Aus der Psychiatrischen Klinik und Hochschulambulanz
der Medizinischen Fakultät der Charité – Universitätsmedizin Berlin

DISSERTATION

Striatal dopamine receptor 2 and 3 availability in alcohol dependence

zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

von

Gianna Spitta

aus Frankfurt am Main

Datum der Promotion: 18.12.2020

Preface

Part of the data on which this work is based on has recently been published in the article of Sebold et al. (2019). More precisely, in the article we used neurobiological data of 39 subjects, whereas in this work we analysed the complete sample of 58 study neuroimaging participants. Furthermore, different clinical scales and questionnaires were used in the article than in this work.

Contents

List of abbreviations

2

2FDG. *2-fluorodeoxy-D-glucose*

3

3D. *three-dimensional*

5

5-HT. *5-hydroxy-tryptamine*
5-HT_3-R. *5-hydroxy-tryptamine type 3 receptor*

A

ACC. *anterior cingulate cortex*
AD. *alcohol dependence, alcohol-dependent*
ADH. *alcohol dehydrogenase*
ADHD. *Attention-deficit/ hyperactivity disorder*
ALDH. aldehyde dehydrogenase
AMPA-R. *α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor*
ANOVA. *univariate analysis of variance*
ARC. *Addiction Research Center*
AS. *associative striatum*
AUD. *alcohol use disorder*
AUDIT. *Alcohol Use Disorder Identification Test*
AWMF. *Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften e.V.*

B

BP. *binding potential*

C

cAMP. *cyclic adenosine monophosphate*
CNS. *central nervous system*
CT. *computed tomography*
CVK. *Charité Virchow Klinikum*

D

DA. *dopamine*
DAT. *dopamine transporter*
DFG. *Deutsche Forschungsgemeinschaft*
DGPPN. *Deutsche Gesellschaft für Psychiatrie, Psychotherapie, Psychosomatik und Nervenheilkunde*
DR2. *Dopamine Receptor 2*
DR3. *Dopamine Receptor 3*,

E

EHI. *Edinburgh Handedness Inventory*
EMA. *European Medicinces Agency*

F

fMRI. *functional Magnetic Resonance Spectroscopy*
FTND. *Fragerström Test for Nicotine Dependence*

G

GABA. *Gamma-aminobutyric acid*
$GABA_A$-R. *Gamma-aminobutyric acid type A receptor*
GHB. *Gamma-hydroxybutyric acid*
GWAS. Genome-wide association studies

H

HC. *healthy control*, *hippocampus*
HR. *high-risk control*

I

ICD-10. *International Statistical Classification of Diseases and Related Health Problems 10th Revision*
IQR. *interquartile range*

K

KAR. *kainate receptor*
kV. *kilovolt*
K-W. *Kruskal-Wallis*

L

LOR. *line of response*
LR. *low-risk control*
LS. *limbic striatum*

M

max. *maximum*
MBq. *megabecquerel*
min. *minutes*, *minimum*
MNI. *Montreal Neurological Institute*
MRI. *Magnetic Resonance Imaging*
MRS. *Magnetic Resonance Spectroscopy*
mSv. *milisievert*

N

NMDA. *N-methyl-D-aspartate*

O

OCD. *obsessive-compulsive disorder*
OCDS. *Obsessive Compulsive Drinking Scale*

P

P1. *Project 1*
P2. *Project 2*
P5. *Project 5*
PTB. *Physikalisch Technische Bundesanstalt*
PTSD. *post traumatic stress disorder*

R

ROI. *region of interest*

S

SD. *standard deviation*
SDSS. *Substance Dependence Severity Scale*
SERT. *serotonin transporter*
SLF. *superior longitudinal fasciculus*
SMS. *sensorimotor striatum*
SNPs. *single nucleotide polymorphisms*
SNR. *signal to noise ratio*
SPECT. *single photon emission computed tomography*
SPM. *Statistic Parametric Mapping*
SUD. *substance use disorder*

T

TACs. *time acitivity curves*

W

WHO. *World Health Organization*

List of figures

List of tables

Abstract

Abstract (English).

Alcohol dependence (AD) is the most common substance use disorder (SUD) both in Germany and worldwide. Yet its specific pathogenesis remains unclear. The aim of this study was to enhance the understanding of the underlying neurochemical processes of AD and thus gain knowledge about the development and maintenance of this disease.

Besides having its well-known GABAergic effects, alcohol interferes directly and indirectly with many other neurotransmitters such as dopamine (DA). In prior studies, a release of DA following acute alcohol intake was often observed in the limbic striatum (LS). Further, DA is strongly associated with positive reinforcement, which is a basic mechanism involved in addiction in general.

Neurobiologically, there are indications that chronic alcohol intake may reduce dopamine D2 and D3 receptor (DR2/3) availability in the striatum. This adaptation process has been interpreted as a compensatory downregulation mechanism of DA receptors, which may provoke certain withdrawal symptoms in AD.

In the present study, we used the highly affine, specific radiotracer ^{18}F-fallypride to quantify the striatal DR2/3 availability via positron emission tomography (PET). The sample consisted of 20 diagnosed alcohol-dependent patients (AD) after alcohol withdrawal, 19 controls with low-risk (LR) and 19 individuals with high-risk alcohol intake (HR). We used three subgroups to reflect different levels of alcohol intake and our aim was to investigate the extent of the dopaminergic impairment within these groups.

We observed significant reductions of the DR2/3 availability of AD subjects compared to LR and HR in the sensorimotor and associative part of the striatum. There were no significant differences between the LR and HR groups. The severity of alcohol dependence as well as the extent of the craving symptoms were inversely correlated with the DR2/3 availability in the associative and sensorimotor striatum in the whole sample.

While earlier studies have mainly focused on the LS, we observed significant differences in DR2/3 availability in the sensorimotor and associative striatum in AD. We did not observe significant differences between our HR and LR individuals, which may be due to power issues.

These findings are in line with the concept that a gradual loss of control over the drinking behavior may be associated with a shift from ventro- to dorsostriatal adaptation processes, which has only been shown in animal studies up to now. Our findings add to a growing body of evidence showing that AD and addiction symptoms such as craving are associated with impaired dorsostriatal DR2/3 availability.

Keywords: Alcohol dependence, dopamine, sensorimotor striatum, associative striatum, PET, ^{18}F-fallypride, DR2/3 availability, craving symptoms.

Abstrakt (German).

Obwohl Alkoholabhängigkeit die häufigste Suchterkrankung, sowohl in Deutschland als auch weltweit ist, konnte die genaue Pathogenese der Erkrankung bisher noch nicht vollständig erklärt werden. Ziel dieser Studie war es nähere Erkenntnisse über die zugrundeliegenden neurobiologischen Grundlagen der Alkoholabhängigkeit zu gewinnen und darüber ein besseres Verständnis über die Entstehung und Aufrechterhaltung dieser Erkrankung zu erlangen.

Neben den bekannten GABAergen Effekten, beeinflusst Alkohol zudem direkt und indirekt noch viele weitere Neurotransmitter, wie zum Beispiel Dopamin (DA). Akuter Substanzkonsum führt möglicherweise zu einer erhöhten Dopaminfreisetzung im limbischen Striatum. DA ist mit positiver Verstärkung assoziiert, einem Lernmechanismus der grundlegend mit Suchtentwicklung verbunden ist.

Neurobiologisch scheint chronischer Alkoholkonsum zu einer verringerten Dopamin- 2 und 3 (D2/3) Rezeptordichte im Striatum zu führen. Dieser Adaptationsvorgang kann als eine kompensatorische Herunterregulation der Rezeptoren interpretiert werden, was wiederum Grundlage klinischer Entzugssymptome von Alkoholabhängigen sein könnte.

In dieser Studie wurde die Dopaminrezeptorverfügbarkeit mittels Positronen-Emissions-Tomographie (PET) bestimmt. ^{18}F-fallypride, ein hoch affiner, spezifischer DR2/3 Antagonist wurde als Radiotracer zur Bestimmung der D2/3 Rezeptorenverfügbarkeiten in striatalen Gehirnregionen genutzt.
Die Stichprobe bestand aus 20 abstinenten Patienten mit diagnostizierter Alkoholabhängigkeit, 19 gesunden Kontrollen mit geringem (Low Risk, LR) und 19 Kontrollen mit riskantem Alkoholkonsum (High Risk, HR). Die drei Referenzgruppen repräsentieren verschiedene Schweregrade von Alkoholkonsum und wurden benötigt, um das Ausmaß der dopaminergen Adaptationsprozesse innerhalb dieser Gruppen zu untersuchen.

Im assoziativen und sensomotorischen Bereich des dorsalen Striatum der alkoholabhängigen Patienten konnte eine signifikant erniedrigte D2/3 Rezeptorenverfügbarkeit im Vergleich zu LR und HR Kontrollen nachgewiesen werden. Es gab keine signifikanten Unteschiede zwischen den LR und HR Gruppe. Desweiteren wurde in der gesamten Stichprobe eine inverse Korrelation zwischen der Dopaminrezeptorverfügbarkeit im assoziativen und sensomotorischen Striatum und der Schwere der Alkoholabhängigkeit sowie „Craving“ Symptomen festgestellt.

Zusammenfassend wurden in dieser Studie signifikante Veränderungen der DR2/3 Verfügbarkeit im assoziativen und sensomotorischen Striatum gefunden, während vorherige Studien vor allem Ergebnisse im limbischen Striatum fanden. Das es keine signifikanten Unterschiede zwischen HR und LR Probanden gab könnte ein Problem der mangelnden Teststärke unserer Analyse sein.

Unsere Befunde sind übereinstimmend mit dem Konzept eines graduellen Kontrollverlusts über das Trinkverhalten, welcher nach Tierstudien mit einem Shift von ventro- zu dorsostriatalen Adaptionsprozessen assoziiert sein könnte. Unsere Ergebnisse fügen sich in eine wachsende Anzahl von Studien ein, die eine Assoziation der Alkoholabhängigkeit mit einer reduzierten dorsostriatalen D2/3 Rezeptorverfügbarkeit zeigen.

Stichworte: Alkoholabhängigkeit, Dopamin, sensomotorisches Striatum, assoziatives Striatum, ^{18}F-fallypride, DR2/3 Verfügbarkeit, Craving Symptome.

Introduction

Alcohol addiction and alcohol dependence are used as functional equivalents based on the definition in the *Diagnostic and Statistical Manual of Mental Disorders*, 4th Edition (American Psychiatric Association, 2000). In this study DSM IV criteria were used to diagnose and include alcohol-dependent (AD) subjects in the sample because the study concept was outlined before the DSM 5 was published. Although the DSM 5 is the current version of the manual, we decided to use DSM IV throughout the text to have a consistent definition for alcohol dependence in the entire work (American Psychiatric Association, 2013, 2000).

Alcohol and ethanol are used as synonyms for better readability. Moreover, LS and ventral striatum are used as functional equivalents. Further, the dorsal striatum is used as the functional equivalent of the sensorimotor striatum (SMS) and associative striatum (AS) as they both form the dorsal striatum.

Alcohol dependence

Definition.

