	DRKS: DRKS00003341
NALCUE (Karl et al., 2021)	1) alcohol use disorder according to the DSM-5; 2) at least 60g for men and 40g for women pure alcohol, at least 5 days/week; 3) non-treatment-seeking.	21	0	ClinicalTrials: NCT02372318
Oxytocin (Hansson et al., 2018)	healthy participants **	0	13	DRKS: DRKS00009253

Total sample size:	238	229	

Abbreviations: diagnostic and statistical manual of mental disorders (DSM), international classification of diseases (ICD), alcohol use disorder (AUD), healthy control (HC).

* General criterion of inclusion and exclusion:

These studies included AUD individuals who 1) between 18 and 75 years, 2) right-handed;

and excluded AUD individuals who 1) comorbid axis-I disorders (other than nicotine dependence) in the last year, 2) treatment with psychotropic or anticonvulsive medications in the last three months, 3) severe neurological or physiological disease (i.e. liver cirrhosis), 4) positive drug screening, 5) ineligibility for MRI scanning (e.g. metal implants), 6) history of severe head trauma.

These studies included healthy participants/light to moderate drinkers who 1) were aged between 18 and 75 years, 2) right-handed, 3) had an average alcohol consumption below 14g pure alcohol;

and excluded healthy participants who 1) comorbid axis-I disorders (other than nicotine dependence) in the last year, 2) treatment with psychotropic or anticonvulsive medications in the last three months, 3) severe neurological or physiological disease (i.e. liver cirrhosis), 4) positive drug screening, 5) ineligibility for MRI scanning (e.g. metal implants), 6) history of severe head trauma.

** light to moderate drinkers were classified as healthy participants.

Supplementary Table 2.2-2 Group characteristics of all participants (N=467).

	AUD Individuals	**Healthy Controls**	**Statistics (T/χ2-value)**	**df**	**p-value**
N	238	229			
Sex (female)	53, 22.3%	50, 21.8%	0	1	0.999
Age (years)	47 ± 10.8	45.7 ± 12.7	1.139	465	0.255
ADS score	13.7 ± 7	2.4 ± 3.6	11.722	171	<0.001
AUQ score	12.8 ± 5.7	10.1 ± 3.5	4.936	328	<0.001
OCDS global[a]	15.4 ± 6.8	2.9 ± 4.1	16.63	279	<0.001

Cumulative amount of alcohol (gram in the last 90 days)[b]	13608.3 ± 12462.5	1095.2 ± 4035.4	8.824	260	<0.001

Abbreviations: Alcohol Dependence Scale (ADS), Alcohol Urge Questionnaire (AUQ) and the Obsessive Compulsive Drinking Scale (OCDS)

[a]The calculation rules of OCDS was based on Mann et al. (Mann & Ackermann, 2000).

[b]The amount of drink was based on FORM90.

Supplementary Table 2.2-3 Different Neural Representation of VOR and RA between AUD and healthy individuals.

ROI Names	**AAL Label (size)**	**Brodmann Label (size)**	**Cluster size**	**MNI coordinate**	**tmax**
VOR1	Supp_Motor_Area_R (111) Frontal_Sup_R (3)	BA6: premotor cortex and supplementary motor cortex (12)	114	10 -4 60	4.9028
VOR2	Precentral_R (74) Postcentral_R (46)	BA4: primary motor cortex (36) BA3: primary somatosensory cortex (27)	120	48 -18 48	4.8782
RA1	Occipital_Sup_L (140) Cuneus_L (67) Occipital_Mid_L (57)	BA18: secondary visual cortex_V2 (35) BA19: associative visual cortex (V3, V4 & V5) (32) BA31: cingulate cortex (5) BA7: visuo-motor coordination (3)	264	-16 -86 26	5.3685

RA2	Temporal_Mid_R (327) Temporal_Sup_R (136) Temporal_Pole_Mid_R (6) Amygdala_R (3) Temporal_Inf_R (1)	BA21: middle temporal gyrus (192)	543	68 -14 -14	5.1199
RA3	Frontal_Inf_Tri_L (185) Frontal_Inf_Orb_L (147) Frontal_Mid_Orb_L (83) Frontal_Mid_L (44)	BA47: pars orbitalis, part of the inferior frontal gyrus (58) BA11: orbitofrontal area (31) BA10: anterior prefrontal cortex (9) BA46: dorsolateral prefrontal cortex (2) BA45: part of Broca area (1)	495	-42 46 -12	4.8378
RA4	Putamen_R (91) Caudate_R (75) Pallidum_R (19) Hippocampus_L (16) Olfactory_R (12) Amygdala_L (12) Frontal_Mid_Orb_R (3) Frontal_Inf_Orb_R (2) Insula_R (2) Frontal_Sup_Orb_R (1) Pallidum_L (1)	BA25: subgenual area (12) BA47: pars orbitalis, part of the inferior frontal gyrus (3) BA11: orbitofrontal area (2) BA13: insular cortex (1) BA34: dorsal entorhinal cortex (1)	512	22 8 -4	4.8361

RA5	Precentral_R (114) Frontal_Inf_Oper_R (105) Rolandic_Oper_R (20) Insula_R (12) Frontal_Mid_R (7)	BA6: premotor cortex and supplementary motor cortex (38) BA9: dorsolateral prefrontal cortex (13) BA13: insular cortex (13) BA44: part of Broca area (6)	374	40 0 30	4.6263
RA6	Frontal_Mid_L (204) Precentral_L (165) Frontal_Sup_L (119) Frontal_Inf_Oper_L (111) Frontal_Inf_Tri_L (60) Frontal_Sup_Medial_L (26) Supp_Motor_Area_L (7)	BA8: frontal eye fields (193) BA9: dorsolateral prefrontal cortex (134) BA6: premotor cortex and supplementary motor cortex (13) BA46: dorsolateral prefrontal cortex (1)	721	-52 12 28	4.6095
RA7	Temporal_Mid_L (109) Temporal_Sup_L (9)	BA21: middle temporal gyrus (55)	121	-64 -4 -16	4.4758
RA8	Calcarine_L (81) Lingual_L (6) Precuneus_L (1)	BA21: middle temporal gyrus (55) BA30: cingulate cortex (30) BA18: secondary visual cortex_V2 (3)	88	-16 -64 6	4.3857
RA9	Lingual_R (57) Calcarine_R (45) Vermis_4_5 (13)	BA30: cingulate cortex (34)	116	18 -54 4	4.3199

		BA18: secondary visual cortex_V2 (12) BA29: cingulate cortex (1)			
RA10	Occipital_Mid_R (67) Occipital_Sup_R (38)	BA19: associative visual cortex_V3, V4 & V5 (16)	105	30 -80 24	4.219
RA11	Lingual_R (77) Vermis_3 (5) Cerebelum_4_5_R (4) ParaHippocampal_R (2) Thalamus_R (2)	BA30: cingulate cortex (12) BA27: piriform cortex (9) BA19: associative visual cortex_V3, V4 & V5 (2)	142	10 -34 -2	4.1889
RA12	Frontal_Inf_Tri_R (114)	BA45: part of Broca area (45) BA46: dorsolateral prefrontal cortex (6)	114	56 38 8	4.0697

Two-sample t-tests were used between Alcohol Use Disorder (N = 238) and healthy (N = 229) individuals, combining voxel-wise-p<0.0005 and FWEc=108 voxels for VOR modelling and 88 voxels for RA modelling, corresponding to cluster-pFWE<0.05. See supplementary Table for the full table with cluster sizes, MNI coordinates and peak T-values of clusters, as well as labels under five voxels.

Supplementary Table 2.2-4 Compare of RDM-fc7 in Visual Object Recognition between AUD and individual.

Lobe	**Brain regions**	**Regions size**	**Cluster size**	**MNI coordinates**	**tmax**
Frontal Lobe	Precentral_R	74	122	48 -16 50	4.579
Parietal Lobe	Postcentral_R	48			

Supplementary Table 2.2-5 Connectivity from the Neural Patterns.

Seeds	AAL Label (size)	Brodmann Label (size)	tmax
	Positive Connectivity		
RA1	Angular_R(125) Lateral_Occipital_superior_R(100)	BA_40: supramarginal gyrus (50) BA_39: angular gyrus (7)	4.196
RA2	Postcentral_R(191) Supramarginal_anterior_R(50)	BA_2: primary somatosensory cortex (postcentral gyrus) (36) BA_40: supramarginal gyrus (19) BA_3: primary somatosensory cortex (postcentral gyrus) (7) BA_4: primary motor cortex (precentral gyrus) (39) BA_3: primary somatosensory cortex (postcentral gyrus) (8) BA_6: premotor cortex and supplementary motor cortex (7)	4.827
RA4	Inferior_Temporal_L(174) Inferior_Temporal_R(99) Lateral_Occipital_inferior_L(97) Temporal_Fusiform_posterior_L(78) Lateral_Occipital_inferior_R(29) Middle_Temporal_L(27) Temporal_Occipital_Fusiform_L(25) Inferior_Temporal_posterior_L(6) Middle_Temporal_R(4) Cerebelum_6_L(4)	BA_37: fusiform gyrus (14) BA_19: associative visual cortex (V3, V4 & V5) (11) BA_37: fusiform gyrus (68) BA_20: inferior temporal gyrus (48) BA_19: associative visual cortex (V3, V4 & V5) (12)	4.701

	Occipital_Fusiform_L(3)		
RA5	Cingulate_posterior(253) Cingulate_anterior(4)	BA_23: cingulate cortex (87) BA_31: dorsal posterior cingulate cortex (10) BA_24: cingulate cortex (4)	4.622
RA6	Parietal_Operculum_L(129) Supramarginal_anterior_L(57) Planum_Temporale_L(22) Superior_Temporal_posterior_L(7)	BA_40: supramarginal gyrus (84) BA_42: primary auditory cortex (Heschl gyrus) (39) BA_13: insular cortex (10)	4.823
RA9	Angular_R(391) Middle_Frontal_R(328) Frontal_Pole_R(289) Cingulate_posterior(101) Supramarginal_posterior_R(61) Lateral_Occipital_superior_R(45) Cingulate_anterior(11)	BA_10: anterior prefrontal cortex (112) BA_9: dorsolateral prefrontal cortex (22) BA_24: cingulate cortex (32) BA_23: cingulate cortex (21) BA_8: frontal eye fields (72) BA_9: dorsolateral prefrontal cortex (71) BA_6: premotor cortex and supplementary motor cortex (4) BA_40: supramarginal gyrus (204) BA_39: angular gyrus (1)	5.146
RA10	Cingulate_posterior(541) Precuneous(434) Lateral_Occipital_superior_L(154) Superior_Parietal_Lobule_L(1)	BA_7: visuo-motor coordination (superior parietal lobule) (212) BA_31: dorsal posterior cingulate cortex (168)	7.410

		BA_23: cingulate cortex (68) BA_24: cingulate cortex (17) BA_7: visuo-motor coordination (superior parietal lobule) (46) BA_39: angular gyrus (4) BA_19: associative visual cortex (V3, V4 & V5) (2) BA_40: supramarginal gyrus (2)	
VOR1	Precentral_R(79) Postcentral_R(34) Central_Opercular_R(5)	BA_6: premotor cortex and supplementary motor cortex (39) BA_4: primary motor cortex (precentral gyrus) (16)	4.092
	Negative Connectivity		
RA1	Lateral_Occipital_superior_R(357) Lateral_Occipital_inferior_R(29)	BA_19: associative visual cortex (V3, V4 & V5) (51) BA_18: secondary visual cortex (V2) (8) BA_31: dorsal posterior cingulate cortex (7) BA_7: visuo-motor coordination (superior parietal lobule) (2)	5.228
RA4	Supramarginal_posterior_R(118) Angular_R(37) Supramarginal_anterior_R(14)	BA_40: supramarginal gyrus (72) BA_2: primary somatosensory cortex (postcentral gyrus) (1)	4.316
RA5	Lateral_Occipital_inferior_L(192)	BA_37: fusiform gyrus (74)	5.280

	Lateral_Occipital_inferior_R(92) Middle_Temporal_L(28) Middle_Temporal_R(24)	BA_39: angular gyrus (16) BA_19: associative visual cortex (V3, V4 & V5) (6) BA_37: fusiform gyrus (24) BA_19: associative visual cortex (V3, V4 & V5) (3)	
RA8	Lateral_Occipital_superior_R(171) Lateral_Occipital_inferior_R(3)	BA_19: associative visual cortex (V3, V4 & V5) (3) BA_39: angular gyrus (3)	5.620
RA9	Cingulate_posterior(204) Lingual_R(189) Lingual_L(155) Vermis_4_5(105) Temporal_Fusiform_posterior_R(65) Parahippocampal_posterior_R(61) Parahippocampal_posterior_L(46) Temporal_Occipital_Fusiform_R(40) Temporal_Fusiform_posterior_L(39) Precuneous(36) Cerebelum_4_5_L(27) Temporal_Occipital_Fusiform_L(22) Thalamus_r(17) Hippocampus_r(14) Cerebelum_4_5_R(14) Hippocampus_l(8) Vermis_3(1)	BA_19: associative visual cortex (V3, V4 & V5) (83) BA_30: part of the cingulate cortex (66) BA_36: perirhinal cortex & ectorhinal area (62) BA_37: fusiform gyrus (43) BA_29: retrosplenial cingulate cortex (30) BA_20: inferior temporal gyrus (11) BA_27: piriform cortex (6)	5.214
RA11	Lingual_R(87) Cingulate_posterior_(56) Temporal_Occipital_Fusiform_R(33) Precuneous(13) Parahippocampal_posterior_R(13) Temporal_Fusiform_posterior_R(2) Hippocampus_r(2)	BA_19: associative visual cortex (V3, V4 & V5) (33) BA_30: part of the cingulate cortex (19) BA_37: fusiform gyrus (15) BA_36: perirhinal cortex & ectorhinal area (8)	4.916

			BA_29: retrosplenial cingulate cortex (3)

Supplementary Table 2.2-6 The support vector weights of ROIs in relapse prediction.