Alcohol dependence (AD) and alcohol abuse are subsumed under the term alcohol use disorder (AUD) in the 4th edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) (American Psychiatric Association, 2000). In DSM-IV AD or rather substance dependence in general is defined as:

> *"…a maladaptive pattern of substance use, leading to clinically significant impairment or distress, as manifested by three (or more) of the following, occurring at any time in the same 12-month period:*
>
> 1. *tolerance, as defined by either of the following:*
>   a. *a need for markedly increased amounts of the substance to achieve intoxication or desired effect*
>   b. *markedly diminished effect with continued use of the same amount of the substance*
>
> 2. *withdrawal, as manifested by either of the following:*
>   a. *the characteristic withdrawal syndrome for the substance*
>   b. *the same (or a closely related) substance is taken to relieve or avoid withdrawal symptoms*

3. *the substance is often taken in larger amounts or over a longer period than was intended*

4. *there is a persistent desire or unsuccessful efforts to cut down or control substance use*

5. *a great deal of time is spent in activities necessary to obtain the substance (e.g., visiting multiple doctors or driving long distances), use the substance (e.g., chain-smoking), or recover from its effects*

6. *important social, occupational, or recreational activities are given up or reduced because of substance use*

7. *the substance 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 the substance (e.g., current cocaine use despite recognition of cocaine-induced depression, or continued drinking despite recognition that an ulcer was made worse by alcohol consumption)"*

(American Psychiatric Association, 2000)

Another influential diagnostic classification is the 10th version of the International Classification of Diseases (ICD-10) published by the WHO (World Health Organization, 2016). The WHO uses the terms "harmful alcohol use" and "alcohol dependence syndrome". Harmful alcohol use is defined here as:

> *"A pattern of psychoactive substance use that is causing damage to health. (…)"*

The definition of "alcohol dependence syndrome" is:

> *"A cluster of behavioral, cognitive, and physiological phenomena that develop after repeated substance use and that typically include a strong desire to take the drug, difficulties in controlling its use, persisting in its use despite harmful consequences, a higher priority given to drug use than to other activities and obligations, increased tolerance, and sometimes a physical withdrawal state. (…)"*
>
> *(World Health Organization, 2016)*

These definitions show that AUD covers a spectrum of diseases and that the boundaries between moderate alcohol consumption, risky alcohol use and alcohol dependence are fluid (Saitz, 2005). For a graphic representation of the spectrum of alcohol use and abuse see *Figure 1*. To reflect different levels of alcohol consumption in our study design, we used three reference groups: subjects with low risk (LR) of developing an AD, individuals at high risk (HR) and abstinent subjects with a diagnosed

AD. LR and HR were classified with the Alcohol Use Disorder Identification Test (AUDIT) (Bush et al., 1998; Saunders et al., 1993).

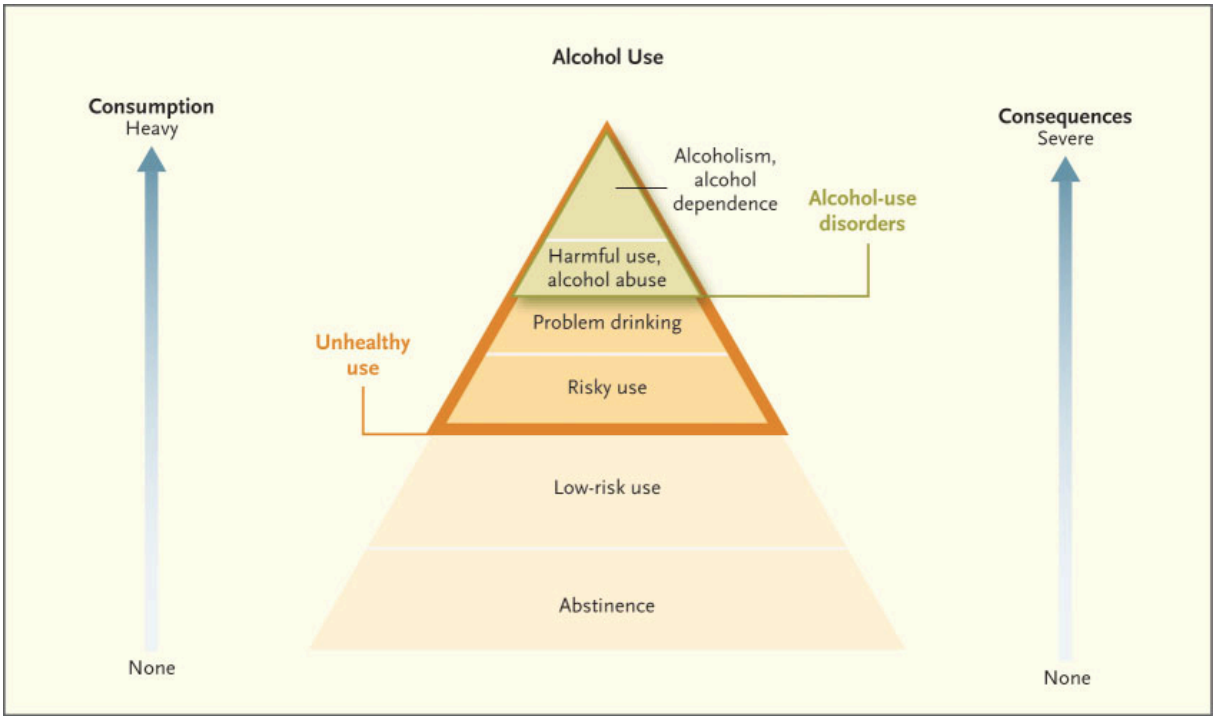

Figure 1. The spectrum of alcohol use, adapted from Saitz (2005).

Epidemiology.

Alcohol abuse is highly common and leads to severe physical and mental health impairments in the consumer (World Health Organisation, 2014). The economic as well as social consequences for society due to health care, prevention and indirectly caused damages are enormous. According to the WHO, 3.3 million people worldwide are killed by alcohol consumption and its consequences every year (World Health Organisation, 2014).

The damages of alcohol on different organ systems and on mental health are well known and well documented in numerous clinical studies (Singer and Batra, 2011). Nearly all organs are affected, and many new findings prove the carcinogenic effect of alcohol intake (Seitz and Müller, 2011; Singer and Batra, 2011). Moreover, Lim et al. showed in 2012 that regular alcohol consumption is one of the major health risk factors (Lim et al., 2012).

Additionally, the WHO's "Global Burden of Disease Study" showed that in Germany alcohol consumption (in men) is the 5th major risk for disease pathogenesis in general (Plass et al., 2014). Furthermore, in Germany 20% of the risk for all diseases is precipitated by alcohol and nicotine intake (Mann et al., 2016).

In Germany the per capita alcohol consumption adds up to just under 10 liters of pure ethanol per year (Batra et al., 2016; Gaertner et al., 2015). This very high level of alcohol consumption has remained relatively constant for several years (Gaertner et al., 2015). 14% of the adult German population (men and women, aged 19 to 64 years) or 7.4 million people are affected by hazardous alcohol consumption (Batra et al., 2016). Further, it is assumed that about 3.1% of the population match the diagnostic criteria of the ICD-10 for harmful alcohol use, while another 3.4% meet the criteria for AD (Batra et al., 2016; Pabst et al., 2013).

The economic impact of alcohol intake and its consequences is immense: at least 30 billion euro are spent each year in Germany (Effertz and Mann, 2013). Hence, AD in Germany – and as well in Europe – is the leading expense factor in the mental health system (Effertz and Mann, 2013).

In summary, this data on AD and its effects show the major importance of research which furthers the understanding of alcoholism and its pathogenesis.

Withdrawal.

Withdrawal – along with tolerance – is a pharmacological criterion for AD, as they are the results of an adjustment process resulting from alcohol use. As with tolerance, withdrawal symptoms vary in regard to quality and quantity, depending on the abused substance. Alcohol is associated with strong withdrawal symptoms in AD or risky consumers (Victor and Adams, 1953). Withdrawal is described in DSM-5 as follows:

> *"Withdrawal is a syndrome that occurs when blood or tissue concentrations of a substance decline in an individual who had maintained prolonged heavy use of the substance. After developing withdrawal symptoms, the individual is likely to consume the substance to relieve the symptoms. Withdrawal symptoms vary greatly across the classes of substances, and separate criteria sets for withdrawal are provided for the drug classes. Marked and generally easily measured physiological signs of withdrawal are common with alcohol, opioids, and sedatives, hypnotics, and anxiolytics. (…)"*
>
> *(American Psychiatric Association, 2013)*

Withdrawal symptoms vary greatly in subjects. Some people describe only harmless symptoms such as a headache and discomfort whereas others may suffer from severe delirium tremens, which might lead to death when not recognized or treated correctly.

There are mild symptoms that are colloquially termed “hangover”. These symptoms normally occur in subjects with moderate alcohol intake. Symptoms range from headache, nausea, diarrhea, feeling weak, tiredness or being impaired in cognitive, visual or spatial skill performance (Wiese et al., 2000).

Moreover, there are early symptoms that usually occur within 48 hours. These might be a hand tremor, different kinds of hallucinations or illusions (visual, auditory, tactile) or generalized tonic-clonic seizures (American Psychiatric Association, 2013; Brust, 2014). Late withdrawal symptoms can be a possible episode of delirium tremens, autonomic hyperactivity, psychomotor agitation, anxiety or insomnia (American Psychiatric Association, 2013; Brust, 2014).

Due to the large variety of symptoms, alcohol withdrawal should in general be medically supervised and, if necessary, pharmacologically treated. Benzodiazepines are the main treatment option for alcohol withdrawal, but there are also other substances such as clomethiazole, which is not available in the United States but very commonly used in Europe (Bonnet et al., 2011). There are also other pharmaceuticals such as phenobarbital, anticonvulsants, baclofen, Gamma-hydroxybutyric acid (GHB), neuroleptics, beta-blockers and alpha-2-agonists, ethanol and N-methyl-d-aspartate (NMDA) receptor blockers, which can be used as second or third line treatment options (Brust, 2014).

Craving symptoms.

Craving is a term used to describe a certain symptom in substance misuse or dependence. There has been a long discussion and dispute about the term and its definition and utility (Drummond et al., 2000; Kozlowski and Wilkinson, 1987; Pickens and Johanson, 1992). In 1991 different international experts discussed the topic at the Addiction Research Center (ARC) of the National Institute on Drug Abuse in the US. They agreed on the importance of the topic and its role in understanding drug dependence but pointed out that there was still a lack of knowledge and further research to do (Pickens and Johanson, 1992). Hence, additional studies followed and examined the theory of craving and the clinical relevance of craving as a major symptom in addiction (Addolorato et al., 2005; Drummond, 2001; Ferguson and Shiffman, 2008).

The definitions of craving are very diverse, but most researchers agree on the assumption that craving is “*a subjective experience of wanting to use a drug”*

(Drummond, 2001; Tiffany et al., 2009). According to Tiffany and Wray (2012), this definition has three elements: *craving is conscious, craving is best captured by an expression of desire, and that desire is directed toward the use of a specific drug.* Since the 5th edition of DSM, craving has been part of the diagnostic criteria for AUD and is described as follows:

> *"Craving is manifested by an intense desire or urge for the drug that may occur at any time but is more likely when in an environment where the drug previously was obtained or used. Craving has also been shown to involve classical conditioning and is associated with activation of specific reward structures in the brain. Craving is queried by asking if there has ever been a time when they had such strong urges to take the drug that they could not think of anything else. Current craving is often used as a treatment outcome measure because it may be a signal of impending relapse."*
>
> *(American Psychiatric Association, 2013)*

The assessment of craving symptoms also varies. For instance, there are questionnaires that contain one item in regard to craving as in the Substance Dependence Severity Scale (SDSS) and on the other hand, there are questionnaires that assess craving as a score out of multiple items as in the Obsessive Compulsive Drinking Scale (OCDS) discussed *in Obsessive Compulsive Drinking Scale (OCDS)*. (Miele et al., 2000). Most questionnaires or questions relating to craving are self-rating questions. Additionally, self-rated craving might also be correlated with corresponding regional brain activation (Brody et al., 2002; Grant et al., 1996; McClernon et al., 2005).

Previous studies have shown a potential link between craving and striatal dopamine receptor density and synthesis capacity (Heinz et al., 2004b, 2005). The clinical importance of craving is its possible role in predicting relapse risk and clinical outcome (Heinz et al., 2010; Schneekloth et al., 2012; Sinha, 2013). The aim of this study was, among other things, to assess the neurobiological correlates of craving and their possible role in relapse prediction.

Abstinence and relapse.

Abstinence is in most cases the aim of the treatment of AD, although, "controlled drinking" might also be considered as an optional treatment goal. The debate about "controlled drinking" has been ongoing for a long time and is still very relevant (Carroll, 1978; Mann et al., 2016). Today "controlled drinking" is being seen more and more as

a second option in the treatment of AD, for example, when the patient refuses abstinence. The new English therapy guidelines from 2011 came to the conclusion that "controlled drinking" should be accepted as an intermediate treatment goal, which led to intense discussions internationally (*NICE. Alcohol-use disorders: diagnosis, assessment and management of harmful drinking and alcohol dependence.*, 2011). The European Medicines Agency (EMA) also agreed with this statement (2011) as did the "Deutsche Gesellschaft für Psychiatrie, Psychotherapie, Psychosomatik und Nervenheilkunde (DGPPN)", a subgroup of the "Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften e.V. (AWMF)" (*EMA (European Medicines Agency). Annual report 2010*, 2011; Mann et al., 2016).

The reason for this acceptance may be the hope that more people with alcohol dependence or harmful use might consider counselling or medical treatment if the reduction of the alcohol intake will be an accepted treatment goal. In the US nearly half of the people with AD in need of treatment reported that they were not willing to cut out drinking completely, which indicates that this hope is realistic (*Substance Abuse and Mental Health Services Administration (SAMHSA). Results from the 2005 National Survey of Drug Use and Health: National Findings.*, 2006). Yet, perhaps "controlled drinking" should not be offered as a second therapeutic option because abstinence seems to be the safest and most effective form of treatment (Batra et al., 2016). In summary, it can be stated that abstinence should be the treatment goal in AD, whereas "controlled drinking" might be an important intermediate step.

Unfortunately, in AD the relapse rate in reformed alcoholics remains at a very high level. Up to 85% of AD patients undergoing detoxification suffer from relapse when they do not receive further treatment such as psychotherapy or psychopharmacotherapy (Boothby and Doering, 2005; Walter et al., 2015). The question how to possibly decrease the relapse rate and how to improve treatment options might be the most important research question in regard to AD.

Relapse to heavy drinking is defined as the alcohol intake of more than 60g of alcohol per occasion in men and more than 48g of alcohol in women after a period of abstinence. The predictors of relapse are a subject of controversial discussion and of great clinical importance. Therefore, one important long-term goal of this study is to contribute to the improvement of relapse prediction.

Pathogenesis.

The exact pathogenesis of AD is not known. It is assumed that a multidimensional development and complex interplay of social, psychological and genetic influences as well as cognitive functioning and reward-dependent learning contribute to the development of AD.

Social factors may be environmental influences such as family situation, parenting practices, educational style, friends and peer groups – especially in young adults (Andrews et al., 2002; Rose et al., 2001; Sher et al., 2005).

Additionally, there are psychological influences such as special personality traits that might be associated with AD. For example, neuroticism, disinhibition and extraversion each seem to have a connection with the development of AD (Sher et al., 2005). Moreover, there are other personality traits which have been found to contribute to an early onset of alcohol consumption, such as extraversion, impulsivity, sensation seeking, and novelty seeking (Nees et al., 2012).

Genetic influences also play a major role in the development of AD. It is estimated that around 50% of the vulnerabilities related to AD are associated with genetic factors (Prescott and Kendler, 1999; Schuckit, 2009). For instance, there are many different genes that may be associated with an increased risk of developing an AD (Goldman et al., 2005). In genome-wide association studies (GWAS), twin studies, linkage studies and candidate gene studies, several potential genetic predisposing factors have been identified (Tawa et al., 2016). The complexity and variance of the genetic factors involved in AD are linked to the concept of intermediate phenotypes (Heinz et al., 2007; Schumann, 2007). Intermediate phenotypes or endophenotypes are defined as intermediate between the observable disorder and its potential biological cause (Schumann, 2007). One example of an intermediate phenotype is the potentially reduced DR sensitivity in AD, which may be modulated through the dopaminergic genes COMTVal58Met and DRD2Taq1A (Schellekens et al., 2012).

Another approach to understanding the pathogenesis of AD takes into account cognitive disorders or dysfunctions, which may also contribute to the development of the disorder. Especially executive dysfunctions might play an important role in the development of addiction and in particular unhealthy alcohol consumption (Corral et al., 2003; Goldstein and Volkow, 2011; Sher et al., 2005). Executive functions are an umbrella term for different cognitive processes such as cognitive inhibition, cognitive flexibility and working memory, and higher order executive functions such as planning,

multi-tasking and fluid intelligence (Chan et al., 2008). Thus, executive dysfunctions may represent an impairment of cognitive control, which seems to be evident in addiction. The brain regions involved in executive functions may be localized in frontal and specifically in prefrontal cortical areas and are potentially impaired in addiction (Goldstein and Volkow, 2011; Volkow and Fowler, 2000).

Moreover, there seems to be a connection between AD and reward-dependent learning, as drugs of abuse seem to increase DA in reward circuits and thus may contribute to its rewarding effects. Hence, a focus of research in this domain is dopaminergic transmission in the midbrain and the basal ganglia, which form a substantial part of the reward system (Di Chiara and Imperato, 1988; Everitt et al., 2001; Wise, 1996). These reward circuits will be discussed further in *Dopaminergic pathways and the reward system.*

All in all, due to the various social, psychological, genetic as well as cognitive factors and their mutual influence, the pathogenesis of alcoholism can be best understood through a multidimensional approach.

Neurobiology.

The numerous neurobiological factors and their interactions in the development of AD are as complex as the underlying neurochemical processes involved in the pathogenesis discussed above. Alcohol directly and indirectly affects many neurotransmitter systems, such as the GABAergic, glutamatergic, serotonergic, opioid and dopaminergic systems (Heinz et al., 2009). It is important to differentiate between the acute effects of ethanol and the results of chronic ethanol intake. In the following, I will predominantly focus on chronic alcohol consumption, as I want to elucidate the underlying neurobiological processes in the disease development of AD.

Gamma-aminobutyric acid.

Alcohol acts as a positive allosteric modulator of the $GABA_A$ receptor (Gamma-aminobutyric acid type A receptor) in the central nervous system (CNS), leading to an opening of chloride channels and hence to a hyperpolarization of the cell (Malenka et al., 2009a). This hyperpolarization reduces the firing rate of the neuron and thus reduces its activity.

Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter, which is

reinforced by alcohol intake mainly through $GABA_A$ receptors. Acute consumption induces the activation of GABA receptors, whereas chronic alcohol intake may lead to a homeostatic downregulation of the $GABA_A$ receptors (Krystal et al., 2006). Thus, in abstinence - after chronic ethanol consumption – the GABAergic system seems to be noticeably impaired (Grobin et al., 1998).

Glutamatergic modulation.

Ethanol modulates ionotropic glutamate receptors, such as the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA-R), the kainate receptor (KAR) and N-methyl-D-aspartate (NMDA) receptor. It is a negative allosteric modulator of these receptors and hence modulates the mainly excitatory acting transmitter glutamate. The effects of ethanol on the $GABA_A$ and the NMDA receptors can be an explanation for the sedating effects of alcohol because the inhibitory transmitter GABA is activated and the excitatory neurotransmitter glutamate is inhibited (Grobin et al., 1998; Spanagel, 2003).

Chronic alcohol consumption leads to a prolonged inhibition of NMDA receptors and thus to a potentially compensatory upregulation of this receptor subtype (Bachteler and Spanagel, 2005; Chastain, 2006; Spanagel, 2003). Moreover, the functionality of the NMDA receptor seems to be optimized through specific structural changes within the receptor, leading to a possibly reinforced sensitivity of the receptor (Spanagel, 2003). If – during withdrawal – alcohol intake is stopped, the activity of those postsynaptic neurons increases and can in the worst-case lead to glutamate-induced excitotoxicity (Tsai et al., 1995). Excitotoxicity means neuronal damage, or rather neurodegenerative processes caused by excessive receptor stimulation (Tsai et al., 1995). Some of the clinical effects of alcoholism, such as withdrawal seizures and delirium tremens, may be consequences of the impaired glutamatergic system in AD (Spanagel, 2003).

Serotonergic impairments.

Subsequently, the serotonergic transmitter system is also mediated by ethanol intake. Ethanol acts as a 5-hydroxytryptamine type 3 receptor ($5\text{-}HT_3$-R) agonist, reinforcing the regulatory monoamine serotonin, also known as 5-hydroxy-tryptamine (5-HT). Serotonin, just like other monoamines, mediates the excitatory (e.g. glutamate)

and inhibitory (e.g. GABA) neurotransmitter systems and thus interacts with cortical information processing (Heinz et al., 2004a). There seems to be an association between high alcohol preference and low endogenous 5-HT functioning (Nishikawa et al., 2009). Furthermore, chronic alcohol consumption may be associated with lowered availability of serotonin transporters (SERT), especially in the brain stem (Heinz et al., 1998).

Moreover, serotonergic impairments seem to be associated with impulsive aggression, negative mood states and lowered response to alcohol intake, which are behavior patterns that may be important for the development and maintenance of AD (Heinz et al., 2001).

All in all, serotonergic impairments play a major role in the development and maintenance of AD and may also indirectly influence GABAergic and glutamatergic neurotransmitter systems. The modulation of serotonergic neurotransmission and its role in AD is complex. It is not yet fully understood, and research so far has produced inconsistent results.

Endogenous opioid system.

The endogenous opioid system is also affected by ethanol intake, leading to increased opioid activity levels (Naber et al., 1981). Alcohol intake leads to the release of beta endorphins, which is conversely linked to the reinforcement of consumption and contributes to dependence (Racz et al., 2008). These alterations in the opioid system due to chronic alcohol consumption are closely linked with the dopaminergic system (Benjamin et al., 1993; Cowen and Lawrence, 1999; Herz, 1997; Koob, 2014).

Furthermore, endogenous opioid release is indirectly influenced by the glutamatergic changes in AD through unidentified mechanisms in the basal ganglia (Malenka et al., 2009a; Möykkynen and Korpi, 2012).

Interactions between glutamate, GABA and dopamine.

Several intoxication and withdrawal symptoms in AD may be explained through the relation between the reduced activity of the NMDA receptor system and the higher activity of the GABAergic receptors in alcohol withdrawal (Schuckit, 2016). Glutamate may subsequently modulate mesolimbic DA release via GABAergic interneurons in AD (Carlsson et al., 1999; Kalivas et al., 2005). Furthermore, Gleich et al. (2015) observed

a negative correlation between frontal glutamate concentration and striatal dopamine synthesis capacity, as well as between striatal glutamate concentration and striatal DA synthesis capacity, leading to the hypothesis of a potential regulatory mechanism of the dopaminergic system through glutamate (Gleich et al., 2015).

Previous animal experiments have shown that prefrontal NMDA receptor blockade leads to an increasing striatal dopamine release (Del Arco et al., 2008; Usun et al., 2013). After withdrawal there might be an increased glutamate concentration in AD because of the omission of alcohol induced NMDA receptor blockade (Kalivas et al., 2005; Spanagel, 2003). This may affect the regulation of the dopaminergic system, which might then result in a potential reinforcement of drug intake (Floresco et al., 2001). A pharmaceutical normalization of elevated glutamate levels with the pharmaceutical acamprosate in AD after detoxification reduces craving symptoms and may thus decrease alcohol intake as Spanagel and others have shown in mice (Sass et al., 1996; Spanagel et al., 2005).

All in all, this shows that the neurochemical interactions and adaptations resulting from alcohol consumption are very complex. We will now focus on DA and its coherences with reward-dependent learning as well as with its role in the development and maintenance of addiction.

Dopamine and alcohol dependence

Alcohol consumption leads to an increased DA release especially in the LS (Di Chiara, 1997). This altered DA release contributes to the rewarding effects of alcohol intake and may provoke reinforcement of the drug intake (Boileau et al., 2003; Di Chiara, 1997; Schultz et al., 1997). Higher levels of striatal DA are of great significance for craving symptoms and relapse prediction (Heinz et al., 2005, 2004b; Schuckit, 2016). In previous AD studies, researchers have been able to show a reduced DR2 availability in the striatum of alcoholics compared to that of healthy controls (Heinz et al., 2004b; Volkow et al., 1996). This finding may be interpreted as a compensatory downregulation of DR due to chronic alcohol intake and thus chronic altered DA release. In the following paragraphs the dopaminergic neurotransmission and its impairments following chronic alcohol consumption will be further discussed.