ROI Names	Weight	AAL Label	Brodmann Label
RA11	0.4802	Lingual_R (77) Vermis_3 (5) Cerebelum_4_5_R (4) ParaHippocampal_R (2) Thalamus_R (2)	BA30: cingulate cortex (12) BA27: piriform cortex (9) BA19: associative visual cortex_V3, V4 & V5 (2)
RA8	0.4131	Calcarine_L (81) Lingual_L (6) Precuneus_L (1)	BA21: middle temporal gyrus (55) BA30: cingulate cortex (30) BA18: secondary visual cortex_V2 (3)
RA9	0.4007	Lingual_R (57) Calcarine_R (45) Vermis_4_5 (13)	BA30: cingulate cortex (34) BA18: secondary visual cortex_V2 (12) BA29: cingulate cortex (1)
VOR1	0.2508	Supp_Motor_Area_R (111) Frontal_Sup_R (3)	BA6: premotor cortex and supplementary motor cortex (12)
VOR2	0.0816	Precentral_R (74) Postcentral_R (46)	BA4: primary motor cortex (36) BA3: primary somatosensory cortex (27)
RA7	-0.0501	Temporal_Mid_L (109) Temporal_Sup_L (9)	BA21: middle temporal gyrus (55)
RA4	-0.0809	Putamen_R (91) Caudate_R (75)	BA25: subgenual area (12)

		Pallidum_R (19) Hippocampus_L (16) Olfactory_R (12) Amygdala_L (12) Frontal_Mid_Orb_R (3) Frontal_Inf_Orb_R (2) Insula_R (2) Frontal_Sup_Orb_R (1) Pallidum_L (1)	BA47: pars orbitalis, part of the inferior frontal gyrus (3) BA11: orbitofrontal area (2) BA13: insular cortex (1) BA34: dorsal entorhinal cortex (1)
RA1	-0.1233	Occipital_Sup_L (140) Cuneus_L (67) Occipital_Mid_L (57)	BA18: secondary visual cortex_V2 (35) BA19: associative visual cortex (V3, V4 & V5) (32) BA31: cingulate cortex (5) BA7: visuo-motor coordination (3)
RA12	-0.1446	Frontal_Inf_Tri_R (114)	BA45: part of Broca area (45) BA46: dorsolateral prefrontal cortex (6)
RA2	-0.1449	Temporal_Mid_R (327) Temporal_Sup_R (136) Temporal_Pole_Mid_R (6) Amygdala_R (3) Temporal_Inf_R (1)	BA21: middle temporal gyrus (192)
RA5	-0.2027	Precentral_R (114) Frontal_Inf_Oper_R (105)	BA6: premotor cortex and supplementary motor cortex (38) BA9: dorsolateral prefrontal cortex (13)

		Rolandic_Oper_R (20) Insula_R (12) Frontal_Mid_R (7)	BA13: insular cortex (13) BA44: part of Broca area (6)
RA3	-0.3025	Frontal_Inf_Tri_L (185) Frontal_Inf_Orb_L (147) Frontal_Mid_Orb_L (83) Frontal_Mid_L (44)	BA47: pars orbitalis, part of the inferior frontal gyrus (58) BA11: orbitofrontal area (31) BA10: anterior prefrontal cortex (9) BA46: dorsolateral prefrontal cortex (2) BA45: part of Broca area (1)
RA10	-1.4421	Occipital_Mid_R (67) Occipital_Sup_R (38)	BA19: associative visual cortex_V3, V4 & V5 (16)
RA6	-1.9085	Frontal_Mid_L (204) Precentral_L (165) Frontal_Sup_L (119) Frontal_Inf_Oper_L (111) Frontal_Inf_Tri_L (60) Frontal_Sup_Medial_L (26) Supp_Motor_Area_L (7)	BA8: frontal eye fields (193) BA9: dorsolateral prefrontal cortex (134) BA6: premotor cortex and supplementary motor cortex (13) BA46: dorsolateral prefrontal cortex (1)

3 DISCUSSION

With datasets from large clinical samples, this work was the first time exploring whole-brain iron accumulation in AUD and its association with compulsive consumption patterns. It was also the first work disentangling the processing of alcohol cues into components of visual object recognition and reward appraisal. Using QSM susceptibility measures, increased iron accumulation in the dorsal striatum is observed in AUD individuals, and with RSA it demonstrates the enhanced neural representation of alcohol cues in specific brain regions. The striatal iron accumulation is associated with compulsive drinking, and the decoding involvements of enhanced neural patterns could contribute to predicting relapse within six months. Moreover, the neural patterns were connected to large-scale functional networks, and also associated with clinical features of AUD.

3.1 Accumulation of brain iron in AUD individuals

As hypothesized (H1), accumulation of brain iron was observed in AUD individuals, especially in the basal ganglia. Alcohol use has a significant and wide-ranging impact on multi-systems/organs and might be associated with systemic iron accumulation in the body. Meanwhile, it is reported that alcohol use disrupts the BBB integrity (Haorah et al., 2005; Pimentel et al., 2020), which could have an impact on iron transport and contributes to brain iron accumulation ((Olmedo-Díaz et al., 2017). What is more, increased brain iron after acute and chronic alcohol exposure was observed in animals (Crews & Nixon, 2009; Rouach et al., 1997; Rouach et al., 1990), which was hypothesized to be related to free radicals and oxidative stress, and consequently in neuroinflammation. Previous studies using priori ROIs have reported brain iron accumulation in the basal ganglia (Juhás et al., 2017; Topiwala, Wang, Ebmeier, Burgess, Bell, Levey, Zhou, McCracken, Roca-Fernández, et al., 2022). The current work based on whole-brain analyses provided further evidence of iron accumulation in specific regions.

A potential reason the iron particularly accumulates in striatal regions might be the high energetic demands from dopaminergic activity, since the striatum holds the highest density in dopaminergic terminals. The dopamine turnover and metabolism are energetically extremely demanding and dopamine forming a potent redox couple (Hare & Double, 2016; Scheurich et al., 2005), which might also underlie the higher sensitivity

of the dorsal vs. ventral striatal regions to neurodegeneration in Parkinson's disease. Meanwhile, iron is a co-factor of tyrosine hydroxylase in dopamine synthesis, which converts tyrosine to dopamine. Tissue culture experiments in peripheral blood cells have shown that dopamine alters cellular iron homeostasis by increasing iron incorporation (Dichtl et al., 2018). Following the dopamine synthesis, molecules from oxidation in the dopamine degradation could be neurotoxic to catecholaminergic cells (Muñoz et al., 2012), and iron was found as a mediator of the neurotoxicity in Parkinson's disease via Fe−dopamine complex (Paris et al., 2005). Thus, regions with high dopaminergic activity appear vulnerable to iron accumulation. This in turn might lead to cognitive and behavioral impairment (Rodrigue et al., 2020; Schröder et al., 2013; Spence et al., 2020; Tonekaboni & Mollamohammadi, 2014). In fact, evidence from human PET and postmortem studies and corresponding animal experiments demonstrated profound alterations in the dopamine system of AUD (Hansson et al., 2019; Hirth et al., 2016).

3.2 Association between the iron accumulation and drinking patterns

The second hypothesis (H2), brain iron accumulation to be associated with the amount of previous drinking, with AUD severity and with previous obsessive-compulsive drinking patterns, could be partially confirmed by correlation analyses between the iron accumulation and clinical features. The current work showed a positive correlation between dorsal striatal iron load and compulsive drinking behavior as measured by the OCDS (Vollstädt‐Klein et al., 2010). AUD is characterized by recurrent compulsive alcohol use, which was also emphasized as a central aspect of addiction. Previous studies demonstrated that the ventral and dorsal striatum play different roles in addiction, and the dorsal striatum is more related to compulsive use. (Ito et al., 2000; Lüscher et al., 2020; Uhl et al., 2019; Vollstädt‐Klein et al., 2010). In animal studies, a large increase in dopamine levels was observed in the dorsal striatum in long-term cocaine-use (Ito et al. 2002), and when inactivating the dorsolateral striatum, the habitual behavior was reduced (Vanderschuren et al., 2005). In addition, a circuit involving the frontal cortex and striatum is suggested to be important for the development of compulsivity. In human imaging studies, a previous study using cue-reactivity tasks indicated that the cue-induced activation of the ventral striatum in social drinkers is higher than in heavy drinkers, while in heavy drinkers it was higher in the dorsal striatum (Vollstädt‐Klein et al., 2010). This suggested that dorsal striatum

became the dominant region in compulsive alcohol use. In 2013, Sjoerds and colleagues also found dysfunction of the anterior putamen in alcohol-dependent patients using an instrumental learning task, which was related to habit control (Sjoerds et al., 2013). The correlation between iron accumulation in the dorsal striatum and OCDS further strengthens the hypothesis that the dorsal striatum is specifically involved with mediating compulsive drinking behavior, and that a potential underlying neural mechanism contributing to this might be iron overload (Tonekaboni & Mollamohammadi, 2014). The AUD participants in the present study had been drinking for 19.8 years, on average, and were therefore likely in the stage of compulsive use, to varying degrees as assessed by the OCDS. Meanwhile, it also found a significant correlation between iron accumulation and the cumulative amount of alcohol consumption in the past three months as hypothesized. However, we did not find significant correlations between dorsal striatal susceptibility and AUQ or ADS scores. It might be not surprising, since the AUQ assesses ‘state’ as opposed to ‘trait’, which reflects a temporary condition and would be unlikely to correlate with a cumulative, chronic indicator like iron-load. The ADS, on the other hand, does in fact measure trait (severity of alcohol dependence), but one which consists of several domains beyond compulsivity, including negative emotion, preoccupation and salience. Therefore, it seems likely that the ADS may associate with neural activity that goes beyond the dorsal striatum. The OCDS is a tool that is specific to the assessment of trait compulsive drinking and its positive correlation with dorsal striatal susceptibility makes a compelling case that increased iron load in the dorsal striatum is directly related to increased compulsive drinking patterns.

3.3 Increased involvements of the neural patterns decoding alcohol cues

With the fMRI alcohol cue-reactivity task, the third hypothesis (H3) could be confirmed by the results of RSA analyses. In AUD individuals, enhanced neural patterns decoding the alcohol cues were observed in the sensory and motor system. The visual cortex is the first gate for visual cues in the cortex, and studies from animals and humans have demonstrated that both primary and higher visual cortices exhibited value-based modulations of their activity responding to reward-mounted cues (Yalachkov et al., 2010). The RA model in RSA shows enhanced neural patterns in higher visual cortices (BA18, 19, and 21), which means that the visual cortex of AUD individuals might represent the reward value of alcohol cues better than in healthy individuals.

Interestingly, the somatosensory cortex of AUD showed higher decoding involvements in the VOR processing. Since the somatosensory cortex is mainly responsible for low-level tactile information, a possible explanation might be that alcohol had powerful impacts on the somatosensory circuits and the exposure to visual cues may meanwhile activate sensory representations in the haptic modality. Besides the somatosensory cortex, AUD individuals showed special neural representation in motor and premotor brain areas in both VOR and RA processes. The underlying mechanism could be that the motor brain regions play a role in the formation of automatized drinking behavior, which is also known as habitual and compulsive drinking (Lüscher et al., 2020). The positive correlation to OCDS scores could also support this interpretation. In many cue-reactivity studies, motor-related areas also have been reported that activated differently towards substance-related stimuli (Vollstädt‐Klein et al., 2010; Yalachkov et al., 2009). In the next section, the involvements of motor-related areas will be discussed in detail. In addition to enhanced neural patterns in the sensory and motor cortex, AUD individuals also showed representation in the middle temporal gyrus. As a part of associate visual cortex, the middle temporal gyrus plays a role as the transit hub of visual attention pathway, whose activity might reflect the sensory analysis of the cue (Corbetta & Shulman, 2002). Meanwhile, the PPI analysis found a connectivity from middle temporal gyrus to the dorsal frontoparietal network (superior parietal lobule), which is involved in top-down control of visual attention (Gilbert & Li, 2013).

In the RA model of RSA, a large network showed higher involvements in AUD individuals compared to healthy participants, especially the orbitofrontal, anterior prefrontal, dorsolateral prefrontal cortex and striatum. These areas could be summarized as three large-scale networks: reward, habit and executive, which reviewed by Zilverstand et al. in 2018 based on iRISA model in addiction research (Zilverstand et al., 2018). Previous studies of substance use disorder demonstrated that the hyperactivation of these three networks was involved in the appraisal of the subjective value of the salient cues, automatization of the reaction, and cognitive control toward processing the cues. Specifically, the reward network was suggested in previous studies of SUD that it supports the appraisal of subjective values by integrating incentive motivational value (Milton & Everitt, 2012), and contributes to computing subjective value and reward expectations (Chase et al., 2015; Koechlin & Hyafil, 2007). Moreover, when drug incentives elicited stronger activation in these three networks in individuals with substance use disorder compared to controls, the aberrant

salience attribution to drug-related stimuli was found to interact with impaired response inhibition in drug addiction (Jasinska et al., 2014; Zeng et al., 2021; Zhukovsky et al., 2020; Zilverstand et al., 2018). A recent study suggested that the interaction of these three key networks may be rebalanced by an opioid receptor antagonist (Grundinger et al., 2022). Besides, the connectivity results showed the interaction among multiple systems. It showed increased connectivity in AUD individuals from the higher visual cortex and attention system to higher cognitive functioning related areas, including regions of dorsolateral prefrontal cortex, supramarginal, angular gyrus and anterior prefrontal cortex, as well as the memory system (Hippocampus and Parahippocampal gyrus). This strengthened the previous findings that even the passive-viewing cue exposure could involve key addiction networks beyond value appraisal, habit learning, and response inhibition to higher cognitive processes (Everitt & Robbins, 2016; Goldstein & Volkow, 2011).

3.4 Clinical relevance of the neural patterns

It was hypothesized that the involvements of neural patterns are correlated with craving, with obsessive-compulsive drinking patterns and with AUD severity (H4). The results from correlation analyses between decoding involvements and psychometrics confirmed this hypothesis. With both VOR and RA model of RSA, the large networks of reward, habit and executive functioning shows positive correlation to severity of dependence and with craving. Neuroimaging studies in drug addiction reported activation levels in the reward network correlating with self-reported craving and urge to use (Kühn & Gallinat, 2011; Wilson & Sayette, 2015). The current work brought new evidence of not only the representation of alcohol-related reward values in the network but also its association with clinical features. Secondly, the habit network has been shown to support habit and stimulus-response learning (Milton & Everitt, 2012)(Milton and Everitt, 2012), which could drive the automatization of behavior (Lüscher et al., 2020). In current results, motor-related areas in the VOR model showed a positive correlation to OCDS scores as mentioned in the earlier section. This might be related to automatized action schemata (Du et al., 2022; Shiffrin & Schneider, 1977; Tiffany, 1990; Yalachkov et al., 2010), which is another underlying complement of automatic behavioral responses besides the habitual stimulus-response mechanisms. As a complementary aspect of automaticity in addiction, the automatic behavioral responses could be related to procedural memory processes in terms of object

manipulation and motor skills after intensive and repetitive motor training. Such automatic behavior might show faster responses and less perception demanding (Logan, 1988; Shiffrin & Schneider, 1977, 1984; Yalachkov et al., 2010). Established motor skills are believed to be important for automatized action in addiction (Yalachkov et al., 2009). The associations between decoding involvements of motor-related areas and clinical features in this work could be new evidence of the automatized action schemata in alcohol cue-reactivity. Here it might be specifically noted that the motor brain represents not only the reward values, but also the cue features from computer vision (dCNN model), which might imply that automatic behavioral responses are deeply embedded and linked to visual information processing. Besides, involvements of the executive network in RA model were also associated with craving, with obsessive-compulsive drinking patterns and with AUD severity. Previous studies demonstrated that the executive network plays a primary role in inhibition of motor, attention redirection and cognitive responses (Voon et al., 2020; Zilverstand et al., 2018). In addiction studies, impaired inhibitory control and cognitive self-regulation have been reported (Luijten et al., 2014; Zilverstand et al., 2017), and some studies integrated drug cue exposure with an inhibitory control task, finding that reward network could be accompanied by a significant increase of activation levels executive work during cue exposure (Arcurio et al., 2015).

3.5 Relapse prediction with the decoding involvement neural patterns

The last hypothesis (H5) regarding relapse prediction could be partly confirmed by the results of SVM model. With linear SVM, the current results showed that the decoding involvement of enhanced neural patterns of cue-reactivity could contribute to predicting relapse within six months. Previous studies of AUD have reported predictors of relapse with various imaging modules (Beck et al., 2012; Charlet et al., 2014; Seo et al., 2015). With voxel-wise univariate approaches, they found risk factors such as atrophy in the bilateral orbitofrontal cortex and the right medial prefrontal and anterior cingulate cortex, and the increased activation of left medial prefrontal cortex in alcohol cue-reactivity task. PPI analyses of cue-reactivity also reported reduced functional connectivity between the midbrain and the left amygdala and between the midbrain and the left orbitofrontal cortex as predictors of relapse (Beck et al., 2012; Voon et al., 2020). However, most previous studies predicted relapse considered neither brain response as neural patterns, nor reward values of the alcohol cues. In the current work,

the decoding involvement of identified neural patterns might be suggested as a novel neuroimaging marker for clinical practice, which reflects the processes decoding alcohol cues in networks, though the accuracy of prediction was moderate. The high-weighted features in the SVM model, the decoding involvements of FEF, dlPFC, had large negative weights towards relapse, which could be considered as protective factors. The FEF and dlPFC could be related to the attention and executive function, which often be discussed in salience and executive networks in addiction studies. The core functions of these networks are attentional and inhibition control. Human image studies found activation levels in the salience network were correlated with self-reported craving, and hypoactivation of the executive network was correlated with impaired inhibition control (Voon et al., 2020; Zilverstand et al., 2018). However, this work focused on neural cue processing did not reach a higher accuracy compared to a previous study, which predicted relapse with cue-reactivity fMRI and structural MRI (Seo et al., 2015), which imply that decoding involvements of fMRI cue-reactivity might not be sufficient as the only predictor.