The dopaminergic system.

Dopamine.

Dopamine (DA, contracted from 3,4-dihydroxyphenethylamine) is an amine synthesized from its precursor chemical L-DOPA, which is produced in the brain as well as in the kidneys. It has several different important functions in the brain and body of the human organism. In the central nervous system (CNS) it functions as a neurotransmitter, which means it is a chemical released by neurons. It is stored in the presynaptic terminal of the neuron within vesicles and released in the synaptic cleft via exocytosis. In the synapse it can be either resumed via dopamine transporters (DAT) or bind to a cell surface dopamine receptor on the postsynaptic terminal of the neuron. There are five different receptor subtypes, which can be divided in two groups, the D1-like receptors (DR1/5) and the D2-like receptors (DR2-4). In the CNS the D1-like receptors lead to a G_s-protein-coupled activation of adenylate cyclase and thus to an increased intracellular level of the second messenger cyclic adenosine monophosphate (cAMP). In this case, the effect on the neuron can be either an activation of the cell through the opening of sodium channels or an inhibition via opening potassium channels. On the other hand, the D2-like receptors lead through a G_i-protein-coupled inhibition of adenylate cyclase to a decreased intracellular level of cAMP. Additionally, there are also dopamine autoreceptors on the presynaptic terminal. Those can be D2 or D3 receptor subtypes, which are responsible for a negative feedback mechanism (Beaulieu and Gainetdinov, 2011). For an overview of a dopaminergic synapse and the receptor types, see *Figure 2*.

Thus, DA may lead to an activation or inhibition of the neuron, depending on the receptor it binds to and the response of the neuron due to the second messenger system (Romanelli et al., 2009). D1 receptors are the most frequent receptor subtype, followed by D2 receptors and then – significantly lower – D3 to D5 receptors (Romanelli et al., 2009). The distribution and the density of the DR vary greatly between different brain regions in the CNS. The striatum is one of the DR-richest regions, whereas white matter and cerebellar tissue have a low availability of DR (Rice et al., 2011).

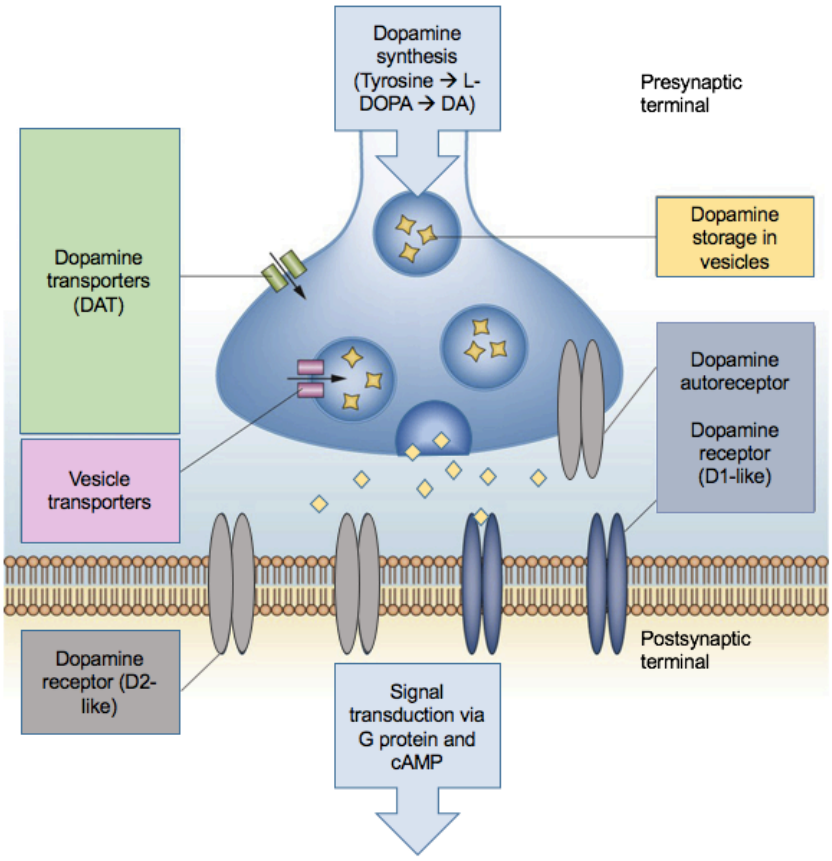

Figure 2. Dopaminergic synapse, adapted from Politis (2014).

Dopaminergic pathways and the reward system.

There are various DA pathways in the brain which play an important role in the extrapyramidal motor system, the hormone system, executive control, arousal, reinforcement, motivation and the reward system. The four major dopaminergic pathways are shown as a schematic structure in *Figure 3.*

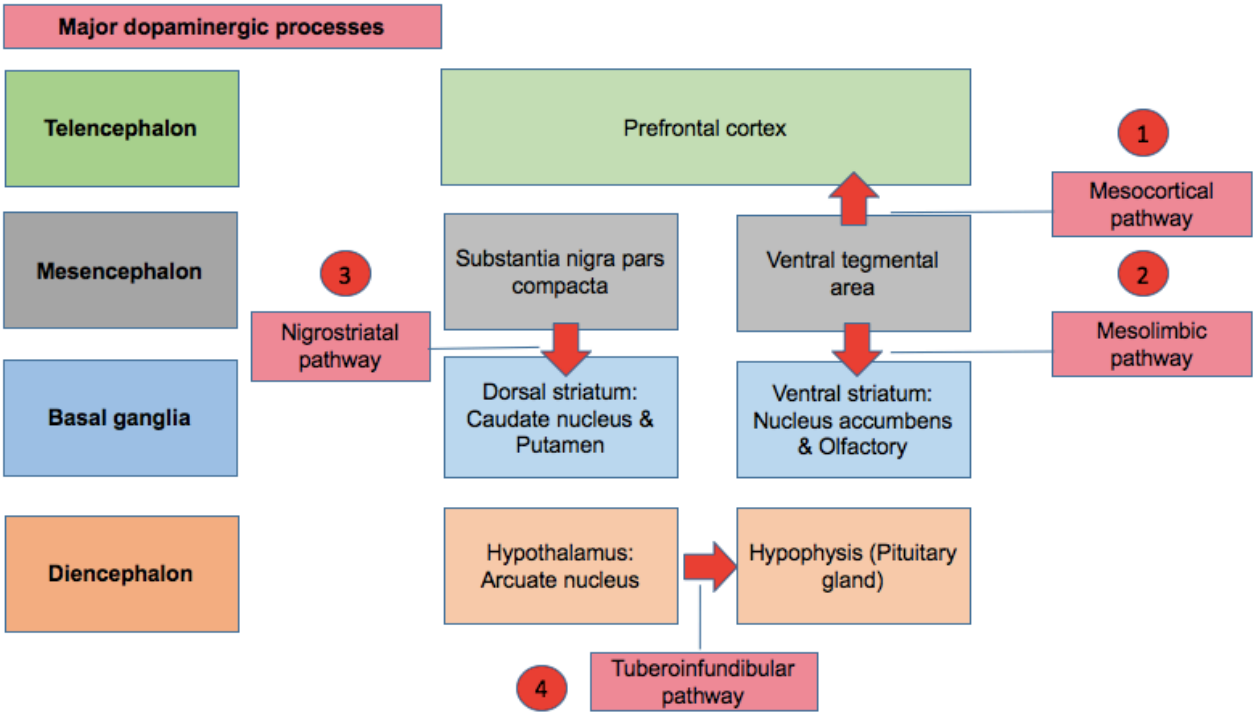

Figure 3. Major dopaminergic pathways (Doyon et al., 2013; Ikemoto, 2010; Malenka et al., 2009b, 2009c; Moal and Simon, 1991).

The diverse roles and tasks of DA in the CNS include its role as a major transmitter of the extrapyramidal motor system (nigrostriatal pathway), the hormonal system (tuberoinfundibular pathway) and its role in regulating arousal and executive control and functioning (mesocortical pathway) (Malenka et al., 2009b). Moreover, DA plays a major role in reward- and aversion-dependent cognition and associative learning (mesolimbic and nigrostriatal pathways (2), (3), see *Figure 3*) (Ikemoto, 2010; Malenka et al., 2009c). These pathways play a key role for reward-dependent learning and thus for understanding the development and maintenance of addiction.

If an activity or behavior is rewarded, it will consequently be reinforced (Olds, 1977; Skinner, 1938). In the paradigm of Skinner, reinforcement is defined as a higher potential for a specific response to a certain behavior. Reward in this case can be defined as a reinforcement, which then increases or decreases the possible behavior. This is part of the concept of operant or instrumental conditioning.

Another approach is the reinforcement paradigm of Pavlov, which was based on his research with dogs (Pavlov, 1927). He introduced the term reinforcement in the context of classical conditioning. Classical conditioning means that a certain cue triggers a specific behavior. In Pavlov's case a ringing bell led to a dog's salivation, because the dog learned beforehand that it would get food when the bell was ringing. Interestingly, both paradigms complementarily help to understand the concept of drug

reward. If related to certain cues, e.g. images or the smell of alcoholic beverages, this may result in clinical symptoms such as craving or drug-seeking behavior analogous to the salivary flow in the dogs of Pavlov's research.

In diverse previous research work it has been suggested that DA might be a neuronal correlate of reward. In 1993 Robinson and Berridge pointed out that the sensitization of the DA system due to learning processes causes incentive salience, which means that a stimulus becomes more attractive. This incentive salience might result in the craving for a substance and hence a possible reinforcement of drug intake (Robinson and Berridge, 1993). Schultz et al. observed in 1997 that the neurobiological correlate of reward may at least partly fulfilled by striatal DA release, depending on the prediction of reward or punishment (Schultz et al., 1997; Wise and Rompre, 1989). The correlate of this DA release is short phasic bursts of dopamine activation, which were shown by Schultz et al. (1997) in rodents. The release is highest when not predicted and there may also be a DA release when the behavior is not followed by a reward (Schultz et al., 1997; Wise and Rompre, 1989). Hence, DA plays a key role in reinforcing behavior and motivational learning (Wise, 2004). Natural reinforcers such as food and others such as substances of abuse or monetary rewards may lead to a subsequently increased DA release and hence to a rewarding effect – neurochemically and behaviorally (Di Chiara, 1997; Schultz, 2015).

The striatum: major part of dopaminergic pathways.

The striatum is an anatomical structure in the forebrain which forms part of the basal ganglia. Functionally, the striatum is a main part of the motor and the reward system. It is a formation of nuclei which can be subdivided in different ways, leading to different classification systems. On the one hand, there are anatomic approaches that classify the different nuclei and their exact position, and on the other hand there are functional approaches that are based on functional entities observed in previous research.

Anatomically, the striatum consists of the nucleus accumbens, the olfactory tubercle, the caudate and the putamen. All the nuclei are located in the forebrain, localized next to the frontal horn of the lateral ventricle and lateral of the thalamus. The nucleus accumbens can additionally be subdivided into a core and a shell.

Moreover, the striatum can be divided in a ventral striatum and a dorsal striatum. This subdivision is controversial among researchers, and different research groups

have proposed alternative subdivisions. In this study, the nomenclature and definitions of Martinez et al. (2003) and Mawlawi et al. (2001) were used. Furthermore, the striatum can be functionally subdivided into a sensorimotor, associative and limbic part. In these functional compartments it forms part of the nigrostriatal and mesolimbic dopaminergic pathways discussed above. The detailed regions of interest which we investigated will be discussed in the Methods section (see *Regions of interest*).

The striatum plays a major role in the reward system and hence in the concept of reinforcement, which explains its importance for addiction. Numerous previous clinical studies have shown the connection between AD, the striatum and alterations in the dopaminergic neurotransmitter system (Beck et al., 2009; Boileau et al., 2003; Deserno et al., 2015; Di Chiara, 1997; Di Chiara and Bassareo, 2007; Heinz et al., 2005, 2004b; Rominger et al., 2012). Striatal dysfunctions play not only a major role in addictive disorders but are also of great importance for other psychiatric diseases such as schizophrenia (Heinz, 2002). Additionally, other psychiatric diseases such as attention-deficit/ hyperactivity disorder (ADHD) or bipolar affective disorders might be associated striatal dysfunctions (Ashok et al., 2017; Biederman, 2005; Chamberlain et al., 2011; Del Campo et al., 2011). Thus, the striatum and its subdivisions are the targeted brain regions we will examine in this work.

Measurement methods of dopamine receptor availability.

Measuring striatal as well as extrastriatal dopamine requires a neuroimaging technique that allows a functional approach on molecular levels. Positron emission tomography (PET) as well as single-photon emission tomography (SPECT) are relatively new nuclear functional imaging techniques that enable researchers to investigate the dopaminergic neurotransmission. With either of them, it is possible to examine presynaptic dopamine activity (including dopamine transporters, vesicle transporters and dopamine storage) as well as the postsynaptic dopaminergic system (DR2/3) (Politis, 2014).

To acquire data, it is necessary to apply a radiolabeled molecule intravenously. This so-called radiotracer allows the exploration of physiological and biochemical functions of the body or the brain. More precisely, PET allows a molecular functional illustration of specific physiological processes, depending on the substance used. An overview of the potential imaging methods for dopamine and the respective radiotracers that can potentially be used in research is shown in *Figure 4.*

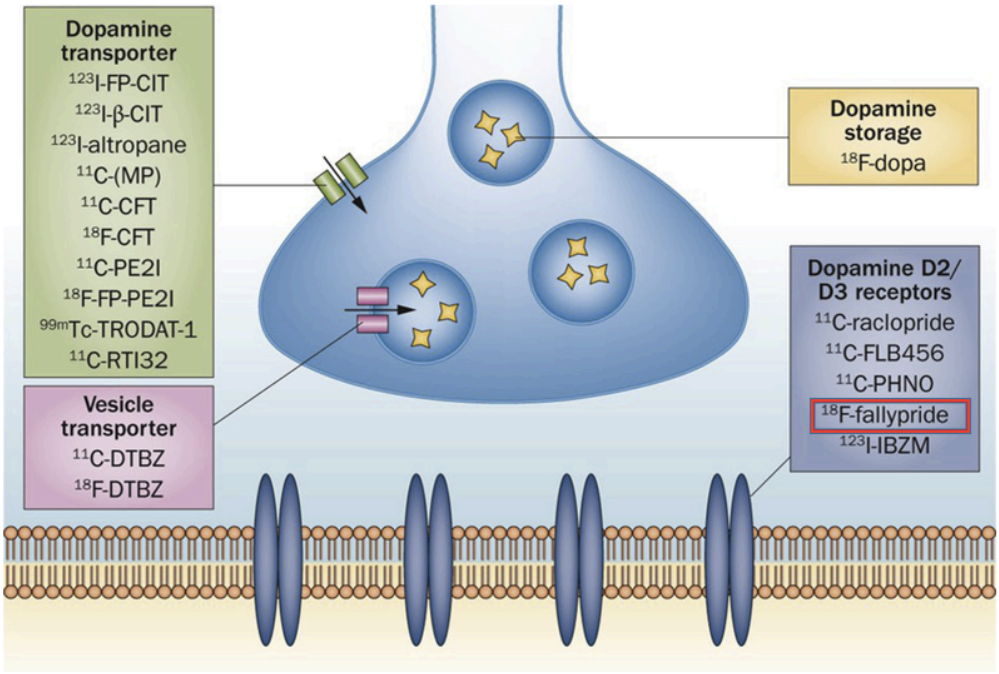

Figure 4. Radiotracers in dopaminergic neuroimaging, reproduced and adapted from Politis (2014).

To be able to perform a PET scan, a radionuclide is injected intravenously into the subject. In the body, the radiotracer emits positrons, which indirectly emit gamma rays. These gamma rays can then be detected by the scanner. The detection and localization of this radioactivity enables a three-dimensional image reconstruction via computational analysis. Typically, a CT scanner is used to contribute structural data to perform this reconstruction. This CT scanner is usually located within the same machine (PET-CT).

In this study our aim was to examine the postsynaptic DR availability of DR2/3 in subregions of the striatum and to investigate the differences between LR and HR controls and AD subjects.

There are different approaches to investigating the postsynaptic DR2/3 status. As shown in *Figure 4,* there are several specific radiotracers that can be used in PET or SPECT measurement for exploring postsynaptic dopamine receptors, such as ^{123}I-epidepride, ^{123}I-IBZM or ^{11}C-PHNO (Erritzoe et al., 2014; Guardia et al., 2000; Plisson et al., 2012; Repo, 1999; Searle et al., 2010; Verhoeff et al., 1993). Another possibility is the amphetamine paradigm, where an examination of the D2/3 receptors with a PET-CT is performed after the in vivo application of amphetamine. This has already been done in clinical studies on alcohol consumption and AD (Martinez et al., 2005; Munro et al., 2006). Other radiotracers which are frequently used in addiction research are ^{11}C-raclopride, a selective D2 receptor antagonist and ^{18}F-fallypride, a high affinity

DR2/3 antagonist. Both can be used in medical research in regard on dopaminergic dysfunctions (Antonini et al., 1996; Farde et al., 1997; Köhler et al., 1985; Mukherjee et al., 1995; Volkow et al., 1996).

^{18}F-fallypride is a high affine DR2/3 ligand measuring striatal as well as extrastriatal DR2/3 availability in the CNS (Mukherjee et al., 1995; Slifstein et al., 2004). It has an affinity similar to that of striatal and extrastriatal DR2/3 and enables a quantitative measurement of the whole brain in one session (Slifstein et al., 2004).

Slifstein et al. (2004) showed that the in vivo affinity of ^{18}F-fallypride for DR2 is lower than its in vitro affinity and also, that there is a similar affinity for the receptors in extrastriatal and striatal brain regions of nonhuman primates (Slifstein et al., 2004). These results suggest that this tracer is not affected very much by regional differences of endogenous DA and thus might be a good tool for investigating regional DR density (Slifstein et al., 2004). This is why we decided to use ^{18}F-fallypride as radiotracer in this study.

^{18}F-fallypride is a radiotracer that is frequently used in research on addiction such as AD, methamphetamine use disorders, cocaine use disorders and nicotine dependence (Lee et al., 2009; Morales et al., 2015; Okita et al., 2016; Robertson et al., 2016; Ballard et al., 2015; Fotros et al., 2013; Fehr et al., 2008).

Dopamine system and alcohol dependence.

The acute consumption of alcohol leads to an increased DA release especially in the striatum (Di Chiara, 1997). This altered DA release contributes to the rewarding effects of ethanol and may provoke reinforcement of the drug intake analogous to the concepts discussed above (Boileau et al., 2003; Di Chiara, 1997; Schultz et al., 1997). In AD, alcohol consumption is continued in spite of negative consequences, which may lead to the assumption that the reward system is impaired by chronic alcohol intake. These alterations consist in a bias towards alcohol and alcohol-related stimuli as opposed to other rewards (Hyman, 2005; Wrase et al., 2007). Neurobiologically, substance-related cues induce higher striatal activation in AD, than conditioned stimuli such as natural or monetary rewards, which induce lower striatal activation (Carelli et al., 2000).

Volkow et al. (2017) reviewed PET studies with regard to acute as well as chronic effects of alcohol on dopaminergic neurotransmission. They concluded that the acute drug effects of the altered DA release, measured via amphetamine application,

had been successfully been investigated in PET studies, whereas the PET studies had yielded only inconsistent results for the chronic effects of alcohol consumption (Boileau et al., 2003; Laruelle et al., 1996; Urban et al., 2010). The clinical PET studies they examined ranged from investigating presynaptic dopamine transporters and dopamine synthesis capacity ([^{18}F]DOPA) to examining of postsynaptic receptors (Heinz et al., 2005, 2004b, 2000; Martinez et al., 2005). Volkow et al. (2017) suggest that chronic alcohol effects have been shown in PET and SPECT studies leading to the common statement that the dopaminergic system seems to be impaired in AD.

Kamp et al. (2018) reviewed a series of PET studies and found that one of the chronic effects of alcohol consumption on the dopaminergic system seems to be a reduced DR availability in AD subjects as compared with healthy subjects whereas DA transporters and DA capacity did not significantly differ in AD and healthy subjects (Kamp et al., 2018).

The first PET study with respect to DR in AD was performed by Volkow et al. (1996), who observed a reduced D2 receptor availability in the striatum of AD compared to nonalcoholics via ^{11}C-raclopride (Volkow et al., 1996). Moreover, Repo et al. (1999) investigated DR2 availability via ^{123}I-epidepride, but did not find significant differences in the receptor levels of AD to HC (Repo et al., 1999). In contrast, Guardia et al. (2000) found higher striatal D2 receptor levels – examined via ^{123}I-IBZM – associated with early relapse in AD subjects. In 2002 Volkow et al. published a^{11}C-raclopride PET study in which reduced DR2 availabilities were observed in the caudate and putamen of AD compared to HC subjects (Volkow et al., 2002b). Heinz et al. (2004) confirmed this finding in another PET study showing lower availability of dopamine receptor 2 and 3 (DR2/ DR3) in the LS (Andreas Heinz, Siessmeier, et al., 2004). In 2014 Erritzoe et al. investigated DR3 availabilities in AD subjects via ^{11}C-PHNO and found the receptor densities to be lowered in the hypothalamus of AD compared to HC (Erritzoe et al., 2014).

Rominger et al. (2011) were the first to use ^{18}F-fallypride to investigate extrastriatal and striatal D2/3R availability in AD (Rominger et al., 2012). They found reductions of the D2/3R availability in extrastriatal brain regions (such as the thalamus and hippocampus) but not in striatal brain regions of AD subjects in comparison to age- and gender-matched controls.

Moreover, Pfeifer et al. (2016) used ^{18}F-fallypride to investigate the D2/3R status in association with acute ethanol intake (Pfeifer et al., 2016). They did not find

acute alterations in the DR2/3 status after alcohol intake and they concluded that alterations might be associated with chronic alcohol intake.

Spreckelmeyer et al. (2011) investigated opiate-induced DA release in AD and healthy controls via ^{18}F-fallypride PET (Spreckelmeyer et al., 2011). The authors observed significantly reduced binding potentials (BP) of ^{18}F-fallypride in the LS in AD and HC, and additionally in the AD subjects, the BP in the LS was positively correlated with the AUDIT score. The LS was defined as the combination of nucleus accumbens, the ventral caudate and the ventral putamen according to the definitions of Mawlawi et al. (2001). We used these definitions of striatal and substriatal regions of interest in our study.

Although the results regarding DR2/3 availability in AD are partly heterogenous, the new meta-analysis of Kamp et al. (2018), which includes 16 in vivo neuroimaging studies, has revealed a significantly lowered DR2/3 availability in AD compared to HC. These impaired DR2/3 receptor densities were observed in the striatum, especially in the caudate as well as in the putamen. To our knowledge the receptor status of individuals at high risk on developing an AD has not yet been examined in any PET study. Thus, it is of great interest to investigate whether the DR2/3 availability of HR subjects lies between that of AD and LR subjects. Therefore, we used this sample with three reference groups representing different levels of alcohol consumption. We aim to elucidate how the receptor status is impaired in subjects with more severe alcohol consumption.