3.6 Novel biomarkers of Alcohol Use Disorder and clinical implications

Iron accumulation could be a novel biomarker of AUD, since its relevance to systemic iron metabolism, BBB integrity, and dopaminergic activity, which could be specifically linked to the compulsive drinking pattern. In clinical assessment, striatal iron concentration from imaging examinations might provide an objective measure associated with recent alcohol exposure and compulsive drinking, which might be helpful for individualized treatment of AUD. The identified neural patterns could be another biomarker as it was shown to be related to relapse, which are also associated with AUD severity, with self-reported craving and with obsessive-compulsive drinking patterns. Alcohol dependence is characterized by a chronic course and a high risk to relapse, especially within the next six months after detoxification, and relapse might be one of the reasons for substantial health problems (Boothby & Doering, 2005; Rehm et al., 2009). Estimating relapse risk could help clinicians optimize the post-detoxification treatment, which meets the idea of precise medicine. From the current work, though the prediction accuracy was moderate, the identified neural patterns could be an add-on biomarker from the aspect of cue-reactivity.

These findings might not only contribute to clinical practice as biomarkers, but also provide new therapeutic targets. Treatments focusing on the metabolism and

elimination of brain iron might consider striatal iron accumulation as a potential target, which could be expected a reduction compulsive drinking on the behavior side. In studies of neurodegeneration with brain iron accumulation, iron chelators was used as the medication, which might be able to remove excess iron from specific brain regions (Ward et al., 2015). Moreover, transcranial magnetic stimulation with the areas such as dlPFC, medial prefrontal cortex and the anterior cingulate cortex has been reported modulating neural activity in brain circuits that mediate cognitive processes relevant to addiction (Gorelick et al., 2014), and as well as obsessive-compulsive disorder, which shared some behavior features with addictive disorders. Some regions in the identified neural patterns from the current work could suggest potential target regions. Furthermore, when deeply embedded action schemata of drinking behavior is observed in cue-reactivity, therapies targeting automatic action tendencies could be add-ons to treatments (Cox et al., 2014). A previous study of AUD used alcohol-avoidance training and reported lower relapse rates at one-year follow-up (Eberl et al., 2013).

3.7 Limitations and future perspectives

Despite the strength and novelty, there were limitations of this work. Since the current work was based on secondary analyses of existing datasets from previous projects using the same inclusion criteria and scanning parameters, the MRI protocols were not optimized to examine brain iron and the spatial resolution of the images for QSM analyses was relatively low, though the GRE sequence appears suitable for standard QSM methods (Haacke et al., 2015). The spatial resolution limited this study to detect iron accumulation in smaller brain regions of the mid and hind brain as previously reported (Juhás et al., 2017; Topiwala, Wang, Ebmeier, Burgess, Bell, Levey, Zhou, McCracken, Roca-Fernandez, et al., 2022) and prevented the exploration of striatal subregions. For further investigation, a 3-D GRE multi-echo sequence is already developed and applied to following studies.

The second limitation was about the systemic iron level. Though systemically altered iron metabolism is well-established knowledge of AUD, this work had no access to blood markers (e.g., iron levels, ferritin and transferrin saturation), and was unable to study the relationship between iron metabolism in whole-body/organs and brain iron accumulation. In a previous brain iron study, cocaine use disorder showed dysregulation of peripheral iron metabolism in the context of levels of iron and

transferrin saturation, when brain iron accumulation was observed in globus pallidus (Ersche et al., 2017). Therefore, investigating the relationship between the peripheral and central iron levels of AUD could be an important area of future research.

Another interesting question is whether brain iron accumulation in the dorsal striatum is a predisposing factor for compulsive behavior and the development of AUD or whether it is the result of long-term alcohol consumption. The present work was limited by its cross-sectional design. Some recent studies have attempted to elucidate the relationship between brain iron, cognitive function, and age in non-AUD populations. Interestingly, in healthy individuals, higher iron load was predictive of deficits in a working memory task, especially in younger and middle-aged participants, when compared to older ones (Rodrigue et al., 2020). However, a different study (Larsen et al., 2020) in which the longitudinal trajectories of striatal iron load were examined came to the conclusion that greater cognitive ability is increasingly associated with greater iron concentration through late adolescence and young-adulthood. In order to explore this question, it would be useful to follow individuals over the trajectory of addiction development, to make a direct intra-individual comparison of iron levels over time.

Moreover, correlation analyses of striatal iron levels and drinking patterns in the current work only found a significant correlation in both groups, but not within the AUD group alone. This might be because the AUD group and control group are not substantially different in this study based on DSM criteria. Some participants in the Control group were also light to moderate alcohol drinkers, but just did not meet enough criteria for the AUD diagnosis. There is already a critical topic in psychiatry for years: the diagnosis of mental disorders is a widely discussed topic that diagnoses based on presenting signs and symptoms have been increasingly shown not to represent valid disease entities (Cuthbert, 2014). So recent studies (including the current work) are trying to find neurobiological associations. Thus restricting the correlation analysis to the AUD group might not be able to find biological and substantial features related to alcohol consumption and describe valid disease entities. However, correlation analysis across both groups was indeed a compromise. To better answer if there are linear relations between iron accumulation and drinking behavior, future studies may need to recruit alcohol users in a wide spectrum, from light to medium, and to heavy drinkers.

Although this work detangled the processing of cues into components of VOR and RA, it was not able to investigate the temporal characteristics of the neural patterns, because of the design of paradigm and the temporal resolution. To answer questions

such as whether the VOR pattern responds to cues before RA pattern or synchronously, a long-event fMRI paradigm design could be used in the future, combined with finite impulse response modelling, or maybe magnetoencephalography or electroencephalogram (MEG/EEG) could also be used to characterize the temporal characters of the neural pattern.

The prediction models, both SVM and lasso penalized logistic regression did not reach a higher accuracy compared to a previous study. The relatively small sample size of participants with relapse data could be a reason, though this study had a relatively large sample size in the baseline. Another reason could be the heterogeneity of different treatments, which were received by the participants providing the relapse data. It was noted that the lasso penalized logistic regression even not better than chance levels, and this could suggest a relatively weak linear relation between neural patterns and relapse.

Interestingly, the findings in study 1 and study 2 converged on several important brain networks, which were linked to the same predominant clinical features in AUD. The brain regions where biomarkers from both studies were derived from, such as the striatum, middle frontal cortex and dlPFC, suggested the important role of frontostriatal circuitries (Feil et al., 2010; Volkow et al., 2013). Dysfunction of frontostriatal circuitries could mediate response-inhibition impairment (Morein-Zamir & Robbins, 2015; Zilverstand et al., 2018). Preclinical evidence indicated that addiction is associated with neuroadaptive changes in frontostriatal networks, which may influence both impulsivity and compulsivity in substance use behavior (Morein-Zamir & Robbins, 2015). In the iRISA model, these regions with increased iron accumulation and decoding involvement are included in almost all six large-scale well-known addiction-related brain networks (reward, habit, salience, executive, memory, and self-directed networks), which could be involved in the appraisal of subjective values by integrating incentive motivational value, supporting habit learning, and selection of a behavioral response. However, it remains unknown yet whether striatal iron levels have associations with the activity of frontostriatal circuitries involved in alcohol cue processing. Moreover, if iron accumulation would have an influence on neural activity, how are the effects on alcohol cue-reactivity of AUD individuals regarding reward appraisal, inhibition control and other cognitive functions? These questions might be investigated in the future.

Besides, there is another open question from a methodological view. Since it was reported that increased brain iron accumulation could lead to signal loss and hence systematic artifacts when acquiring fMRI images because of the static field inhomogeneity (Puckett et al., 2018; Song, 2001), it is still unclear whether the iron accumulation has effects on fMRI alcohol cue-reactivity. In an ongoing follow-up study, the correlation between BOLD susceptibility and contrast values derived from a cue-reactivity task is explored to examine iron effects on task-based fMRI.

4 SUMMARY

Alcohol Use Disorder, as a chronically relapsing disorder, is a worldwide public health problem. Neuroimaging studies reported alterations related to alcohol use from various aspects, which could be potential biomarkers in Alcohol Use Disorder. However, neuroimaging features of Alcohol Use Disorder need further description, and the underlying neural mechanisms are still not fully understood. This study aimed to identify neuroimaging biomarkers in Alcohol Use Disorder from the aspects of brain iron accumulation and neural patterns decoding alcohol cues, and their clinical relevance and relapse prediction using these novel biomarkers.

This study was based on secondary analyses of previous datasets. To examine brain iron accumulation, 186 individuals with Alcohol Use Disorder and 274 healthy participants were included. Quantitative Susceptibility Mapping, an emerging MRI technique developed for quantifying tissue magnetic susceptibility, was performed to measure the whole-brain iron level. Using an alcohol cue-reactivity task, functional magnetic resonance imaging data from 238 Alcohol Use Disorder individuals and 229 healthy participants were used to identify neural patterns during processing visual cues of alcohol. The processes of visual object recognition and reward appraisal of alcohol cues were separately modeled using Representational Similarity Analysis. To develop biomarkers in Alcohol Use Disorder, whole-brain iron levels and decoding involvements during cue-reactivity task were compared between Alcohol Use Disorder individuals and healthy participants. The relationship between drinking patterns and biomarkers was explored, and the decoding involvements during a cue-reactivity task were used for predicting relapse within six months. Moreover, connectivity analyses of functional magnetic resonance imaging data were conducted to investigate communications between neural networks within the brain.

Whole-brain analyses of Quantitative Susceptibility Mapping showed that the susceptibility in dorsal striatum (putamen and caudate) among Alcohol Use Disorder individuals was higher than in healthy participants, and was positively related to the Obsessive Compulsive Drinking Scale scores and the amount of drinking in the past three months. During decoding alcohol cues, individuals with Alcohol Use Disorder, compared to healthy individuals, showed higher involvement of motor-related brain regions in the process of visual object recognition, and their reward, habit and executive networks were more engaged in appraising reward values. Connectivity

analyses showed that the involved neural systems were widely connected with higher cognitive networks during alcohol cue processing in Alcohol Use Disorder individuals, and decoding involvements of frontal eye fields and dorsolateral prefrontal cortex could contribute to relapse prediction.

Higher iron accumulation in the dorsal striatum was observed in Alcohol Use Disorder. This surrogate for the brain iron level was linearly associated with compulsive drinking patterns and the recent amount of drinking, which justified to provide a new biomarker in relation to brain iron accumulation for clinical practice. The decoding neural patterns during alcohol cue-reactivity provide insight into how Alcohol Use Disorder individuals differently decode alcohol cues compared to healthy participants, from the componential processes of visual object recognition and reward appraisal. These identified patterns are suggested as biomarkers and potential therapeutic targets in Alcohol Use Disorder.

Die Alkoholkonsumstörung ist als eine chronisch-rezidivierende Erkrankung ein weltweites Problem der öffentlichen Gesundheit. Neuroimaging-Studien berichteten Veränderungen im Zusammenhang mit Alkoholkonsum unter verschiedenen Aspekten, die als potentielle Biomarker für Alkoholkonsumstörung dienen können. Die Merkmale der Neuroimaging-Befunde bei Alkoholkonsumstörung bedürfen jedoch einer weiteren Beschreibung, und die zugrundeliegenden neuronalen Mechanismen sind noch nicht vollständig geklärt. Ziel dieser Studie ist die Identifizierung von Neuroimaging-Biomarkern bei Alkoholkonsumstörung unter den Aspekten der Eisenablagerung im Gehirn und neuronaler Muster, die Alkoholhinweisreize dekodieren, sowie deren klinische Relevanz und die Rückfallvorhersage anhand dieser Biomarker.

Die vorliegende Studie basiert auf Sekundäranalysen von Datensätzen. Zur Untersuchung der Eisenablagerung im Gehirn wurden 186 Proband*innen mit Alkoholkonsumstörung und 274 gesunde Proband*innen eingeschlossen. Zur Messung des Eisengehalts im gesamten Gehirn wurde Quantitative-Susceptibility-Mapping, eine neue Magnetic-Resonance-Imaging Technik zur Quantifizierung der magnetischen Suszeptibilität von Gewebe, durchgeführt. Mit einer Cue-Reaktivitäts-Aufgabe wurden functional-Magnetic-Resonance-Imaging-Daten von 238 Proband*innen mit Alkoholkonsumstörung und 229 gesunden Proband*innen verwendet, um neuronale Muster bei der Verarbeitung visueller Hinweisreize auf Alkohol zu identifizieren. Die Prozesse der visuellen Objekterkennung und der Belohnungsbewertung von Alkohol-Hinweisreizen wurden mithilfe der Representational Similarity Analysis separat modelliert. Um Biomarker für Alkoholkonsumstörung zu entwickeln, wurden die Eisenwerte im gesamten Gehirn und die Dekodierungsbeteiligung während der Cue-Reaktivitäts-Aufgabe zwischen Proband*innen mit Alkoholkonsumstörung und gesunden Proband*innen verglichen. Der Zusammenhang zwischen dem Trinkverhalten und den Biomarkern wurde untersucht, und die Dekodierungsbeteiligung während der Cue-Reaktivitäts-Aufgabe wurde zur Vorhersage eines Rückfalls innerhalb von sechs Monaten verwendet. Darüber hinaus wurden Konnektivitätsanalysen der functional magnetic resonance imaging durchgeführt, um die Kommunikation zwischen neuronalen Netzwerken in gesamten Gehirn zu untersuchen.

Ganzkopfanalysen des Quantitative-Susceptibility-Mapping zeigten, dass die Suszeptibilität im dorsalen Striatum (Putamen und Caudat) bei Proband*innen mit

Alkoholkonsumstörung höher war als bei gesunden Proband*innen und in einem positiven Zusammenhang mit den Scores der Obsessive-Compulsive-Drinking-Scale und der Trinkmenge der letzten drei Monate stand. Bei der Dekodierung von Alkohol-Hinweisreizen waren bei Proband*innen mit Alkoholkonsumstörung im Vergleich zu gesunden Personen motorische Hirnregionen stärker in den Prozess der visuellen Objekterkennung involviert, und ihre Belohnungs-, Gewohnheits- und Exekutivnetzwerke waren stärker an der Bewertung von Belohnungswerten beteiligt. Konnektivitätsanalysen zeigten, dass die beteiligten neuronalen Systeme während der Verarbeitung von Alkoholhinweisreizen bei Proband*innen mit Alkoholkonsumstörung in hohem Maße mit höheren kognitiven Netzwerken verbunden waren, und die Dekodierung der frontalen Augenfelder und des dorsolateralen präfrontalen Kortex zur Rückfallvorhersage beitragen konnte.

Bei Alkoholkonsumstörung wurde eine höhere Eisenablagerung im dorsalen Striatum beobachtet. Dieses Surrogat für den Eisengehalt des Gehirns stand in linearem Zusammenhang mit dem zwanghaften Trinkverhalten und der vorherigen Trinkmenge, was die Nutzung als einen neuen Biomarker in Bezug auf die Eisenablagerung im Gehirn für die klinische Praxis rechtfertigen könnte. Die neuronalen Dekodierungsmuster während der Reaktion auf Alkoholhinweisreize geben Aufschluss darüber, wie Proband*innen mit Alkoholkonsumstörung Alkoholhinweisreize anders dekodieren als gesunde Teilnehmer, und zwar auf der Grundlage der Teilprozesse der visuellen Objekterkennung und der Belohnungsbewertung. Diese identifizierten Muster könnten als Biomarker und potenzielle therapeutische Ziele bei Alkoholkonsumstörung vorgeschlagen werden.

5 REFERENCE

Acosta-Cabronero, J., Williams, G. B., Cardenas-Blanco, A., Arnold, R. J., Lupson, V., & Nestor, P. J. (2013). In Vivo Quantitative Susceptibility Mapping (QSM) in Alzheimer's Disease. *PLOS ONE, 8*(11), e81093. https://doi.org/10.1371/journal.pone.0081093

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders (DSM-5®)*. American Psychiatric Pub.

Anton, R. F., Moak, D. H., & Latham, P. (1995). The Obsessive Compulsive Drinking Scale: a self‐rated instrument for the quantification of thoughts about alcohol and drinking behavior. *Alcoholism: Clinical and Experimental Research, 19*(1), 92-99.

Arcurio, L. R., Finn, P. R., & James, T. W. (2015, 2015/03/01). Neural mechanisms of high-risk decisions-to-drink in alcohol-dependent women [https://doi.org/10.1111/adb.12121]. *Addiction Biology, 20*(2), 390-406. https://doi.org/https://doi.org/10.1111/adb.12121

Bach, P., Hill, H., Reinhard, I., Gädeke, T., Kiefer, F., & Leménager, T. (2021, 2021/07/17). Reliability of the fMRI-based assessment of self-evaluation in individuals with internet gaming disorder. *European Archives of Psychiatry and Clinical Neuroscience*. https://doi.org/10.1007/s00406-021-01307-2

Bach, P., Zois, E., Vollstädt-Klein, S., Kirsch, M., Hoffmann, S., Jorde, A., Frank, J., Charlet, K., Treutlein, J., Beck, A., Heinz, A., Walter, H., Rietschel, M., & Kiefer, F. (2019, 2019/01/01). Association of the alcohol dehydrogenase gene polymorphism rs1789891 with gray matter brain volume, alcohol consumption, alcohol craving and relapse risk [https://doi.org/10.1111/adb.12571]. *Addiction Biology, 24*(1), 110-120. https://doi.org/https://doi.org/10.1111/adb.12571

Baxter, B. W., & Hinson, R. E. (2001). Is smoking automatic? Demands of smoking behavior on attentional resources. *Journal of Abnormal Psychology, 110*(1), 59.

Beck, A., Wüstenberg, T., Genauck, A., Wrase, J., Schlagenhauf, F., Smolka, M. N., Mann, K., & Heinz, A. (2012). Effect of Brain Structure, Brain Function, and Brain Connectivity on Relapse in Alcohol-Dependent Patients. *Archives of General Psychiatry, 69*(8), 842-852. https://doi.org/10.1001/archgenpsychiatry.2011.2026

Belin, D., Belin-Rauscent, A., Murray, J. E., & Everitt, B. J. (2013, 2013/08/01/). Addiction: failure of control over maladaptive incentive habits. *Current Opinion*

in Neurobiology, 23(4), 564-572. https://doi.org/https://doi.org/10.1016/j.conb.2013.01.025

Ben-Shachar, D., Livne, E., Spanier, I., Zuk, R., & Youdim, M. B. (1993, Sep). Iron modulates neuroleptic-induced effects related to the dopaminergic system. *Isr J Med Sci, 29*(9), 587-592.

Ben-Shachar, D., & Youdim, M. B. (1990, Apr). Neuroleptic-induced supersensitivity and brain iron: I. Iron deficiency and neuroleptic-induced dopamine D2 receptor supersensitivity. *J Neurochem, 54*(4), 1136-1141. https://doi.org/10.1111/j.1471-4159.1990.tb01940.x

Bohn, M. J., Krahn, D. D., & Staehler, B. A. (1995, 1995/06/01). Development and Initial Validation of a Measure of Drinking Urges in Abstinent Alcoholics [https://doi.org/10.1111/j.1530-0277.1995.tb01554.x]. *Alcoholism: Clinical and Experimental Research, 19*(3), 600-606. https://doi.org/https://doi.org/10.1111/j.1530-0277.1995.tb01554.x

Boothby, L. A., & Doering, P. L. (2005, 2005/06/01/). Acamprosate for the treatment of alcohol dependence. *Clinical Therapeutics, 27*(6), 695-714. https://doi.org/https://doi.org/10.1016/j.clinthera.2005.06.015

Bühler, M., & Mann, K. (2011, 2011/10/01). Alcohol and the Human Brain: A Systematic Review of Different Neuroimaging Methods [https://doi.org/10.1111/j.1530-0277.2011.01540.x]. *Alcohol: Clinical and Experimental Research, 35*(10), 1771-1793. https://doi.org/https://doi.org/10.1111/j.1530-0277.2011.01540.x

Carter, B. L., & Tiffany, S. T. (1999, 1999/03/01). Meta-analysis of cue-reactivity in addiction research [https://doi.org/10.1046/j.1360-0443.1999.9433273.x]. *Addiction, 94*(3), 327-340. https://doi.org/https://doi.org/10.1046/j.1360-0443.1999.9433273.x

Carvalho, A. F., Heilig, M., Perez, A., Probst, C., & Rehm, J. (2019, 2019/08/31/). Alcohol use disorders. *The Lancet, 394*(10200), 781-792. https://doi.org/https://doi.org/10.1016/S0140-6736(19)31775-1

Chang, C.-C., & Lin, C.-J. (2011). LIBSVM: a library for support vector machines. *ACM transactions on intelligent systems and technology (TIST), 2*(3), 1-27.

Charlet, K., Beck, A., Jorde, A., Wimmer, L., Vollstädt-Klein, S., Gallinat, J., Walter, H., Kiefer, F., & Heinz, A. (2014, 2014/05/01). Increased neural activity during high working memory load predicts low relapse risk in alcohol dependence [https://doi.org/10.1111/adb.12103]. *Addiction Biology, 19*(3), 402-414. https://doi.org/https://doi.org/10.1111/adb.12103

Chase, H. W., Kumar, P., Eickhoff, S. B., & Dombrovski, A. Y. (2015, 2015/06/01). Reinforcement learning models and their neural correlates: An activation likelihood estimation meta-analysis. *Cognitive, Affective, & Behavioral Neuroscience, 15*(2), 435-459. https://doi.org/10.3758/s13415-015-0338-7

Chen, T., Tan, H., Lei, H., Li, X., Wu, Q., Xu, X., Ye, Y., Zhong, N., Du, J., Jiang, H., Su, H., & Zhao, M. (2021, 2021/09/30/). Nature of glutamate alterations in substance dependence: A systematic review and meta-analysis of proton magnetic resonance spectroscopy studies. *Psychiatry Research: Neuroimaging, 315*, 111329. https://doi.org/https://doi.org/10.1016/j.pscychresns.2021.111329

Cohen, J. D., Perlstein, W. M., Braver, T. S., Nystrom, L. E., Noll, D. C., Jonides, J., & Smith, E. E. (1997, 1997/04/01). Temporal dynamics of brain activation during a working memory task. *Nature, 386*(6625), 604-608. https://doi.org/10.1038/386604a0

Coller, J. K., & Hutchinson, M. R. (2012, 2012/05/01/). Implications of central immune signaling caused by drugs of abuse: Mechanisms, mediators and new therapeutic approaches for prediction and treatment of drug dependence. *Pharmacology & Therapeutics, 134*(2), 219-245. https://doi.org/https://doi.org/10.1016/j.pharmthera.2012.01.008

Corbetta, M., & Shulman, G. L. (2002, 2002/03/01). Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews Neuroscience, 3*(3), 201-215. https://doi.org/10.1038/nrn755

Cox, W. M., Fadardi, J. S., Intriligator, J. M., & Klinger, E. (2014). Attentional bias modification for addictive behaviors: clinical implications. *CNS spectrums, 19*(3), 215-224.

Crews, F. T., & Nixon, K. (2009). Mechanisms of neurodegeneration and regeneration in alcoholism. *Alcohol & Alcoholism, 44*(2), 115-127.

Cuthbert, B. N. (2014, 2014/02/01). The RDoC framework: facilitating transition from ICD/DSM to dimensional approaches that integrate neuroscience and psychopathology [https://doi.org/10.1002/wps.20087]. *World Psychiatry, 13*(1), 28-35. https://doi.org/https://doi.org/10.1002/wps.20087

de Rochefort, L., Liu, T., Kressler, B., Liu, J., Spincemaille, P., Lebon, V., Wu, J., & Wang, Y. (2010). Quantitative susceptibility map reconstruction from MR phase data using bayesian regularization: validation and application to brain imaging. *Magnetic Resonance in Medicine: An Official Journal of the International Society for Magnetic Resonance in Medicine, 63*(1), 194-206.

De Santis, S., Bach, P., Pérez-Cervera, L., Cosa-Linan, A., Weil, G., Vollstädt-Klein, S., Hermann, D., Kiefer, F., Kirsch, P., & Ciccocioppo, R. (2019). Microstructural white matter alterations in men with alcohol use disorder and rats with excessive alcohol consumption during early abstinence. *JAMA Psychiatry, 76*(7), 749-758.

Deistung, A., Schweser, F., & Reichenbach, J. R. (2017). Overview of quantitative susceptibility mapping. *NMR in Biomedicine, 30*(4), e3569.

Deng, J., Dong, W., Socher, R., Li, L.-J., Li, K., & Fei-Fei, L. (2009). Imagenet: A large-scale hierarchical image database.

Detre, J. A., & Wang, J. (2002, 2002/05/01/). Technical aspects and utility of fMRI using BOLD and ASL. *Clinical Neurophysiology, 113*(5), 621-634. https://doi.org/https://doi.org/10.1016/S1388-2457(02)00038-X

Dichtl, S., Haschka, D., Nairz, M., Seifert, M., Volani, C., Lutz, O., & Weiss, G. (2018, Feb). Dopamine promotes cellular iron accumulation and oxidative stress responses in macrophages. *Biochem Pharmacol, 148*, 193-201. https://doi.org/10.1016/j.bcp.2017.12.001

Drummond, D. C. (2000, 2000/08/01). What does cue-reactivity have to offer clinical research? [https://doi.org/10.1046/j.1360-0443.95.8s2.2.x]. *Addiction, 95*(8s2), 129-144. https://doi.org/https://doi.org/10.1046/j.1360-0443.95.8s2.2.x

Du, Y., Krakauer, J. W., & Haith, A. M. (2022). The relationship between habits and motor skills in humans. *Trends in Cognitive Sciences*.

Duane, P., Raja, K. B., Simpson, R. J., & Peters, T. J. (1992). Intestinal iron absorption in chronic alcoholics. *Alcohol and Alcoholism, 27*(5), 539-544.

Eberl, C., Wiers, R. W., Pawelczack, S., Rinck, M., Becker, E. S., & Lindenmeyer, J. (2013, 2013/04/01/). Approach bias modification in alcohol dependence: Do clinical effects replicate and for whom does it work best? *Developmental Cognitive Neuroscience, 4*, 38-51. https://doi.org/https://doi.org/10.1016/j.dcn.2012.11.002

Eickenberg, M., Gramfort, A., Varoquaux, G., & Thirion, B. (2017). Seeing it all: Convolutional network layers map the function of the human visual system. *NeuroImage, 152*, 184-194.

Ersche, K. D., Acosta-Cabronero, J., Jones, P. S., Ziauddeen, H., van Swelm, R. P., Laarakkers, C. M., Raha-Chowdhury, R., & Williams, G. B. (2017, Feb 21). Disrupted iron regulation in the brain and periphery in cocaine addiction. *Transl Psychiatry, 7*(2), e1040. https://doi.org/10.1038/tp.2016.271

Everitt, B. J., & Robbins, T. W. (2016). Drug addiction: updating actions to habits to compulsions ten years on. *Annu Rev Psychol, 67*(1), 23-50.

Feil, J., Sheppard, D., Fitzgerald, P. B., Yücel, M., Lubman, D. I., & Bradshaw, J. L. (2010, 2010/11/01/). Addiction, compulsive drug seeking, and the role of frontostriatal mechanisms in regulating inhibitory control. *Neuroscience & Biobehavioral Reviews, 35*(2), 248-275. https://doi.org/https://doi.org/10.1016/j.neubiorev.2010.03.001

Friston, K. J., Buechel, C., Fink, G. R., Morris, J., Rolls, E., & Dolan, R. J. (1997). Psychophysiological and modulatory interactions in neuroimaging. *NeuroImage, 6*(3), 218-229.

Fritz, M., Klawonn, A. M., & Zahr, N. M. (2022, 2022/05/01). Neuroimaging in alcohol use disorder: From mouse to man [https://doi.org/10.1002/jnr.24423]. *Journal of Neuroscience Research, 100*(5), 1140-1158. https://doi.org/https://doi.org/10.1002/jnr.24423

Garrison, K. A., & Potenza, M. N. (2014, 2014/10/12). Neuroimaging and Biomarkers in Addiction Treatment. *Current psychiatry reports, 16*(12), 513. https://doi.org/10.1007/s11920-014-0513-5

Gerchen, M. F., Weiss, F., Kirsch, M., Rentsch, A., Halli, P., Kiefer, F., & Kirsch, P. (2021, 2021/01/01). Dynamic frontostriatal functional peak connectivity (in alcohol use disorder) [https://doi.org/10.1002/hbm.25201]. *Human Brain Mapping, 42*(1), 36-46. https://doi.org/https://doi.org/10.1002/hbm.25201

Gilbert, C. D., & Li, W. (2013, 2013/05/01). Top-down influences on visual processing. *Nature Reviews Neuroscience, 14*(5), 350-363. https://doi.org/10.1038/nrn3476

Goldstein, R. Z., & Volkow, N. D. (2011). Dysfunction of the prefrontal cortex in addiction: neuroimaging findings and clinical implications. *Nature Reviews Neuroscience, 12*(11), 652-669.

Gorelick, D. A., Zangen, A., & George, M. S. (2014). Transcranial magnetic stimulation in the treatment of substance addiction. *Annals of the New York Academy of Sciences, 1327*(1), 79-93.

Grundinger, N., Gerhardt, S., Karl, D., Mann, K., Kiefer, F., & Vollstädt-Klein, S. (2022, 2022/08/01). The effects of nalmefene on the impulsive and reflective system in alcohol use disorder: A resting-state fMRI study. *Psychopharmacology, 239*(8), 2471-2489. https://doi.org/10.1007/s00213-022-06137-1

Haacke, E. M., Liu, S., Buch, S., Zheng, W., Wu, D., & Ye, Y. (2015, 2015/01/01/). Quantitative susceptibility mapping: current status and future directions. *Magnetic Resonance Imaging, 33*(1), 1-25. https://doi.org/https://doi.org/10.1016/j.mri.2014.09.004

Hansson, A. C., Gründer, G., Hirth, N., Noori, H. R., Spanagel, R., & Sommer, W. H. (2019, 2019/11/01/). Dopamine and opioid systems adaptation in alcoholism revisited: Convergent evidence from positron emission tomography and postmortem studies. *Neuroscience & Biobehavioral Reviews, 106*, 141-164. https://doi.org/https://doi.org/10.1016/j.neubiorev.2018.09.010

Hansson, A. C., Koopmann, A., Uhrig, S., Bühler, S., Domi, E., Kiessling, E., Ciccocioppo, R., Froemke, R. C., Grinevich, V., Kiefer, F., Sommer, W. H., Vollstädt-Klein, S., & Spanagel, R. (2018, 2018/05/01). Oxytocin Reduces Alcohol Cue-Reactivity in Alcohol-Dependent Rats and Humans. *Neuropsychopharmacology, 43*(6), 1235-1246. https://doi.org/10.1038/npp.2017.257

Haorah, J., Heilman, D., Knipe, B., Chrastil, J., Leibhart, J., Ghorpade, A., Miller, D. W., & Persidsky, Y. (2005). Ethanol‐induced activation of myosin light chain kinase leads to dysfunction of tight junctions and blood‐brain barrier compromise. *Alcoholism: Clinical and Experimental Research, 29*(6), 999-1009.