The lowered DR2/3 availability in AD has up to now often been interpreted as an adaptational downregulation process in AD due to an excess of DA release because of a more frequent ethanol consumption. The neuroadaptational processes underlying the development and maintenance of AD involve certain steps and brain regions, as reviewed by Koob and Volkow (2010). The authors argue that with a more automatized drinking pattern the neuronal adaptation mechanisms shift from ventrostriatal to dorsostriatal brain regions. This has been shown in several preclinical studies (Corbit et al., 2012; Haber et al., 2000; Ikemoto, 2007). Thus, both the ventral and the dorsal striatum may be involved in the development and maintenance of AD. Hence, it will be interesting to investigate the DR2/3 availabilities in the dorsal as well as ventral regions of the striatum in subjects with different alcohol consumption patterns as this has not yet been performed in humans.

For this purpose, we used the precise functional subdivisions of the striatum into

a sensorimotor, associative and limbic part along with the definition of Martinez et al. (Martinez et al., 2003; Mawlawi et al., 2001). Further, we wanted to investigate if there is a difference in the striatal location of the potential dopaminergic impairments of individuals at high risk from AD patients compared to the LR controls.

Moreover, the severity of the impairment of the dopaminergic system may be correlated with the extent of the bias of the reward system. (Di Chiara and Bassareo, 2007). This might also contribute to withdrawal symptoms in subjects who remain abstinent after a long time of chronic ethanol consumption. This correlation might thus be an important connection to clinical scales such as craving symptoms and clinical outcome parameters (Volkow et al., 1996; Heinz et al., 2004b; Grüsser et al., 2004; Beck et al., 2009; Wrase et al., 2007). For example, Volkow et al. (2002) observed an association of lowered DR2 availabilities with higher ratings of “drug-wanting” in AD (Volkow et al., 2002a). This finding was supported by the study of Heinz et al. (2004b), who found a correlation of craving symptoms with impaired DR2/3 availabilities in the ventral striatum of AD. Additionally, there may be a potential link between the reduced DR2 sensitivity and availability and the prediction of relapse in AD (Heinz et al., 1996, 1995). Therefore, we would like to link our investigations of the DR availabilities to clinical symptoms such as the severity of symptoms caused by alcohol and especially craving symptoms. To our knowledge this has not yet been investigated in HR subjects. Further, this may help us to potentially link DR availabilities and clinical symptoms such as craving to relapse prediction.

All in all, one new aspect of this work is the representation of alcohol consumption on different levels. This dimensional approach has not yet been considered in any PET study regarding alcohol addiction.

Hypotheses

With regard to the above-mentioned clinical studies and recently published reviews (Heinz et al., 2004b; Kamp et al., 2018; Volkow et al., 2002b, 1996), we assume a reduced striatal availability of D2 and 3 receptors in AD compared to LR controls, which leads to our first hypothesis:

(H1) There is a significant reduction in the binding potential of ^{18}F-fallypride – measuring striatal DR2/3 availability – in AD compared to LR.

Along with the above-mentioned preclinical studies suggesting a shift from ventro to dorsostriatal dopaminergic impairments in a more habituated alcohol consumption (Corbit et al., 2012; Haber et al., 2000; Ikemoto, 2010), we aim to investigate the striatal D2/3 receptor availability in the ventral as well as dorsal part of the striatum. For this, we will use the functional subdivisions of the striatum in the sensorimotor, associative and limbic parts as defined by Martinez et al. (Martinez et al., 2003; Mawlawi et al., 2001).

According to the above-mentioned concept of addiction as a spectrum disorder, with AD representing the upper part of a spectrum of different levels of alcohol use, we decided to do our investigations in a sample with three study participant groups (Saitz, 2005). As planned in our study design, we recruited a group of abstinent AD patients and two matched control groups: one group with low risk of developing an AD (LR) and one group with high risk of developing an AD (HR), classified via the AUDIT score (Barbor et al., 1989; Saunders et al., 1993). Thus, we hypothesize, that

(H2) Striatal DR2/3 availability in HR lies intermediately between that of AD and LR with significant reductions in striatal DR2/3 availability in HR compared to LR controls (H2a) and significantly higher striatal DR2/3 availability in HR compared to AD patients (H2b).

Moreover, to link the potential differences on the receptor level with clinical symptoms and look at their potential clinical relevance we have chosen the Obsessive-Compulsive Drinking Scale (OCDS) and the Alcohol Dependence Scale (ADS).

As discussed above, craving symptoms have been associated with dopaminergic impairments on a receptor level in AD (Heinz et al., 2005, 2004b). Yet the severity of the alcohol addiction and the extent of the craving symptoms in association with DR2/3 availability have to our knowledge not been investigated in a dimensional sample with a subgroup of individuals at high risk. Thus, we will investigate the severity of alcohol dependence, measured with the ADS, as well as craving symptoms, measured with the OCDS, and with their potential correlation with the impaired dopaminergic system. This leads us to our 3rd and last hypothesis:

(H3) Higher scores in the ADS and in the OCDS correlate negatively with striatal DR2/3 availability.

Methods

Learning and alcohol dependence (LeAD) study.

The current study is part of a larger project of numerous clinical studies about learning mechanisms and their connection with AD (see *Figure 5* and www.lead-studie.de for further information, clinical trial number: NCT01679145). It is a *"Deutsche Forschungsgemeinschaft"* (DFG) funded project (DFG-FOR 16/17) and a collaboration between the *"Technische Universität Dresden"* and the *"Psychiatrische Universitätsklinik der Charité Berlin"*.

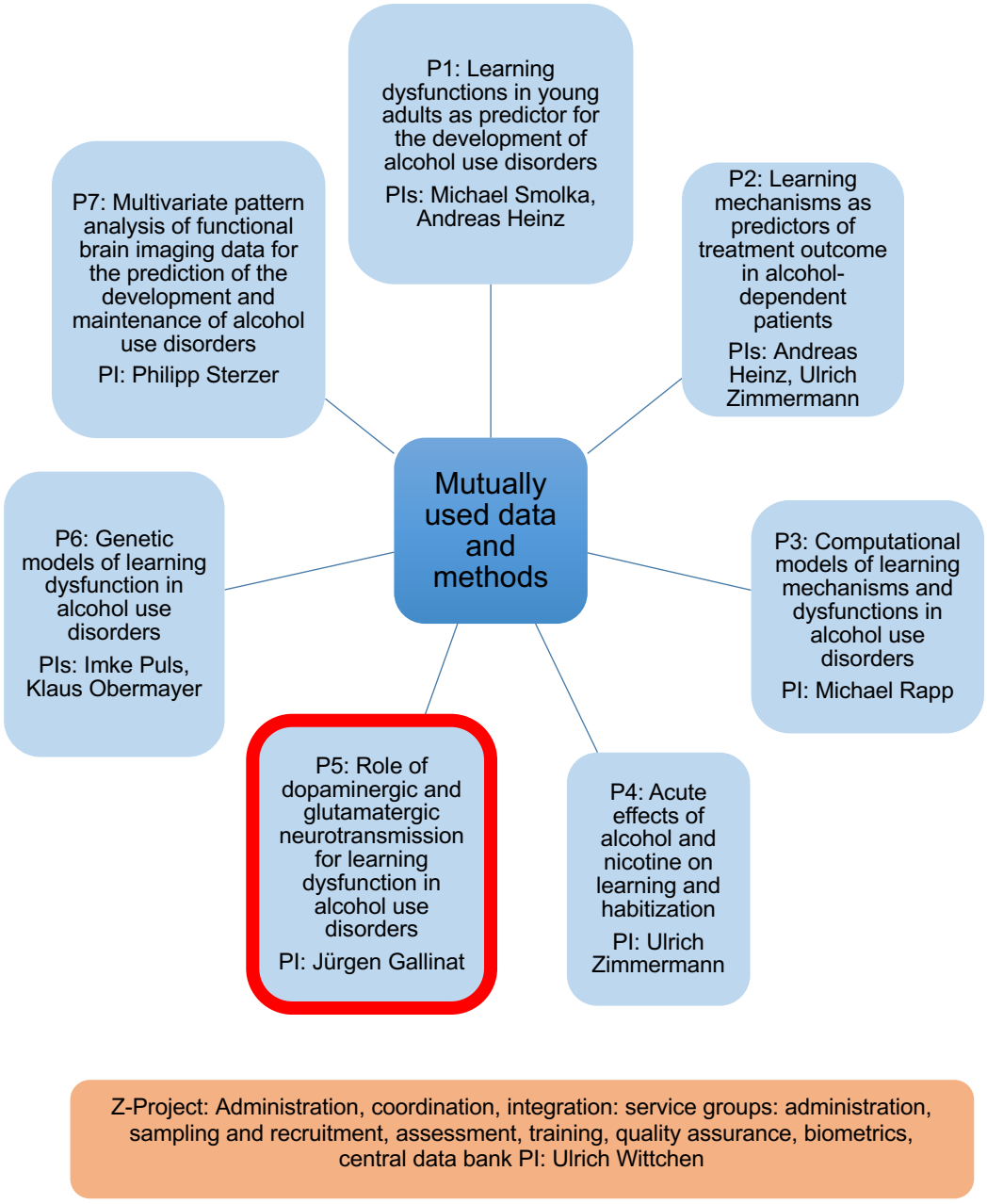

Figure 5. Learning and alcohol dependence (LeAD) study, 1st funding period.

In the 1st funding period, there were seven different projects involved, among which Project 1 (P1) and Project (P2) were the main projects. Thus, P1 and P2 had a central role in regard to the recruitment process, and the other projects used their sample and some of their data with different methods and research questions for their own objectives. This work is about Project 5 (P5), which collaborated closely with P2 and also shared some of their data, as further explained in *Figure 12.*

P5 was approved by the local ethics committee and supervised by Prof. Jürgen Gallinat under the title "*Role of dopaminergic and glutamatergic neurotransmission for learning dysfunction in alcohol use disorders*". The focus of this work was in particular dopaminergic neurotransmission in AD in the context of clinical scales and was thus only an extract of the whole project itself. The data storage and process were executed by project *Z* (see *Figure 5*), which coordinated all the. research groups and administrated the data acquisition and storage.

Study process.

At first all potential study participants were contacted via telephone or in person and underwent a standardized screening. All subjects that were included in the study after the screening were informed about the aim of the study, the imaging methods and in particular about the radiation exposure of about 5.8 mSv due to the PET/CT and signed informed consent forms. After that, the study participants were invited to our standardized clinical assessment, which included a drug and alcohol test, a battery of questionnaires, impulsivity tasks and several neuropsychological tests. After having taken part in the assessment, the study participants got their appointments for the neuroimaging slots.

At a first appointment at the *"Physikalisch Technische Bundesanstalt"* (PTB), study participants underwent magnetic resonance imaging (MRI) in a 3 Tesla scanner (Siemens TRIO). Magnetic resonance spectroscopy (MRS) was performed in the anterior cingulate cortex (ACC) and the hippocampus (HC) to measure different concentrations of transmitters such as GABA and glutamate. Additionally, functional magnetic resonance imaging (fMRI) was performed during the resting state and while the study participants were playing Tetris in the scanner. The MRS and fMRI data will not be a subject of this work, but the T1-weighted MR images were partly essential for acquiring the PET data. Specifically, those images were used to have a higher quality

image as a template for the emission signals of the PET (see *Processing of PET data.*). The PET scan was performed on another scheduled date at *"Charité Campus Virchow Klinikum"* (CVK).

Furthermore, we contacted the study participants 6 months afterwards via telephone for our standardized telephone assessment, which consisted of several questionnaires investigating the drinking behavior, mental and physical health, hospital or medical consults in the meantime and the current living situation of the subjects. After 12 months we contacted them again to invite them for another clinical assessment in person. It was similar to our baseline assessment and included various questionnaires, impulsivity tasks and neuropsychological tasks as well as another blood sample collection.

After their participation in the study, all subjects received an appropriate expense allowance for the baseline assessment and neuroimaging appointments as well as for the follow-ups. An overview of the study process is shown in *Figure 6.*

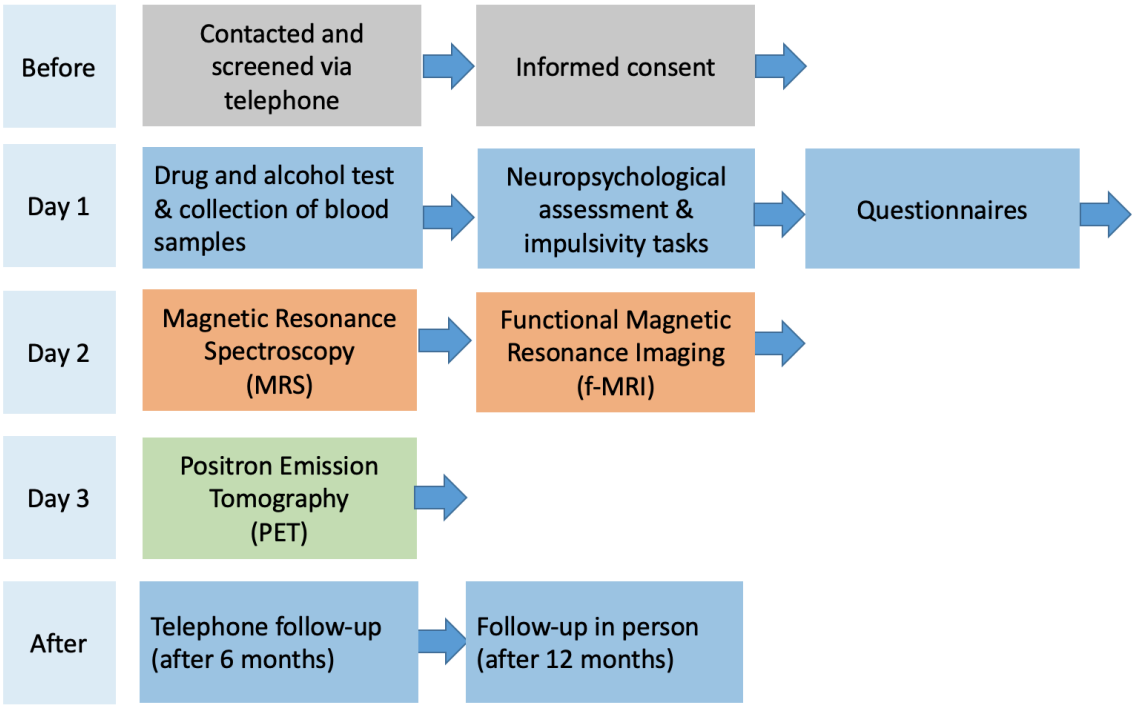

Figure 6. Study process.

Subjects

We successfully recruited 58 study participants: 20 patients with alcohol dependence (AD), 19 controls with low risk (LR) and 19 controls with high risk (HR) of developing an AD. Due to a high number of drop-outs and technical difficulties such as problems with the tracer synthesis and with the PET scanner itself, we had to recruit a higher number of subjects in each group to reach a sufficient number of PET participants. For an overview of the sample size and the reasons for drop-outs see *Figure 7*.

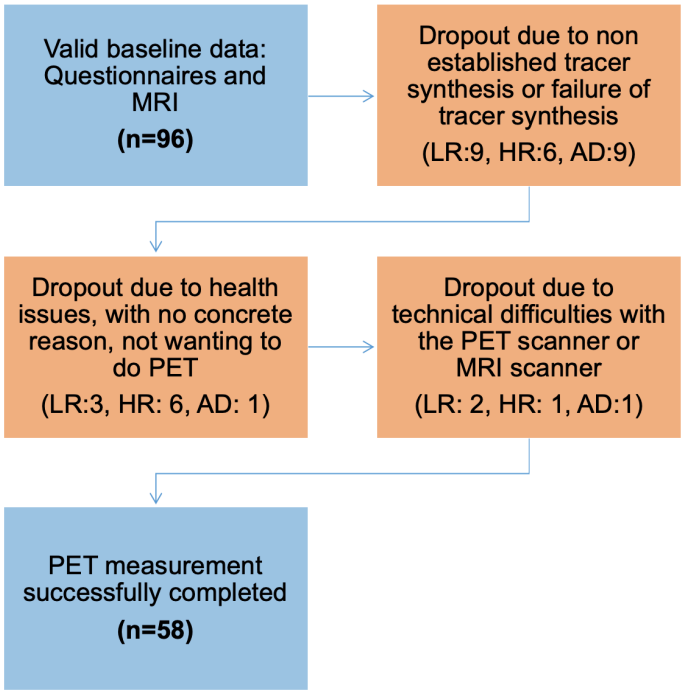

Figure 7. Sample size and reasons for drop-outs.

Recruitment.

We recruited patients with a diagnosed alcohol dependence according to DSM-IV who were undergoing alcohol withdrawal in an alcohol detoxification treatment unit (American Psychiatric Association, 2000). Patients were recruited from inpatient and outpatient facilities in Berlin. Different hospitals were involved in the recruitment process: *Jüdisches Krankenhaus Berlin, Bundeswehrkrankenhaus, St. Hedwig Krankenhaus, Psychiatrische Universitätsklinik Charité Berlin Campus Mitte*. The AD was diagnosed by the independent, respective practitioner in the institutions and later confirmed within our assessment through the *Composite International Diagnostic Interview* (CIDI) (Jacobi et al., 2013; Wittchen and Pfister, 1997).

The LR and HR controls were both recruited via the local online platform "*Ebay Kleinanzeigen"* (https://www.ebay-kleinanzeigen.de) as well as in the regional community (advertisements in supermarkets, newspapers). The announcements included information about the aim and conditioning of the study as well as some of the inclusion and exclusion criteria. Everyone who was interested could contact us for further information and – if interested – undergo the standardized telephone screening. We recruited mostly men and only few women to match the gender with our patient group, which consisted mostly of men. We additionally tried to match the age and educational level between the three groups.

Exclusion criteria.

Subjects with a lifetime history of DSM-IV bipolar or psychotic disorder were excluded, as well as subjects with a current DSM-IV diagnosis of major depressive disorder, generalized anxiety disorder, post-traumatic stress disorder (PTSD), borderline personality disorder, or obsessive-compulsive disorder (OCD) (American Psychiatric Association, 2000). Other exclusion criteria were a history of substance dependence other than alcohol or nicotine dependence and a current substance use other than alcohol or nicotine. A urine screening was performed to check other potentially abused substances. If the screening was positive for any substance, it led directly to exclusion. Additionally, subjects with a history of severe head trauma or central neurological disorders such as dementia, Parkinson's disease, multiple sclerosis or strokes were excluded. Moreover, pregnant or nursing/ breastfeeding women were excluded from the study participation.

Especially for the experimental part (assessment, MRS and PET scan) there were other exclusion criteria such as alcohol intake during the last 24 hours and use of medication or drugs which are known to interact with the central nervous system (CNS) during the previous 10 days or at least four half-lives after the last intake, except for detoxification treatment such as benzodiazepines or chlomethiazole.

Additionally, subjects who had participated in another clinical study with radiation exposure (e.g. PET, SPECT, CT) during the last 3 months were not included in this project. Moreover, all study participants had to have no contraindications with regard to undergoing an MRI scan. For MRI capability, study participants had to have no metal in their body such as screws, metal clips, implants, dental prostheses, insulin pumps etc. Furthermore, large tattoos and colored tattoos were not allowed as they

may heat during the scan. We will now further discuss the inclusion criteria of our three subgroups.

Inclusion criteria.

As mentioned above, we tried to carefully match our study participants from the different groups with regard to age, gender, educational attainment and other variables such as handedness.

Low-risk and high-risk controls.

LR and HR had to be mainly right-handed and smokers. Furthermore, they should not have had a critical consumption of any other drug other than nicotine and alcohol. To be able to pool LR subjects from our project (P5) and our partner project (P2), we had to use slightly different instruments to classify our participants.

In P5, the subjects were categorized via the AUDIT score (Bush et al., 1998; Saunders et al., 1993). An AUDIT score equal to or below 8 led to study participation as a LR control, whereas an AUDIT score of more then 8 led to participation as a HR control. The HR subjects were not undergoing any kind of treatment for their substance misuse. In contrast, the AD patients were undergoing alcohol withdrawal in an alcohol detoxification treatment unit before their participation in the study.

The LR subjects who were recruited by our partner project (P2) were not categorized by the AUDIT, but instead were given the *Composite International Diagnostic Interview* (CIDI) in which an alcohol dependence and an alcohol abuse are excluded (Jacobi et al., 2013; Wittchen and Pfister, 1997). We did not include any HR subjects from P2.

Alcohol-dependent patients.

We included patients who had suffered from AD or symptoms of AD consistently for at least the last three years. Moreover, AD subjects had to be abstinent for a minimum of 3 days (72h).

Besides the exclusion criteria discussed above, patients had to have a low severity of withdrawal symptoms. We used the CIWA (Clinical Institute Withdrawal Assessment for Alcohol) Scale to objectify the withdrawal symptoms (Saitz et al., 1994;

Sullivan et al., 1989). The CIWA score had to be below 3, indicating that they were physically and mentally able to participate. Moreover, the subjects needed to be able to provide a fully informed consent and to use self-rating scales.

Clinical assessment

All study participants underwent a standardized clinical assessment including a battery of questionnaires, a neuropsychological assessment and a drug and alcohol test, which - if positive - led to exclusion. Additionally, we collected blood samples from all our study participants to investigate markers of neuroplasticity. The assessment took place at least one day before the MRS measurement and a minimum of two days before the PET in a special testing room at *St Hedwig Krankenhaus Berlin* and at *Charité Campus Mitte* (see *Study process.*)*.*

The assessment took about 3 hours all in all. First, we informed our participants conscientiously about the aim of the study and the procedure. After the consent, the drug and alcohol test (respiratory alcohol test and urine screening for drugs) followed. After that, we did a neuropsychological testing session (paper pencil) and a rapid visual processing task at the computer (RVP), and then we continued with the impulsivity tasks (gambling tasks with possible wins of money in cash). Moreover, the participants answered a battery of validated and standardized questionnaires at a computer. After the successful completion of the assessment, we arranged the appointments for the MRS and PET scans during the following days.

The Fragerström Test for Nicotine Dependence (FTND) was used to assess the smoking behavior of the study participants (Fagerström, 1978; Fagerstrom and Schneider, 1989; Heatherton et al., 1991). Further, the Edinburgh Handedness Inventory (EHI) was used to assess handedness and to exclude left-handed subjects (Oldfield, 1971). In the following paragraphs, we will discuss the AUDIT score which was part of the screening and the ADS and OCDS scales, which were the main clinical scales used in this work.

Alcohol Use Disorders Identification Test (AUDIT).

The AUDIT is a screening instrument for AD consisting of 10 items: three core questions (known as AUDIT-C) and 7 additional items (Bush et al., 1998; Saunders et al., 1993). The first questions quantify the alcohol consumption and the other questions

ask about risky drinking behavior and other characteristics of substance abuse. The AUDIT was developed by the World Health Organization (WHO), first published in 1989 and then subsequently updated in 1992 (Barbor et al., 1989). In 2001 the second version of the manual was published, containing the version of the AUDIT Score that we used in the screening to differentiate the LR from the HR controls (Babor et al., 2001). It is a tool that is used internationally for both clinical and research purposes and is known for its validity and reliability. We used the cut-off score of 8 points, which was evaluated with respect to sensitivity and specificity for harmful alcohol intake by Saunders et al. (1993) and Conigrave et al. (1995). We included the study participants with an AUDIT score over 8 points in the HR control group.

Alcohol Dependence Scale (ADS).