Hare, D. J., & Double, K. L. (2016). Iron and dopamine: a toxic couple. *Brain, 139*(4), 1026-1035. https://doi.org/10.1093/brain/aww022

Heinz, A., Reimold, M., Wrase, J., Hermann, D., Croissant, B., Mundle, G., Dohmen, B. M., Braus, D. H., Schumann, G., Machulla, H.-J., Bares, R., & Mann, K. (2005). Correlation of Stable Elevations in Striatal μ-Opioid Receptor Availability in Detoxified Alcoholic Patients With Alcohol Craving: A Positron Emission Tomography Study Using Carbon 11–Labeled Carfentanil. *Archives of General Psychiatry, 62*(1), 57-64. https://doi.org/10.1001/archpsyc.62.1.57

Hirth, N., Meinhardt, M. W., Noori, H. R., Salgado, H., Torres-Ramirez, O., Uhrig, S., Broccoli, L., Vengeliene, V., Roßmanith, M., Perreau-Lenz, S., Köhr, G., Sommer, W. H., Spanagel, R., & Hansson, A. C. (2016). Convergent evidence from alcohol-dependent humans and rats for a hyperdopaminergic state in protracted abstinence. *Proceedings of the National Academy of Sciences, 113*(11), 3024. https://doi.org/10.1073/pnas.1506012113

Hubertus, S., Thomas, S., Cho, J., Zhang, S., Wang, Y., & Schad, L. R. (2019a, Oct). Comparison of gradient echo and gradient echo sampling of spin echo sequence for the quantification of the oxygen extraction fraction from a combined quantitative susceptibility mapping and quantitative BOLD

(QSM+qBOLD) approach. *Magn Reson Med, 82*(4), 1491-1503. https://doi.org/10.1002/mrm.27804

Hubertus, S., Thomas, S., Cho, J., Zhang, S., Wang, Y., & Schad, L. R. (2019b, Dec). Using an artificial neural network for fast mapping of the oxygen extraction fraction with combined QSM and quantitative BOLD. *Magn Reson Med, 82*(6), 2199-2211. https://doi.org/10.1002/mrm.27882

Ioannou, G. N., Dominitz, J. A., Weiss, N. S., Heagerty, P. J., & Kowdley, K. V. (2004, 2004/05/01/). The effect of alcohol consumption on the prevalence of iron overload, iron deficiency, and iron deficiency anemia. *Gastroenterology, 126*(5), 1293-1301. https://doi.org/https://doi.org/10.1053/j.gastro.2004.01.020

Ito, R., Dalley, J. W., Howes, S. R., Robbins, T. W., & Everitt, B. J. (2000). Dissociation in Conditioned Dopamine Release in the Nucleus Accumbens Core and Shell in Response to Cocaine Cues and during Cocaine-Seeking Behavior in Rats. *The Journal of Neuroscience, 20*(19), 7489. https://doi.org/10.1523/JNEUROSCI.20-19-07489.2000

Ito, R., Dalley, J. W., Robbins, T. W., & Everitt, B. J. (2002). Dopamine release in the dorsal striatum during cocaine-seeking behavior under the control of a drug-associated cue. *Journal of Neuroscience, 22*(14), 6247-6253.

Jasinska, A. J., Stein, E. A., Kaiser, J., Naumer, M. J., & Yalachkov, Y. (2014). Factors modulating neural reactivity to drug cues in addiction: a survey of human neuroimaging studies. *Neuroscience and biobehavioral reviews, 38*, 1-16. https://doi.org/10.1016/j.neubiorev.2013.10.013

Juhás, M., Sun, H., Brown, M. R. G., MacKay, M. B., Mann, K. F., Sommer, W. H., Wilman, A. H., Dursun, S. M., & Greenshaw, A. J. (2017). Deep grey matter iron accumulation in alcohol use disorder. *NeuroImage, 148*, 115-122.

Karl, D., Bumb, J. M., Bach, P., Dinter, C., Koopmann, A., Hermann, D., Mann, K., Kiefer, F., & Vollstädt-Klein, S. (2021, 2021/08/01). Nalmefene attenuates neural alcohol cue-reactivity in the ventral striatum and subjective alcohol craving in patients with alcohol use disorder. *Psychopharmacology, 238*(8), 2179-2189. https://doi.org/10.1007/s00213-021-05842-7

Kempuraj, D., Thangavel, R., Natteru, P. A., Selvakumar, G. P., Saeed, D., Zahoor, H., Zaheer, S., Iyer, S. S., & Zaheer, A. (2016). Neuroinflammation Induces Neurodegeneration. *Journal of neurology, neurosurgery and spine, 1*(1), 1003.

Kivlahan, D. R., Sher, K. J., & Donovan, D. M. (1989). The Alcohol Dependence Scale: a validation study among inpatient alcoholics. *Journal of studies on alcohol, 50*(2), 170-175.

Koechlin, E., & Hyafil, A. (2007, 2007/10/26). Anterior Prefrontal Function and the Limits of Human Decision-Making. *Science, 318*(5850), 594-598. https://doi.org/10.1126/science.1142995

Kohgo, Y., Ohtake, T., Ikuta, K., Suzuki, Y., Torimoto, Y., & Kato, J. (2008). Dysregulation of systemic iron metabolism in alcoholic liver diseases. *Journal of gastroenterology and hepatology, 23*, S78-S81.

Koob, G. F., & Volkow, N. D. (2010, 2010/01/01). Neurocircuitry of Addiction. *Neuropsychopharmacology, 35*(1), 217-238. https://doi.org/10.1038/npp.2009.110

Koob, G. F., & Volkow, N. D. (2016, 2016/08/01/). Neurobiology of addiction: a neurocircuitry analysis. *The Lancet Psychiatry, 3*(8), 760-773. https://doi.org/https://doi.org/10.1016/S2215-0366(16)00104-8

Kriegeskorte, N., Goebel, R., & Bandettini, P. (2006, 2006/03/07). Information-based functional brain mapping. *Proceedings of the National Academy of Sciences, 103*(10), 3863-3868. https://doi.org/10.1073/pnas.0600244103

Kriegeskorte, N., Mur, M., & Bandettini, P. (2008). Representational similarity analysis - connecting the branches of systems neuroscience [Original Research]. *Frontiers in Systems Neuroscience, 2*.

Kühn, S., & Gallinat, J. (2011, 2011/04/01). Common biology of craving across legal and illegal drugs – a quantitative meta-analysis of cue-reactivity brain response [https://doi.org/10.1111/j.1460-9568.2010.07590.x]. *European Journal of Neuroscience, 33*(7), 1318-1326. https://doi.org/https://doi.org/10.1111/j.1460-9568.2010.07590.x

Kurz, F. T., Buschle, L. R., Rotkopf, L. T., Herzog, F. S., Sterzik, A., Schlemmer, H. P., Kampf, T., Bendszus, M., Heiland, S., & Ziener, C. H. (2021, 2021/03/06/). Dependence of the frequency distribution around a sphere on the voxel orientation. *Zeitschrift für Medizinische Physik*. https://doi.org/https://doi.org/10.1016/j.zemedi.2021.01.005

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1997). International affective picture system (IAPS): Technical manual and affective ratings. *NIMH Center for the Study of Emotion and Attention, 1*(39-58), 3.

Langkammer, C., Schweser, F., Krebs, N., Deistung, A., Goessler, W., Scheurer, E., Sommer, K., Reishofer, G., Yen, K., & Fazekas, F. (2012). Quantitative susceptibility mapping (QSM) as a means to measure brain iron? A post mortem validation study. *NeuroImage, 62*(3), 1593-1599.

Larsen, B., Bourque, J., Moore, T. M., Adebimpe, A., Calkins, M. E., Elliott, M. A., Gur, R. C., Gur, R. E., Moberg, P. J., Roalf, D. R., Ruparel, K., Turetsky, B. I., Vandekar, S. N., Wolf, D. H., Shinohara, R. T., & Satterthwaite, T. D. (2020, Feb 26). Longitudinal Development of Brain Iron Is Linked to Cognition in Youth. *J Neurosci, 40*(9), 1810-1818. https://doi.org/10.1523/jneurosci.2434-19.2020

Liu, T., Liu, J., De Rochefort, L., Spincemaille, P., Khalidov, I., Ledoux, J. R., & Wang, Y. (2011). Morphology enabled dipole inversion (MEDI) from a single‐angle acquisition: comparison with COSMOS in human brain imaging. *Magnetic Resonance in Medicine, 66*(3), 777-783.

Logan, G. D. (1988). Toward an instance theory of automatization. *Psychological review, 95*(4), 492.

Lucantonio, F., Caprioli, D., & Schoenbaum, G. (2014, 2014/01/01/). Transition from 'model-based' to 'model-free' behavioral control in addiction: Involvement of the orbitofrontal cortex and dorsolateral striatum. *Neuropharmacology, 76*, 407-415. https://doi.org/https://doi.org/10.1016/j.neuropharm.2013.05.033

Luijten, M., Machielsen, M. W. J., Veltman, D. J., Hester, R., de Haan, L., & Franken, I. H. A. (2014). Systematic review of ERP and fMRI studies investigating inhibitory control and error processing in people with substance dependence and behavioural addictions. *Journal of Psychiatry and Neuroscience, 39*(3), 149. https://doi.org/10.1503/jpn.130052

Lüscher, C., Robbins, T. W., & Everitt, B. J. (2020, 2020/05/01). The transition to compulsion in addiction. *Nature Reviews Neuroscience, 21*(5), 247-263. https://doi.org/10.1038/s41583-020-0289-z

Makris, N., Oscar-Berman, M., Jaffin, S. K., Hodge, S. M., Kennedy, D. N., Caviness, V. S., Marinkovic, K., Breiter, H. C., Gasic, G. P., & Harris, G. J. (2008, 2008/08/01/). Decreased Volume of the Brain Reward System in Alcoholism. *Biological Psychiatry, 64*(3), 192-202. https://doi.org/https://doi.org/10.1016/j.biopsych.2008.01.018

Mann, K., & Ackermann, K. (2000). Die OCDS-G: Psychometrische Kennwerte der deutschen Version der obsessive compulsive drinking scale. *Sucht, 46*(2), 90-100.

Matthews, P. M., & Jezzard, P. (2004). Functional magnetic resonance imaging. *Journal of Neurology, Neurosurgery & Psychiatry, 75*(1), 6.

Mechelli, A., Henson, R. N., Price, C. J., & Friston, K. J. (2003, Mar). Comparing event-related and epoch analysis in blocked design fMRI. *NeuroImage, 18*(3), 806-810. https://doi.org/10.1016/s1053-8119(02)00027-7

Melega, W. P., Laćan, G., Harvey, D. C., & Way, B. M. (2007, Oct 29). Methamphetamine increases basal ganglia iron to levels observed in aging. *Neuroreport, 18*(16), 1741-1745. https://doi.org/10.1097/WNR.0b013e3282f0d4f4

Milton, A. L., & Everitt, B. J. (2012, 2012/04/01/). The persistence of maladaptive memory: Addiction, drug memories and anti-relapse treatments. *Neuroscience & Biobehavioral Reviews, 36*(4), 1119-1139. https://doi.org/https://doi.org/10.1016/j.neubiorev.2012.01.002

Moeller, S. J., & Goldstein, R. Z. (2014). Impaired self-awareness in human addiction: deficient attribution of personal relevance. *Trends in Cognitive Sciences, 18*(12), 635-641.

Möller, H. E., Bossoni, L., Connor, J. R., Crichton, R. R., Does, M. D., Ward, R. J., Zecca, L., Zucca, F. A., & Ronen, I. (2019). Iron, myelin, and the brain: Neuroimaging meets neurobiology. *Trends in neurosciences, 42*(6), 384-401.

Morein-Zamir, S., & Robbins, T. W. (2015, 2015/12/02/). Fronto-striatal circuits in response-inhibition: Relevance to addiction. *Brain Research, 1628*, 117-129. https://doi.org/https://doi.org/10.1016/j.brainres.2014.09.012

Muñoz, P., Huenchuguala, S., Paris, I., & Segura-Aguilar, J. (2012, 2012/08/26). Dopamine Oxidation and Autophagy. *Parkinson’s Disease, 2012*, 920953. https://doi.org/10.1155/2012/920953

Necus, J., Smith, F. E., Thelwall, P. E., Flowers, C. J., Sinha, N., Taylor, P. N., Blamire, A. M., Wang, Y., & Cousins, D. A. (2019, Nov 21). Quantification of brain proton longitudinal relaxation (T(1)) in lithium-treated and lithium-naïve patients with bipolar disorder in comparison to healthy controls. *Bipolar Disord*. https://doi.org/10.1111/bdi.12878

Nili, H., Wingfield, C., Walther, A., Su, L., Marslen-Wilson, W., & Kriegeskorte, N. (2014). A Toolbox for Representational Similarity Analysis. *PLOS Computational Biology, 10*(4), e1003553. https://doi.org/10.1371/journal.pcbi.1003553

O'Reilly, J. X., Woolrich, M. W., Behrens, T. E. J., Smith, S. M., & Johansen-Berg, H. (2012). Tools of the trade: psychophysiological interactions and functional connectivity. *Social Cognitive and Affective Neuroscience, 7*(5), 604-609. https://doi.org/10.1093/scan/nss055

Olmedo-Díaz, S., Estévez-Silva, H., Orädd, G., af Bjerkén, S., Marcellino, D., & Virel, A. (2017, 2017/10/24/). An altered blood–brain barrier contributes to brain iron accumulation and neuroinflammation in the 6-OHDA rat model of Parkinson's disease. *Neuroscience, 362*, 141-151. https://doi.org/https://doi.org/10.1016/j.neuroscience.2017.08.023

Pando-Naude, V., Toxto, S., Fernandez-Lozano, S., Parsons, C. E., Alcauter, S., & Garza-Villarreal, E. A. (2021, 2021/01/11). Gray and white matter morphology in substance use disorders: a neuroimaging systematic review and meta-analysis. *Translational Psychiatry, 11*(1), 29. https://doi.org/10.1038/s41398-020-01128-2

Paris, I., Martinez-Alvarado, P., Cárdenas, S., Perez-Pastene, C., Graumann, R., Fuentes, P., Olea-Azar, C., Caviedes, P., & Segura-Aguilar, J. (2005, 2005/03/01). Dopamine-Dependent Iron Toxicity in Cells Derived from Rat Hypothalamus. *Chemical Research in Toxicology, 18*(3), 415-419. https://doi.org/10.1021/tx0497144

Pérez-Ramírez, Ú., López-Madrona, V. J., Pérez-Segura, A., Pallarés, V., Moreno, A., Ciccocioppo, R., Hyytiä, P., Sommer, W. H., Moratal, D., & Canals, S. (2022). Brain Network Allostasis after Chronic Alcohol Drinking Is Characterized by Functional Dedifferentiation and Narrowing. *The Journal of Neuroscience, 42*(21), 4401. https://doi.org/10.1523/JNEUROSCI.0389-21.2022

Petrakis, I. L., Ralevski, E., Gueorguieva, R., Sloan, M. E., Devine, L., Yoon, G., Arias, A. J., & Sofuoglu, M. (2019, 2019/10/01). Targeting neuroinflammation with minocycline in heavy drinkers. *Psychopharmacology, 236*(10), 3013-3021. https://doi.org/10.1007/s00213-019-05205-3

Pimentel, E., Sivalingam, K., Doke, M., & Samikkannu, T. (2020). Effects of Drugs of Abuse on the Blood-Brain Barrier: A Brief Overview [Mini Review]. *Frontiers in Neuroscience, 14*.

Puckett, A. M., Bollmann, S., Poser, B. A., Palmer, J., Barth, M., & Cunnington, R. (2018, 2018/05/15/). Using multi-echo simultaneous multi-slice (SMS) EPI to improve functional MRI of the subcortical nuclei of the basal ganglia at ultra-high field (7T). *NeuroImage, 172*, 886-895. https://doi.org/https://doi.org/10.1016/j.neuroimage.2017.12.005

Ravanfar, P., Loi, S. M., Syeda, W. T., Van Rheenen, T. E., Bush, A. I., Desmond, P., Cropley, V. L., Lane, D. J. R., Opazo, C. M., Moffat, B. A., Velakoulis, D., & Pantelis, C. (2021). Systematic Review: Quantitative Susceptibility Mapping (QSM) of Brain Iron Profile in Neurodegenerative Diseases [Systematic Review]. *Frontiers in Neuroscience, 15*.

Rehm, J., Mathers, C., Popova, S., Thavorncharoensap, M., Teerawattananon, Y., & Patra, J. (2009). Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use disorders. *The Lancet, 373*(9682), 2223-2233. https://doi.org/10.1016/S0140-6736(09)60746-7

Rehm, J., & Shield, K. D. (2019). Global burden of disease and the impact of mental and addictive disorders. *Current psychiatry reports, 21*, 1-7.

Rinck, M., Wiers, R. W., Becker, E. S., & Lindenmeyer, J. (2018). Relapse prevention in abstinent alcoholics by cognitive bias modification: Clinical effects of combining approach bias modification and attention bias modification. *Journal of Consulting and Clinical Psychology, 86*(12), 1005.

Rodrigue, K. M., Daugherty, A. M., Foster, C. M., & Kennedy, K. M. (2020, 2020/04/15/). Striatal iron content is linked to reduced fronto-striatal brain function under working memory load. *NeuroImage, 210*, 116544. https://doi.org/https://doi.org/10.1016/j.neuroimage.2020.116544

Rouach, H., Houzé, P., Gentil, M., Orfanelli, M.-T., & Nordmann, R. (1997, 1997/02/21/). Changes in some pro-and antioxidants in rat cerebellum after chronic alcohol intake. *Biochemical Pharmacology, 53*(4), 539-545. https://doi.org/https://doi.org/10.1016/S0006-2952(96)00770-8

Rouach, H., Houze, P., Orfanelli, M.-T., Gentil, M., Bourdon, R., & Nordmann, R. (1990, 1990/03/15/). Effect of acute ethanol administration on the subcellular distribution of iron in rat liver and cerebellum. *Biochemical Pharmacology, 39*(6), 1095-1100. https://doi.org/https://doi.org/10.1016/0006-2952(90)90289-W

Rubio‐Araiz, A., Porcu, F., Pérez‐Hernández, M., García‐Gutiérrez, M. S., Aracil‐Fernández, M. A., Gutierrez‐López, M. D., Guerri, C., Manzanares, J., O'Shea, E., & Colado, M. I. (2017). Disruption of blood‐brain barrier integrity in postmortem alcoholic brain: preclinical evidence of TLR4 involvement from a binge‐like drinking model. *Addiction Biology, 22*(4), 1103-1116.

Samokhvalov, A. V., Popova, S., Room, R., Ramonas, M., & Rehm, J. (2010, 2010/11/01). Disability Associated With Alcohol Abuse and Dependence [https://doi.org/10.1111/j.1530-0277.2010.01275.x]. *Alcohol: Clinical and Experimental Research, 34*(11), 1871-1878. https://doi.org/https://doi.org/10.1111/j.1530-0277.2010.01275.x

Scheurich, A., Müller, M. J., Anghelescu, I., Lörch, B., Dreher, M., Hautzinger, M., & Szegedi, A. (2005). Reliability and validity of the form 90 interview. *Eur Addict Res, 11*(1), 50-56. https://doi.org/10.1159/000081417

Schröder, N., Figueiredo, L. S., & de Lima, M. N. (2013). Role of brain iron accumulation in cognitive dysfunction: evidence from animal models and human studies. *J Alzheimers Dis, 34*(4), 797-812. https://doi.org/10.3233/jad-121996

Seeliger, K., Fritsche, M., Güçlü, U., Schoenmakers, S., Schoffelen, J. M., Bosch, S. E., & van Gerven, M. A. J. (2018, 2018/10/15/). Convolutional neural network-based encoding and decoding of visual object recognition in space and time. *NeuroImage, 180*, 253-266. https://doi.org/https://doi.org/10.1016/j.neuroimage.2017.07.018

Seo, S., Mohr, J., Beck, A., Wüstenberg, T., Heinz, A., & Obermayer, K. (2015, 2015/11/01). Predicting the future relapse of alcohol-dependent patients from structural and functional brain images [https://doi.org/10.1111/adb.12302]. *Addiction Biology, 20*(6), 1042-1055. https://doi.org/https://doi.org/10.1111/adb.12302

Shiffrin, R. M., & Schneider, W. (1977). Controlled and automatic human information processing: II. Perceptual learning, automatic attending and a general theory. *Psychological review, 84*(2), 127.

Shiffrin, R. M., & Schneider, W. (1984). Automatic and controlled processing revisited.

Simonyan, K., & Zisserman, A. (2014). Very deep convolutional networks for large-scale image recognition. *arXiv preprint arXiv:1409.1556*.

Sjoerds, Z., de Wit, S., van den Brink, W., Robbins, T. W., Beekman, A. T. F., Penninx, B. W. J. H., & Veltman, D. J. (2013, 2013/12/01). Behavioral and neuroimaging evidence for overreliance on habit learning in alcohol-dependent patients. *Translational Psychiatry, 3*(12), e337-e337. https://doi.org/10.1038/tp.2013.107

Smith, S. M. (2004, 2004/12/01). Overview of fMRI analysis. *The British Journal of Radiology, 77*(suppl_2), S167-S175. https://doi.org/10.1259/bjr/33553595

Smolka, M. N., Bühler, M., Klein, S., Zimmermann, U., Mann, K., Heinz, A., & Braus, D. F. (2006). Severity of nicotine dependence modulates cue-induced brain activity in regions involved in motor preparation and imagery. *Psychopharmacology, 184*(3), 577-588.

Song, A. W. (2001, 2001/08/01). Single-shot EPI with signal recovery from the susceptibility-induced losses [https://doi.org/10.1002/mrm.1205]. *Magnetic Resonance in Medicine, 46*(2), 407-411. https://doi.org/https://doi.org/10.1002/mrm.1205

Spence, H., McNeil, C. J., & Waiter, G. D. (2020). The impact of brain iron accumulation on cognition: A systematic review. *PLOS ONE, 15*(10), e0240697. https://doi.org/10.1371/journal.pone.0240697

Sun, H., & Wilman, A. H. (2014). Background field removal using spherical mean value filtering and Tikhonov regularization. *Magnetic Resonance in Medicine, 71*(3), 1151-1157.

Tan, H., Gerchen, M. F., Bach, P., Lee, A. M., Hummel, O., Sommer, W., Kirsch, P., Kiefer, F., & Vollstädt-Klein, S. (2023). Decoding fMRI alcohol cue reactivity and its association with drinking behaviour. *BMJ Mental Health, 26*(1), e300639. https://doi.org/10.1136/bmjment-2022-300639

Tan, H., Hubertus, S., Thomas, S., Lee, A. M., Gerhardt, S., Gerchen, M. F., Sommer, W. H., Kiefer, F., Schad, L., & Vollstädt-Klein, S. (2023, 2023/02/01). Association between iron accumulation in the dorsal striatum and compulsive drinking in alcohol use disorder. *Psychopharmacology, 240*(2), 249-257. https://doi.org/10.1007/s00213-022-06301-7

Tavill, A. S., & Qadri, A. M. (2004, Aug). Alcohol and iron. *Semin Liver Dis, 24*(3), 317-325. https://doi.org/10.1055/s-2004-832943

Tiffany, S. T. (1990). A cognitive model of drug urges and drug-use behavior: role of automatic and nonautomatic processes. *Psychological review, 97*(2), 147.

Tonekaboni, S. H., & Mollamohammadi, M. (2014, Fall). Neurodegeneration with brain iron accumulation: an overview. *Iranian journal of child neurology, 8*(4), 1-8.

Topiwala, A., Wang, C., Ebmeier, K. P., Burgess, S., Bell, S., Levey, D. F., Zhou, H., McCracken, C., Roca-Fernandez, A., & Petersen, S. (2022). Impact of moderate alcohol consumption on brain iron and cognition: observational and genetic analyses.

Topiwala, A., Wang, C., Ebmeier, K. P., Burgess, S., Bell, S., Levey, D. F., Zhou, H., McCracken, C., Roca-Fernández, A., Petersen, S. E., Raman, B., Husain, M., Gelernter, J., Miller, K. L., Smith, S. M., & Nichols, T. E. (2022). Associations between moderate alcohol consumption, brain iron, and cognition in UK Biobank participants: Observational and mendelian randomization analyses. *PLOS Medicine, 19*(7), e1004039. https://doi.org/10.1371/journal.pmed.1004039

Uhl, G. R., Koob, G. F., & Cable, J. (2019). The neurobiology of addiction. *Annals of the New York Academy of Sciences, 1451*(1), 5.

Ulmer, S. (2013). Introduction. In S. Ulmer & O. Jansen (Eds.), *fMRI: Basics and Clinical Applications* (pp. 3-5). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-34342-1_1

Vanderschuren, L. J. M. J., Di Ciano, P., & Everitt, B. J. (2005). Involvement of the dorsal striatum in cue-controlled cocaine seeking. *Journal of Neuroscience, 25*(38), 8665-8670.

Volkow, N. D., Wang, G.-J., Tomasi, D., & Baler, R. D. (2013, 2013/08/01/). Unbalanced neuronal circuits in addiction. *Current Opinion in Neurobiology, 23*(4), 639-648. https://doi.org/https://doi.org/10.1016/j.conb.2013.01.002

Vollstädt-Klein, S., Gerhardt, S., Lee, A., Strosche, A., Sharafi, G., Nuriyeva, R., Seidt, J., Hennig, O., Alm, B., Hermann, D., Sommer, W. H., Kiefer, F., Luderer, M., & Sobanski, E. (2020, Jun). Interaction between behavioral inhibition and neural alcohol cue-reactivity in ADHD and alcohol use disorder. *Psychopharmacology (Berl), 237*(6), 1691-1707. https://doi.org/10.1007/s00213-020-05492-1

Vollstädt-Klein, S., Loeber, S., Kirsch, M., Bach, P., Richter, A., Bühler, M., von der Goltz, C., Hermann, D., Mann, K., & Kiefer, F. (2011, 2011/06/01/). Effects of Cue-Exposure Treatment on Neural Cue Reactivity in Alcohol Dependence: A Randomized Trial. *Biological Psychiatry, 69*(11), 1060-1066. https://doi.org/https://doi.org/10.1016/j.biopsych.2010.12.016

Vollstädt‐Klein, S., Wichert, S., Rabinstein, J., Bühler, M., Klein, O., Ende, G., Hermann, D., & Mann, K. (2010). Initial, habitual and compulsive alcohol use is characterized by a shift of cue processing from ventral to dorsal striatum. *Addiction, 105*(10), 1741-1749.

Voon, V., Grodin, E., Mandali, A., Morris, L., Doñamayor, N., Weidacker, K., Kwako, L., Goldman, D., Koob, G. F., & Momenan, R. (2020, 2020/06/01/). Addictions NeuroImaging Assessment (ANIA): Towards an integrative framework for alcohol use disorder. *Neuroscience & Biobehavioral Reviews, 113*, 492-506. https://doi.org/https://doi.org/10.1016/j.neubiorev.2020.04.004

Wang, Y., & Liu, T. (2015, 2015/01/01). Quantitative susceptibility mapping (QSM): Decoding MRI data for a tissue magnetic biomarker [https://doi.org/10.1002/mrm.25358]. *Magnetic Resonance in Medicine, 73*(1), 82-101. https://doi.org/https://doi.org/10.1002/mrm.25358

Ward, R. J., Dexter, D. T., & Crichton, R. R. (2015). Neurodegenerative diseases and therapeutic strategies using iron chelators. *Journal of Trace Elements in Medicine and Biology, 31*, 267-273.

Westman, J., Wahlbeck, K., Laursen, T. M., Gissler, M., Nordentoft, M., Hällgren, J., Arffman, M., & Ösby, U. (2015, 2015/04/01). Mortality and life expectancy of people with alcohol use disorder in Denmark, Finland and Sweden [https://doi.org/10.1111/acps.12330]. *Acta Psychiatrica Scandinavica, 131*(4), 297-306. https://doi.org/https://doi.org/10.1111/acps.12330

Whitfield-Gabrieli, S., & Nieto-Castanon, A. (2012). Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain connectivity, 2*(3), 125-141.

Whitfield, J. B., Zhu, G., Heath, A. C., Powell, L. W., & Martin, N. G. (2001, Jul). Effects of alcohol consumption on indices of iron stores and of iron stores on alcohol intake markers. *Alcohol Clin Exp Res, 25*(7), 1037-1045.

Wiers, R. W., Eberl, C., Rinck, M., Becker, E. S., & Lindenmeyer, J. (2011, 2011/04/01). Retraining Automatic Action Tendencies Changes Alcoholic Patients' Approach Bias for Alcohol and Improves Treatment Outcome. *Psychological Science, 22*(4), 490-497. https://doi.org/10.1177/0956797611400615

Wilson, S. J., & Sayette, M. A. (2015, 2015/02/01). Neuroimaging craving: urge intensity matters [https://doi.org/10.1111/add.12676]. *Addiction, 110*(2), 195-203. https://doi.org/https://doi.org/10.1111/add.12676

World Health Organization. (2004). *ICD-10 : international statistical classification of diseases and related health problems*. World Health Organization.

World Health Organization. (2019). *Global status report on alcohol and health 2018*. World Health Organization.

World Health Organization. (2020). *Global Health Estimates 2020: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2019.*

Xie, S., Kaiser, D., & Cichy, R. M. (2020, 2020/07/06/). Visual Imagery and Perception Share Neural Representations in the Alpha Frequency Band. *Current Biology, 30*(13), 2621-2627.e2625. https://doi.org/https://doi.org/10.1016/j.cub.2020.04.074

Yalachkov, Y., Kaiser, J., Görres, A., Seehaus, A., & Naumer, M. J. (2013). Sensory modality of smoking cues modulates neural cue reactivity. *Psychopharmacology, 225*(2), 461-471.

Yalachkov, Y., Kaiser, J., & Naumer, M. J. (2009). Brain regions related to tool use and action knowledge reflect nicotine dependence. *Journal of Neuroscience, 29*(15), 4922-4929.

Yalachkov, Y., Kaiser, J., & Naumer, M. J. (2010). Sensory and motor aspects of addiction. *Behavioural brain research, 207*(2), 215-222.

Yalachkov, Y., Kaiser, J., & Naumer, M. J. (2012, 2012/02/01/). Functional neuroimaging studies in addiction: Multisensory drug stimuli and neural cue reactivity. *Neuroscience & Biobehavioral Reviews, 36*(2), 825-835. https://doi.org/https://doi.org/10.1016/j.neubiorev.2011.12.004

Yao, S., Zhong, Y., Xu, Y., Qin, J., Zhang, N., Zhu, X., & Li, Y. (2017). Quantitative Susceptibility Mapping Reveals an Association between Brain Iron Load and Depression Severity. *Front Hum Neurosci, 11*, 442. https://doi.org/10.3389/fnhum.2017.00442

Zeng, J., Yu, S., Cao, H., Su, Y., Dong, Z., & Yang, X. (2021, 2021/09/01/). Neurobiological correlates of cue-reactivity in alcohol-use disorders: A voxel-wise meta-analysis of fMRI studies. *Neuroscience & Biobehavioral Reviews, 128*, 294-310. https://doi.org/https://doi.org/10.1016/j.neubiorev.2021.06.031

Zhou, B., Bau, D., Oliva, A., & Torralba, A. (2018). Interpreting deep visual representations via network dissection. *IEEE transactions on pattern analysis and machine intelligence, 41*(9), 2131-2145.

Zhou, D., Liu, T., Spincemaille, P., & Wang, Y. (2014, Mar). Background field removal by solving the Laplacian boundary value problem. *NMR Biomed, 27*(3), 312-319. https://doi.org/10.1002/nbm.3064

Zhukovsky, P., Morein-Zamir, S., Meng, C., Dalley, J. W., & Ersche, K. D. (2020, 2020/06/01). Network failures: When incentives trigger impulsive responses [https://doi.org/10.1002/hbm.24941]. *Human Brain Mapping, 41*(8), 2216-2228. https://doi.org/https://doi.org/10.1002/hbm.24941

Zilverstand, A., Huang, A. S., Alia-Klein, N., & Goldstein, R. Z. (2018, 2018/06/06/). Neuroimaging Impaired Response Inhibition and Salience Attribution in Human Drug Addiction: A Systematic Review. *Neuron, 98*(5), 886-903. https://doi.org/https://doi.org/10.1016/j.neuron.2018.03.048

Zilverstand, A., Parvaz, M. A., & Goldstein, R. Z. (2017, 2017/05/01/). Neuroimaging cognitive reappraisal in clinical populations to define neural targets for enhancing emotion regulation. A systematic review. *NeuroImage, 151*, 105-116. https://doi.org/https://doi.org/10.1016/j.neuroimage.2016.06.009

6 TABULAR APPENDIX

6.1 Questionnaire: Alcohol Dependence Scale (ADS)

Lesen Sie bitte die Fragen und alle Antworten aufmerksam durch und markieren Sie den Buchstaben derjenigen Antwort, die am besten für Sie zutrifft. Das Wort „Trinken“ bezieht sich immer auf das Trinken von alkoholischen Getränken. Bitte überlegen Sie nicht allzu lange und lassen Sie keine Frage aus.

Diese Fragen beziehen sich auf die letzten 12 Monate

1. Wie viel haben Sie das letzte Mal getrunken?

[ ] Genug, um gute Laune zu bekommen oder weniger
[ ] Genug, um betrunken zu sein
[ ] Genug, um die Besinnung zu verlieren

2. Hatten Sie Sonntag/Montag morgens häufig einen Kater?

[ ] nein
[ ] ja

3. Fühlten Sie sich „zittrig“, als Sie nüchtern wurden (Händezittern, innere Unruhe)?

[ ] nein
[ ] manchmal
[ ] oft

4. Ging es Ihnen nach dem Trinken körperlich schlecht (z.B. Magenkrämpfe, Erbrechen)?

[ ] nein
[ ] manchmal
[ ] fast immer

5. **Hatten Sie jemals ein Delirium, d.h. haben Sie Dinge gesehen, gehört oder gefühlt, die nicht wirklich vorhanden waren, und fühlten Sie sich dabei sehr ängstlich, unruhig und sehr aufgeregt?**

[ ] nein
[ ] gelegentlich
[ ] mehrmals

6. Hatten Sie nach dem Trinken Mühe, noch geradeaus zu gehen und das Gleichgewicht zu halten?

[ ] Nein
[ ] manchmal
[ ] oft

7. Fühlten Sie sich infolge des Trinkens erhitzt und verschwitzt (fiebrig)?

[ ] nein
[ ] einmal
[ ] mehrmals

8. Hatten Sie infolge des Trinkens Dinge gesehen, die nicht wirklich vorhanden waren?

[ ] nein
[ ] einmal
[ ] mehrmals

9. Geraten Sie in Panik, wenn Sie befürchten müssen, nichts zu trinken zu bekommen, obwohl Sie es brauchen?

[ ] nein
[ ] ja

10. Hatten Sie als Folge des Trinkens „Blackouts“ (Erinnerungslücken, ohne dass Sie das Bewusstsein verloren hatten)?

[ ] nie
[ ] manchmal
[ ] oft
[ ] fast immer

11. Hatten Sie immer etwas zu Trinken bei sich oder in Ihrer Nähe?

[ ] nein
[ ] manchmal
[ ] die meiste Zeit

12. Hatten Sie Phasen der Abstinenz beendet, indem Sie wieder besonders viel getrunken haben?

[ ] nein
[ ] manchmal
[ ] fast immer

13. Hatten Sie in den letzten 12 Monaten infolge des Trinkens das Bewusstsein verloren?

[ ] nein
[ ] einmal
[ ] mehr als einmal

14. Hatten Sie einen Krampfanfall?

[ ] nein
[ ] ja
[ ] mehrmals

15. Tranken Sie auch über den ganzen Tag verteilt?

[ ] nein
[ ] ja

16. War Ihr Denken verwirrt oder unklar, nachdem Sie viel getrunken hatten?

[ ] nein
[ ] ja, aber nur wenige Stunden
[ ] ja, ein oder zwei Tage lang
[ ] ja, mehrere Tage lang

17. Fühlten Sie infolge des Trinkens Ihr Herz schneller schlagen?

[ ] nein
[ ] ja
[ ] mehrmals

18. Dachten Sie praktisch ständig über Alkohol nach?

[ ] nein
[ ] ja

19. Hatten Sie infolge des Trinkens „Dinge gehört", die nicht wirklich vorhanden waren?

[ ] nein
[ ] ja
[ ] mehrmals

20. Hatten Sie ungewöhnliche oder erschreckende Empfindungen während des Trinkens?

[ ] nein
[ ] einmal oder zweimal
[ ] mehrmals

21. Hatten Sie infolge des Trinkens das Gefühl, dass etwas auf Ihrer Haut krabbelt (z.B. Spinnen, Käfer)?

[ ] nein
[ ] ja
[ ] mehrmals

22. Diese Frage bezieht sich auf „Blackouts" (Erinnerungslücken):

[ ] ich hatte niemals einen Blackout
[ ] ich hatte Blackouts, die weniger als eine Stunde andauerten
[ ] ich hatte Blackouts, die mehrere Stunden andauerten
[ ] ich hatte Blackouts, die einen Tag oder länger andauerten

23. Hatten Sie ohne Erfolg versucht weniger zu trinken?

[ ] nein
[ ] einmal
[ ] mehrmals

24. Tranken Sie sehr schnell (Sturztrinken)?

[ ] nein
[ ] ja

25. Konnten Sie nach ein oder zwei Gläsern normalerweise mit dem Trinken aufhören?

[ ] ja
[ ] nein

6.2 Questionnaire: Alcohol Urge Questionnaire (AUQ)

Im Folgenden werden Ihnen Aussagen vorgegeben, die sich auf Ihre Gefühle zum Trinken beziehen. Die Wörter „trinken“ und „einen Drink haben“ beziehen sich auf alkoholische Getränke wie Bier, Wein oder Schnaps.
Bitte markieren Sie das Feld ([ ]) zwischen „stimmt gar nicht“ und „stimmt völlig“, das am besten angibt, wie stark Sie mit der Aussage übereinstimmen bzw. nicht übereinstimmen. Je mehr das von Ihnen markierte Feld in der Nähe des Anfangs bzw. Endes liegt, desto mehr drückt das Ihre Ablehnung bzw. Zustimmung aus.
Bitte beantworten Sie jede Aussage. Wir interessieren uns, wie Sie gerade in diesem Moment, wo Sie den Fragebogen ausfüllen, denken oder fühlen.

1. Alles was ich jetzt will, ist Trinken.

stimmt gar nicht	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	stimmt völlig

2. Ich brauche jetzt wirklich keinen Alkohol.

stimmt gar nicht	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	stimmt völlig

3. Es würde mir schwer fallen, in diesem Moment Alkohol abzulehnen.

stimmt gar nicht	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	stimmt völlig

4. Jetzt zu trinken, würde die Dinge einfach perfekt erscheinen lassen.

stimmt gar nicht	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	stimmt völlig

5. Ich möchte so sehr trinken, dass ich es fast schmecken kann.

stimmt gar nicht	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	stimmt völlig

6. Nichts wäre besser als jetzt zu trinken.

stimmt gar nicht	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	stimmt völlig

7. Wenn ich die Möglichkeit hätte, jetzt einen Drink zu bekommen, glaube ich nicht, dass ich trinken würde.

stimmt gar nicht	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	stimmt völlig

8. Ich habe jetzt Verlangen nach Alkohol.

stimmt gar nicht	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	stimmt völlig

6.3 Questionnaire: FORM 90

1. Einleitung und Festlegung des Untersuchungszeitraums

„Zunächst einmal möchte ich Ihnen versichern, dass alles was Sie hier sagen, wie auch in den vorherigen Interviews vertraulich behandelt wird. Ich werde Ihnen einige Fragen stellen zum Zeitraum von der letzten Visite

am _____ bis zum gestrigen Tag.“

- *Zeigen Sie dem Patienten den Kalender, den Sie aus dem Anhang entnehmen.*
- *Falls das Interview telefonisch durchgeführt wird, soll der Patient einen regulären Kalender verwenden.*

2. Erhebung wichtiger Ereignisse

„Ich weiß, dass ist eine lange Zeit. Um Ihnen zu helfen, alle Ereignisse in dieser Zeit zu erinnern, werden wir diesen Kalender benutzen.

„Gab es einige besondere Ereignisse in dieser Zeit für Sie, die sie mit Ihren Alkoholkonsum in Verbindung bringen wie Geburtstage, Krankheiten oder Unfälle, Jahrestage, Festlichkeiten, Krankenhausaufenthalte, Ferien, Veränderungen Ihrer Berufstätigkeit oder auch Veränderungen zu Hause?“

- *Tragen Sie die Ereignisse in den Kalender ein.*

3. Erhebung der Abstinenzzeiten

„Nehmen Sie sich zur Beantwortung der Fragen ausreichend Zeit, denn es ist wichtig, dass Sie sich an die Dinge so gut und so genau wie möglich erinnern. Wenn Sie mal eine Frage nicht verstehen oder sich nicht sicher sind, was die Frage meint, fragen Sie mich. OK?“

- *Zunächst erfragen Sie die Abstinenzzeiten.*

„Ich möchte Sie über Ihren Alkoholkonsum während dieser Zeit fragen. Zuallererst möchte ich Sie jedoch fragen, ob es Tage oder eine längere Zeit (Jahre oder Monate) gab, in der Sie nichts getrunken haben?“

- *Markieren Sie bitte im Kalender alle abstinenten Tage mit „A“. Falls das bei dem langen Untersuchungszeitraum zu kompliziert ist. Notieren die die abstinenten Zeiträume (Datum Beginn und Ende) auf ein Blatt, um später die Anzahl abstinenter Tage ausrechnen zu können.*
- *Wenn der Patient die meiste Zeit abstinent war, ist es möglicherweise einfacher ihn zuerst über die Trinktage zu fragen, und diese in den Kalender einzutragen.*

4. Erhebung des Alkoholkonsums für Zeiten mit Trinkmustern

„Während dieser Zeit, in der Sie getrunken haben, war Ihr Trinkmuster von einer zur nächsten Woche ähnlich, zumindest für einige Wochen? Ich weiß, dass das Trinken jeden Tag und jede Woche unterschiedlich sein kann, aber mich interessiert jetzt, ob die Wochen bezüglich Ihres Trinkverhaltens ähnlich waren. Gab es in Ihrem Trinkverhalten Übereinstimmungen von Woche zu Woche?“

- *Falls nicht, fahren Sie mit 5. (Erhebung des Alkoholkonsums für einzelne Tage) auf der nächsten Seite fort.*
  Falls ja, setzen Sie bitte hier fort und notieren Sie dabei auf der übernächsten Seite die Trinkmenge bezüglich der Tageszeiten in die Tabelle P1.

„Beschreiben Sie mir bitte Ihre übliche oder typische Trink-Woche. In einer typischen Woche, lassen Sie uns mit den Wochentagen beginnen – Montag bis Freitag – was trinken Sie normalerweise am Morgen, von der Zeit nach dem Aufstehen bis zum Mittag?“

- *Notieren Sie in der Tabelle.*

„Wie sieht es mit den Nachmittagen unter der Woche aus? Was tranken Sie beim Mittagessen, über den Nachmittag bis zum Abend-Essen? Was tranken Sie normalerweise an den Nachmittagen in der Woche, von Montag bis Freitag?“

- *Notieren Sie in der Tabelle.*

„Was tranken Sie am Abend in der Woche? Was trinken Sie normalerweise zum Abendbrot und was danach in der Zeit bis zum Schlafengehen?“

- *Notieren Sie in der Tabelle.*
- *Prüfen Sie die Freitagabende, hier ist das Trinkverhalten häufig unterschiedlich zu den anderen Wochentagen.*
  Wiederholen Sie die gleichen Instruktionen für die Wochenenden (Samstag und Sonntag).
- *Bestimmen Sie nun die P1 Wochen.*

„ Nun, an welchen Wochen hier auf diesem Kalender haben sie in dieser Weise getrunken?“

- *Markieren Sie diese Wochen mit P1 im Kalender.*

- *Manchmal benötigen Sie eine zweite Trinkmustertabelle (P2). In diesem Falle wiederholen Sie die obige Prozedur für P2 und markieren diese Wochen mit P2 auf dem Kalender*

5. Erhebung des Alkoholkonsums für einzelne Tage

- *Wenn Sie die Trinkmuster dokumentiert haben, tragen Sie bitte die ungefähre Trinkmenge und Getränkeart in die entsprechenden restlichen Tage des Kalenders ein, die nicht mit P1 oder P2 gekennzeichnet wurden. Errechnen Sie später mittels der Umrechnungstabelle (im Anhang) für jeden Tag die Alkoholmenge in Gramm und tragen sie in den Kalender ein.*

„Nun haben wir Ihr übliches Trinkmuster festgehalten. Jetzt möchte ich gerne noch etwas über die Zeiten während dieser Periode wissen, an denen Ihr Trinkverhalten anders war. Schauen Sie noch mal auf den Kalender, und denken Sie an die Zeit zurück. Wann waren die Tage oder Zeiten, in denen Sie mehr oder weniger Alkohol als gewöhnlich getrunken haben?“

- *oder*

„Wenn Sie kein typisches Trinkverhalten über Wochen haben, beschreiben Sie mir Tage oder andere Zeiträume in dem ursprünglichen Zeitraum hier im Kalender, an denen Sie getrunken haben.“

Trinkmuster 1 (P1)

	Morgens Getränkart und Menge	Mittags Getränkeart und Menge	Abends Getränkeart und Menge	Gramm Alkohol
Montag				
Dienstag				
Mittwoch				
Donnerstag				
Freitag				
Samstag				
Sonntag				
Summe Gramm Alkohol pro Woche				

- *Markieren Sie alle Tage mit diesem Trinkmuster mit P1 im Kalender.*
  Falls das oben beschriebene Muster nicht für alle Trinkwochen zutrifft, fragen Sie:

„In den anderen Wochen, in denen Sie getrunken haben, war Ihr Trinkmuster von Woche zu Woche gleich?“

- *Falls „ja“, vervollständigen Sie Tabelle P2. Falls „nein“ gehen Sie zu 5. (Erhebung des Alkoholkonsums für einzelne Tage)*

Trinkmuster 2 (P2)

	Morgens Getränkart und Menge	Mittags Getränkeart und Menge	Abends Getränkeart und Menge	Gramm Alkohol
Montag				☐☐☐
Dienstag				☐☐☐
Mittwoch				☐☐☐
Donnerstag				☐☐☐
Freitag				☐☐☐
Samstag				☐☐☐
Sonntag				☐☐☐
Summe Gramm Alkohol pro Woche				☐☐☐☐

◙ *Markieren Sie alle Tage mit diesem Trinkmuster mit P2 im Kalender.*

Form 90 Dokumentationsbogen

- *Bitte tragen Sie abschließend auf diesem Bogen alle erhobenen Daten ein*

1. Zeitraum vom ☐☐.☐☐.☐☐ bis zum gestrigen Tag

2. Anzahl der Tage im Untersuchungszeitraum: ☐☐☐☐☐☐

3. Interviewsituation: [ ] persönlich in der Klinik/Institut/Einrichtung

[ ] telefonisch

4. Erster Tag mit Alkoholkonsum im Untersuchungszeitraum: ☐☐.☐☐.☐☐

5. Letzter Tag mit Alkoholkonsum im Untersuchungszeitraum: ☐☐.☐☐.☐☐

6. Anzahl abstinente Tage im Untersuchungszeitraum: ☐☐☐☐☐☐

7. Anzahl Trinktage im Untersuchungszeitraum: ☐☐☐☐☐☐

8. Anzahl Tage mit mehr als 48/60g (Frauen/Männer) Alkohol im Untersuchungszeitraum: ☐☐☐☐☐☐

9. Kumulierte Alkoholmenge im Untersuchungszeitraum: ☐☐☐☐☐☐☐☐ (Gramm)

6.4 Questionnaire: Obsessive Compulsive Drinking Scale (OCDS)

Die folgenden Fragen beziehen sich auf Ihren Alkoholkonsum und auf Gedanken, Vorstellungen, Impulse oder Bilder, die mit dem Trinken von Alkohol zusammenhängen. Bitte kreuzen Sie jeweils die Aussage an, die am ehesten auf Sie zutrifft.
Falls nicht anders angegeben, beziehen sich die Fragen auf den Zeitraum der vergangenen sieben Tage.

1. Wenn Sie keinen Alkohol trinken, wie viel Ihrer Zeit wird dann von Vorstellungen, Gedanken, Impulsen oder Bildern beansprucht, die etwas mit dem Trinken zu tun haben?

[ ] Keine
[ ] Weniger als eine Stunde am Tag
[ ] 1-3 Stunden am Tag
[ ] 4-8 Stunden am Tag
[ ] Mehr als 8 Stunden am Tag

2. Wie häufig treten diese Gedanken oder Vorstellungen auf?

[ ] Niemals
[ ] Nicht häufiger als achtmal am Tag
[ ] Häufiger als achtmal am Tag, aber die meisten Stunden des Tages sind frei davon
[ ] Mehr als achtmal am Tag und während der meisten Stunden des Tages
[ ] Die Gedanken treten so häufig auf, dass man sie nicht mehr zählen kann, und es vergeht kaum eine Stunde, in der sie nicht auftreten

3. Wie stark werden Ihre berufliche Tätigkeit oder Ihr soziales Verhalten von diesen Vorstellungen, Gedanken, Impulsen oder Bildern beeinflusst? Gibt es etwas, was sie deswegen nicht tun oder nicht können?
Falls sie gerade nicht berufstätig sind: Wie sehr wäre Ihre berufliche Tätigkeit beeinflusst, falls Sie arbeiten würden?

[ ] Die Gedanken an Alkohol beeinflussen mich überhaupt nicht - ich arbeite oder verhalte mich völlig normal.
[ ] Die Gedanken an Alkohol beeinflussen mein soziales Verhalten oder meine beruflichen Tätigkeiten in geringem Maße, mein Auftreten oder meine Leistung insgesamt ist jedoch nicht beeinträchtigt.
[ ] Die Gedanken an Alkohol beeinflussen mein soziales Verhalten oder meine berufliche Leistung eindeutig, ich komme aber noch damit zurecht.
[ ] Die Gedanken an Alkohol beeinträchtigen mein soziales Verhalten oder meine berufliche Leistung ganz erheblich.
[ ] Die Gedanken an Alkohol beeinträchtigen mein soziales Verhalten oder meine Arbeitsleistung vollständig.

4. Wenn Sie keinen Alkohol trinken, wie sehr leiden Sie dann unter den Vorstellungen, Gedanken, Impulsen oder Bildern, die mit dem Trinken zu tun haben bzw. wie sehr werden Sie dadurch gestört.

[ ] Keine Belastung oder Störung

[ ] Geringfügig, selten und nicht besonders störend
[ ] Mäßig, häufig und störend; ich kann aber noch damit zurechtkommen
[ ] Stark, sehr häufig und sehr störend
[ ] Extrem stark, fast durchgängig, alles andere wird beeinträchtigt.

5. Wenn Sie keinen Alkohol trinken, wie sehr bemühen Sie sich dann, diesen Gedanken zu widerstehen, Sie nicht zu beachten oder Ihre Aufmerksamkeit auf etwas Anderes zu lenken? (Geben Sie das Ausmaß Ihrer Bemühungen um Widerstand gegen diese Gedanken an, nicht den Erfolg oder Misserfolg, den Sie dabei haben.)

[ ] Ich habe so selten derartige Gedanken, dass es nicht notwendig ist, dagegen anzugehen. Wenn Sie aber auftauchen, bemühe ich mich immer, diesen Gedanken zu widerstehen.
[ ] Ich versuche meistens, diesen Gedanken zu widerstehen.
[ ] Ich unternehme einige Anstrengungen, um diesen Gedanken zu widerstehen.
[ ] Ich lasse allen derartigen Gedanken freien Lauf, ohne zu versuchen, sie zu kontrollieren. Dabei habe ich allerdings ein ungutes Gefühl.
[ ] Ich lasse diesen Gedanken freien Lauf.

6. Wenn Sie keinen Alkohol trinken, wie erfolgreich können Sie dann diese Gedanken beenden oder sie zerstreuen?

[ ] Es gelingt mir stets vollkommen, diese Gedanken zu beenden oder sie zu zerstreuen.
[ ] Gewöhnlich kann ich diese Gedanken mit einiger Anstrengung und Konzentration beenden oder zerstreuen.
[ ] Manchmal kann ich diese Gedanken beenden oder sie zerstreuen.
[ ] Ich kann diese Gedanken nur ganz selten beenden und sie nur sehr schwerlich zerstreuen.
[ ] Selbst für kurze Momente kann ich solche Gedanken nur selten zerstreuen.

7. Wie viele „drinks“ nehmen Sie täglich zu sich?
Denken Sie an die letzten Wochen, in denen Sie Alkohol getrunken haben.

[ ] Keinen
[ ] Weniger als einen „drink“ (entspricht weniger als 0,33 Liter Bier oder 1/8 Liter Wein oder 30 ml Schnaps)
[ ] 1-2 „drinks“ täglich (entspricht maximal 0,66 Liter Bier oder 1/4 Liter Wein oder 60 ml Schnaps)
[ ] 3-7 „drinks“ täglich (entspricht bis 2,5 Liter Bier oder bis 1 Liter Wein oder bis 200 ml Schnaps)
[ ] 8 oder mehr „drinks“ täglich (entspricht mehr als 2,5 Liter Bier oder mehr als 1 Liter Wein oder mehr als 200 ml Schnaps)

8. An wie vielen Tagen in der Woche trinken Sie Alkohol?
Denken Sie an die letzten Wochen, in denen Sie Alkohol getrunken haben.

[ ] An keinem
[ ] An nicht mehr als einem Tag
[ ] An 2-3 Tagen die Woche
[ ] An 4-5 Tagen die Woche

[ ] An 6-7 Tagen die Woche

9. Wie stark wird Ihre berufliche Tätigkeit durch das Trinken von Alkohol beeinflusst? Gibt es etwas, was Sie wegen Ihres Trinkens nicht machen oder nicht können?
(Falls Sie gerade nicht berufstätig sind: Wie sehr wäre Ihre berufliche Tätigkeit beeinflusst, falls Sie arbeiten würden?)

[ ] Das Trinken beeinflusst mich beruflich überhaupt nicht – ich arbeite völlig normal.
[ ] Das Trinken beeinflusst meine beruflichen Tätigkeiten in geringem Maße, meine Arbeitskraft insgesamt ist jedoch nicht beeinträchtigt.
[ ] Das Trinken beeinflusst meine berufliche Leistung eindeutig, ich komme aber noch damit zurecht.
[ ] Das Trinken beeinträchtigt meine berufliche Leistung ganz erheblich.
[ ] Das Trinken beeinträchtigt meine Arbeitsleistung völlig.

10. Wie stark wird Ihr soziales Verhalten durch das Trinken von Alkohol beeinflusst? Gibt es etwas, was Sie wegen Ihres Trinkens nicht machen oder nicht können?

[ ] Das Trinken beeinflusst mein soziales Verhalten überhaupt nicht – ich verhalte mich völlig normal.
[ ] Das Trinken beeinflusst mein soziales Verhalten in geringem Maße, mein Auftreten insgesamt ist jedoch nicht beeinträchtigt.
[ ] Das Trinken beeinflusst mein soziales Verhalten eindeutig, ich komme aber noch damit zurecht.
[ ] Das Trinken beeinträchtigt mein soziales Verhalten ganz erheblich.
[ ] Das Trinken beeinträchtigt mein soziales Verhalten völlig.

11. Wenn Sie ein alkoholisches Getränk trinken möchten, aber daran gehindert wären, wie ängstlich oder ungehalten würden Sie dann werden?

[ ] Ich würde überhaupt nicht ängstlich oder gereizt.
[ ] Ich würde in geringem Maße ängstlich oder gereizt.
[ ] Angst oder Reizbarkeit würden ansteigen, aber noch zu beherrschen sein.
[ ] Angst oder Reizbarkeit würden für mich sehr stark und störend.
[ ] Angst oder Reizbarkeit würden mich völlig überwältigen.

12. Wie sehr bemühen Sie sich, dem Trinken von Alkohol zu widerstehen?
(Geben Sie das Ausmaß Ihrer Bemühungen um Widerstand gegen das Trinken an, nicht den Erfolg oder Misserfolg, den Sie dabei haben.)

[ ] Ich trinke so minimal, dass es nicht notwendig ist, dagegen anzugehen. Wenn ich doch trinke, bemühe ich mich immer, dem Trinken zu widerstehen.
[ ] Ich versuche meistens, dem Trinken zu widerstehen.
[ ] Ich unternehme einige Anstrengungen, um dem Trinken zu widerstehen.
[ ] Ich lasse dem Trinken meistens freien Lauf, ohne zu versuchen, es zu kontrollieren. Dabei habe ich ein ungutes Gefühl.
[ ] Ich lasse dem Trinken völlig freien Lauf.

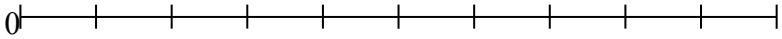
13. Wie stark ist Ihr Drang, Alkohol zu trinken?

[ ] Ich verspüre keinen Drang.
[ ] Ich verspüre etwas Drang, Alkohol zu trinken.
[ ] Ich verspüre starken Drang, Alkohol zu trinken.
[ ] Ich verspüre sehr starken Drang, Alkohol zu trinken.
[ ] Der Drang zum Trinken ist völlig überwältigend und nicht zu beeinflussen.

14. Wie viel Kontrolle haben Sie über Ihr Trinkverhalten?

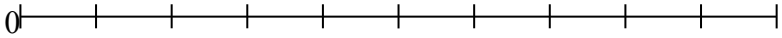
[ ] Ich habe mein Trinkverhalten völlig unter Kontrolle.
[ ] Gewöhnlich kann ich mein Trinkverhalten unter willentlicher Kontrolle halten.
[ ] Ich kann mein Trinkverhalten nur unter Schwierigkeiten kontrollieren.
[ ] Ich muss trinken und kann es nur unter Schwierigkeiten hinausschieben.
[ ] Ich bin kaum in der Lage, das Trinken auch nur für kurze Zeit hinauszuschieben.

15. Wie stark war während der letzten sieben Tage Ihr Verlangen nach Alkohol (der Wunsch nach Alkohol, während der Zeit, in der sie nicht tranken) im Durchschnitt?



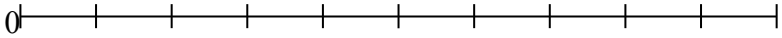
nicht vorhanden sehr stark

16. Denken Sie bitte einmal an den Moment innerhalb der letzten sieben Tage zurück, als das Verlangen nach Alkohol am stärksten war. Wie stark war dieses Verlangen?



nicht vorhanden sehr stark

17. Wie häufig hatten Sie während der letzten sieben Tage Verlangen nach Alkohol (den Wunsch nach Alkohol, während der Zeit, in der sie nicht tranken)?



nie immer

18. Wann haben Sie zuletzt Alkohol getrunken?

. Vor ☐☐☐ Tagen

7 CURRICULUM VITAE

PERSONAL INFORMARTION

Name:	Haoye Tan
Date of Birth:	13.10.1993
Place of Birth:	Zhejiang China
Nationality:	Chinese

ACADEMIC EDUCATION

10.2019 – present	Doctoral student, Department of Addictive Behaviour and Addiction Medicine, Central Institute of Mental Health, Medical Faculty of Mannheim, Heidelberg University; Mannheim, Germany Thesis: Neuroimaging biomarkers in Alcohol Use Disorder: clinical relevance and relapse prediction. Supervisor: Prof. Dr. Sabine Vollstädt-Klein
09.2016 – 06.2019	Master of Science in Medicine, Department of Drug Dependence, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine; Shanghai, China Thesis: Craving assessments in Methamphetamine Use Disorder with virtual reality (VR), eye-tracking and neuroimaging. Supervisor: Prof. Dr. Min Zhao
09.2011 – 06.2016	Bachelor of Medicine, The Second Military Medical University; Shanghai, China
09.2008 – 06.2011	Wenling high school; Zhejiang, China Certificate

PUBLICATION LIST

Included publications

Tan, H., Hubertus, S., Thomas, S., Lee, A. M., Gerhardt, S., Gerchen, M. F., Sommer, W. H., Kiefer, F., Schad, L., & Vollstädt-Klein, S. (2023). Association between iron accumulation in the dorsal striatum and compulsive drinking in alcohol use disorder. Psychopharmacology, 240(2), 249-257. https://doi.org/10.1007/s00213-022-06301-7.

Tan, H., Gerchen, M. F., Bach, P., Lee, A. M., Hummel, O., Sommer, W., Kirsch, P., Kiefer, F., & Vollstädt-Klein, S. (2023). Decoding fMRI alcohol cue reactivity and its association with drinking behaviour. BMJ Mental Health, 26(1), e300639. https://doi.org/10.1136/bmjment-2022-300639.

Other publications

Gerhardt, S., Hoffmann, S., **Tan, H.**, Gerchen, M. F., Kirsch, P., Vollstädt-Klein, S., Kiefer, F., Bach, P., & Lenz, B. (2022). Neural cue reactivity is not stronger in male than in female patients with alcohol use disorder. Frontiers in Behavioral Neuroscience. https://doi.org/https://doi.org/10.3389/fnbeh.2022.1039917

Hornoiu, I. L., Lee, A. M., **Tan, H.**, Nakovics, H., Bach, P., Mann, K., Kiefer, F., Sommer, W., & Vollstädt-Klein, S. (2022). The Role of Unawareness, Volition and Neural Hyperconnectivity in Alcohol Use Disorder: A Functional Magnetic Resonance Imaging Study. Biological Psychiatry: Cognitive Neuroscience and Neuroimaging. https://doi.org/https://doi.org/10.1016/j.bpsc.2022.12.008

Tan, H., Nakovics, H., Zeng, H., Copello, A., Akhtar, S., Lee, A. M., Kiefer, F., & Vollstädt-Klein, S. (2022). Assessment of automated craving across substances and across cultures: stability-analysis of the Craving Automated Scale (CAS). Journal of Addictive Diseases, 40(3), 405-414. https://doi.org/10.1080/10550887.2021.2015053

Türkmen, C., Machunze, N., **Tan, H.**, Gerhardt, S., Kiefer, F., & Vollstädt-Klein, S. (2022). Vulnerability for alcohol use disorder after adverse childhood experiences (AUDACE): protocol for a longitudinal fMRI study assessing neuropsychobiological risk factors for relapse. BMJ Open, 12(6), e058645. https://doi.org/10.1136/bmjopen-2021-058645

Zhang, J., Chen, T., **Tan, H**., Wu, Q., Chen, L., Yuan, C., Ding, X., Zhang, L., Du, C., Li, J., Lu, E., Wu, Y., Zhao, M., & Du, J. (2022). Mindfulness-Based Intervention on Chinese Patients with Amphetamine-Type Stimulant Use Disorders: an EEG Functional Connectivity Study. Mindfulness, 13(5), 1320-1332. https://doi.org/10.1007/s12671-022-01882-y

Chen, T., **Tan, H.**, Lei, H., Li, X., Wu, Q., Xu, X., Ye, Y., Zhong, N., Du, J., Jiang, H., Su, H., & Zhao, M. (2021). Nature of glutamate alterations in substance dependence: A systematic review and meta-analysis of proton magnetic resonance spectroscopy studies. Psychiatry Research: Neuroimaging, 315, 111329. https://doi.org/https://doi.org/10.1016/j.pscychresns.2021.111329

Chen, T., Su, H., Zhong, N., **Tan, H.**, Li, X., Meng, Y., Duan, C., Zhang, C., Bao, J., Xu, D., Song, W., Zou, J., Liu, T., Zhan, Q., Jiang, H., & Zhao, M. (2020). Disrupted brain network dynamics and cognitive functions in methamphetamine use disorder: insights from EEG microstates. BMC Psychiatry, 20(1), 334. https://doi.org/10.1186/s12888-020-02743-5

Chen, T., **Tan, H.**, Lei, H., Su, H., & Zhao, M. (2020). Proton magnetic resonance spectroscopy in substance use disorder: recent advances and future clinical applications. Science China Information Sciences, 63(7), 170101. https://doi.org/10.1007/s11432-019-2818-5

Tan, H., Chen, T., Du, J., Li, R., Jiang, H., Deng, C.-l., Song, W., Xu, D., & Zhao, M. (2019). Drug-related Virtual Reality Cue Reactivity is Associated with Gamma Activity in Reward and Executive Control Circuit in Methamphetamine Use Disorders. Archives of Medical Research, 50(8), 509-517. https://doi.org/https://doi.org/10.1016/j.arcmed.2019.09.003

Tan, H., Liang, D., Zhong, N., Zhao, Y., Chen, Z., Zhao, M., & Jiang, H. (2019). History of Alcohol and Opioid Use Impacts on the Long-Term Recovery Trajectories of Methamphetamine-Dependent Patients [Original Research]. Frontiers in Psychiatry, 10.

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