The ADS is a scale containing 29 items, which was developed in 1982 by Skinner et al. (Skinner and Allen, 1982; Skinner and Horn, 1984). The scale allows a quantification of alcohol dependence in different degrees of abusive alcohol consumption. In addition, it directly correlates with a high number of psychopathological symptoms and physical consequences resulting from alcohol consumption (Skinner and Allen, 1982; Skinner and Horn, 1984). Whereas the AUDIT is more frequently used in identifying individuals with alcohol abuse and alcohol dependence, the ADS is used in subjects with problematic alcohol intake to quantify their symptom severity. The ADS was used in this study to search for a potential association of the symptom severity with the striatal DR2/3 availability and to investigate the clinical importance of the possible dopaminergic impairment in HR and AD subjects.

Obsessive Compulsive Drinking Scale (OCDS).

The OCDS is a self-rating instrument which asks questions about the thoughts, images and pictures of alcohol consumption and how the individual deals with them. There are 14 questions about the thoughts and behaviors that might occur with regard to alcohol intake during the time when the individual is not drinking alcoholic beverages (Anton et al., 1995). There OCDS consists of two subscales. The first 7 questions deal with obsessions and the following questions 8 to 14 with compulsions. Obsessions are defined as uncontrollable unwanted intrusive reoccurring thoughts, whereas

compulsions are defined as repetitive unstoppable behavior patterns or mental acts (American Psychiatric Association, 2013). The assessment of craving symptoms was performed to link potential impairments of the dopaminergic system with clinical symptoms as has been done before in several clinical studies in addiction research (Heinz et al., 2004b, 2005; Morales et al., 2015; Volkow et al., 2006b). Up to now craving symptoms and DR2/3 availability has not been investigated in individuals at high risk of developing an AD. As craving symptoms are associated with a potentially higher relapse risk in alcohol-dependent subjects, they are of great clinical importance (Heinz et al., 2010; Paliwal et al., 2008; Potgieter et al., 1999; Schneekloth et al., 2012).

Positron Emission Tomography

Development and history.

Positron emission tomography (PET) is part of the relatively young medical field of nuclear medicine. In the United States the Society of Nuclear Medicine was founded in 1954, and in 1960 the first papers were published in their *Journal of Nuclear Medicine*. In 1971 nuclear medicine was officially recognized by the American Medical Association and in 1972 the American Board of Nuclear Medicine was founded. The concept of emission and transmission tomography was formed in the late 1950s by David E. Kuhl, Luke Chapman and Roy Edwards. Additionally, the work of Gordon Brownell, Charles Burnham and others at the Massachusetts General Hospital on annihilation radiation, the use of light pipes and volumetric analysis in medical imaging contributed greatly to the development of PET imaging (Brownell, 1999).

PET imaging became more accepted in clinical medicine with the development of radiopharmaceuticals such as primarily labeled 2-fluorodeoxy-D-glucose analogs (2FDG), developed by Ido et al. (1977 and 1978) and the Brookhaven group (Gallagher et al., 1977). In 1986 the work of Hamacher et al. made it possible to produce higher amounts of tracer, which was also a big step in the history of PET (Hamacher et al., 1986). The in vivo application of ^{18}F-fallypride, a high affinity D2/D3 receptor antagonist and the specific radiotracer used in our study, was first performed in 1997 by Mukherjee et al. in nonhuman primates and in 2002 primarily in healthy volunteers (Mukherjee et al., 1997, 2002).

Principles of PET.

A radiotracer is a radiolabeled chemical compound that takes part in the metabolic processes in the body and thus allows diverse investigations. Radiolabeled means that one or more atoms are replaced by a radioisotope. Thus, a radiopharmaceutical has two parts: a molecular structure and a positron emitting radioisotope (Wadsak and Mitterhauser, 2010).

The iotopes of a chemical element differ only in the mass number, which is written on the top left side of the element, e.g. ^{1}H, ^{2}H etc. for hydrogen. The mass number is the total number of protons and neutrons in the atomic nucleus (also called nucleons). An isotope is radioactive if its atomic nucleus is unstable due to its excess nuclear energy. The radioisotopes of hydrogen, carbon, phosphorus and iodine are frequently used to better understand biochemical reactions, or to track the distribution of a substance or fluid flow (Rennie, 1999). The tracer is designed to have properties similar to those of a physiological molecule and can thus participate equally in the metabolism/ functioning of the biological substance without significantly altering in the physiological process. Hence, these characteristics allow the substance to be tracked through the body (Wadsak and Mitterhauser, 2010).

Various radiotracers used in different fields of medicine, for example ^{18}F-fluorodeoxyglucose (FDG) in oncology, cardiology and infectiology and ^{11}C-raclopride or ^{18}F-fallypride in neuroimaging, as well as other tracers in pharmacokinetics to track a radiolabeled drug in preclinical studies. The radionuclides that are used in PET normally have short half-lives, for instance carbon-11 about 20 min or fluorine-18 about 110 min (Carlson, 1998). They can be classified according to the radionuclide used (e.g. ^{11}C, ^{18}F), the field of application (e.g. oncology, neuroimaging), the status of development (e.g. preclinical status) or the target site (e.g. glucose transporter) (Wadsak and Mitterhauser, 2010).

After selection of the appropriate radiotracer for the respective research question, the tracer is usually injected intravenously into the subject. Further, it distributes, depending on its molecular structure, to a certain receptor type or metabolic process in the brain or body. The tracer emits positrons, which means that because of its unstable atomic nucleus and excess of protons it may undergo β^+ decay (positron emission).

β^+ decay is defined as the decay of nuclides which have more protons in their nucleus than neutrons. The excessive proton (p) of this nuclide is thus converted into

a neutron (n), a positron (e^+) and an electron neutrino (neutrino, v_e):

$$p \rightarrow n + e^+ + v_e$$

The underlying mechanisms of emission are a result of the transformation of quarks, which are elementary particles participating in the formation of protons and neutrons.

The result of the β^+ decay is a more stable nuclide, due to the conversion of the proton (p) into the neutron (n). The positron as well as the electron neutrino (neutrino) are emitted (positron emission). The positron is by definition a positively charged antiparticle of an electron (antielectron, e^+), which means that it has the same mass and spin, but its electric charge is +1. The neutrino (v_e) is electrically neutral and has a very small mass. It escapes without interaction in contrast to the positron, which interacts with an electron of the subject. This interaction is called annihilation radiation and leads to the conversion of both the electron and the positron into two photons. As a result of this transformation of their entire mass, these high energy photons (511keV, 120EHz, long-wave gamma-emission) are distributed in opposite directions (at about a 180° angle) (Mikla and Mikla, 2014). This gamma radiation can be detected by the PET. In the following paragraph, I will discuss the detection and processing of signals in the PET system.

Functioning of the PET system.

Signal detection and data collection.

A PET-CT scanner possesses circular detectors that register the coincidences of the annihilation radiation of both sides. These coincidence processing units localize the source of the radiation nearly simultaneously through a straight line (line of response, LOR). The timing resolution of this process determines the signal-to-noise ratio (SNR) and thus the image quality. These coincidence events represent the “raw data”, which is then grouped into projection images called sonograms. They are analogous to CT images and are reconstructed similarly, yet they are noisier and have a lot fewer counts than the usual CT scans.

Signal noise and side effects.

Another side effect arises if at least one of two photons interacts with matter and thus gets deflected from its path. This is called “scatter” and subsequently leads to an incorrect LOR. Furthermore, there are so-called “random events”, where photons are detected as coincidence pairs, although they actually originate from different events. Additionally, in the short time after the detection of the event, the detector cannot detect new events, and is called “dead time”.

Data processing.

Due to the noise, scatter, random events etc., a substantial preprocessing of the data is essential afterwards. Moreover, the spatial and temporal registration of the decay process is performed, which allows interferences to be made about the distribution of the radiotracer in the body. The preferred method for the reconstruction is a statistical, likelihood-based approach with iterative expectation maximization algorithms, which was also used in this study (see *Measurement*.) (Lange and Carson, 1984; Vardi et al., 1985).

Attenuation correction.

Another important step in the data acquisition is the attenuation correction. Attenuation happens when the emitted photons are absorbed by intervening tissue on their way through the body before they are detected. The intensity of the attenuation differs with the thickness of tissue in the path of the LORs. To correct the attenuation, a CT scan is performed to estimate the attenuation. Because of potential artifacts, the corrected and uncorrected images are added and reconstructed together.

Computational reconstruction.

A computational reconstruction allows the computation of sectional and thus three-dimensional (3D) images. Multiple circular detectors are needed to be able to do a 3D reconstruction, and the coincidences have to be detected between rings as well as within rings. The 3D images have a better sensitivity in comparison to 2D images whereas, as a side effect, scatter and random events are slightly enhanced.

Coregistration.

The resulting PET data can then be combined with CT or MRI images to unify the functional with the structural imaging of the subject. In our study the PET images were coregistered with the individual T1-weighted MRI scans. By using the unified segmentation approach, the T1 images and the subjects map of the BP_{ND} of ^{18}F-fallypride were spatially normalized into the anatomical space of the Montreal Neurological Institute (MNI) (Ashburner and Friston 2005). The result was a functional as well as structural image on a molecular level, depending on the respective radiotracer. We will now discuss the specific tracer that was used in this study.

Measurement.

^{18}F-fallypride as radiotracer.

^{18}F-fallypride, a high affinity DR2/3 antagonist, was used as radiotracer in this trial. It is a benzamide radiolabeled with Fluorine-18 (^{18}F), which is a fluorine radioisotope (Mukherjee et al., 1995). It is substituted for a hydroxyl group in fallypride (the parent molecule). The molecular formula of ^{18}F-fallypride is $C_{20}H_{29}{}^{18}FN_2O_3$ ((S)-N-((1-Allyl-2-pyrrolidinyl)methyl)-5-(3-[18F]fluoropropyl)-2,3-dimethoxybenzamide); its chemical structure is shown in *Figure 8.* ^{18}F-fallypride has a short half-life (109.7min) and in 97% of the time emits positrons during its decay (β+ radiation) (Fowler and Wolf, 1973) .

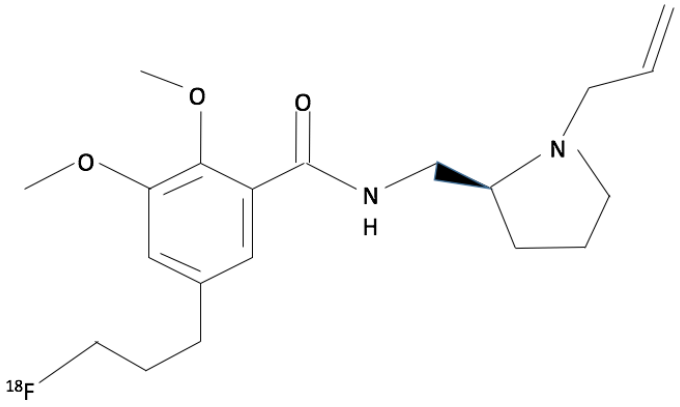

Figure 8. Chemical structure of ^{18}F-fallypride ($C_{20}H_{29}{}^{18}FN_2O_3$).

Procedure.

The D2/3 receptor status was examined via ^{18}F-fallypride PET by a time-of-flight PET/CT system Philips Gemini TF 16 (Surti et al., 2007). The effective radiation dose for our study participants from the injection of this radiotracer was 4.3 mSv, and the radiation exposure from the low dose CT scans was <0.5 mSv each. Altogether, there was a radiation exposure of about 5.8 mSv for each study participant. They were informed in detail about this method beforehand, particularly about the radiation exposure, and gave their consent.

The procedure started with intravenously injecting two hundred MBq (megabecquerel) of the radiotracer (^{18}F-fallypride) over 30 seconds. After this, the data was acquired in 3 consecutive blocks and a resulting time of approximately 240 minutes (Slifstein et al., 2010a). The procedure was structured as follows: 50 min list-mode emission scan, 30 min break, 60 min list-mode emission scan, 60 min break, 40 min list-mode emission scan (Slifstein et al., 2010b). Scanning was continued until 4h after the injection, because, as Laruelle et al. discovered in 2003, the radiotracer reaches the wash-out phase in the striatum within this time period and hence allows a precise quantification of DR2/3 receptor density (Laruelle et al., 2003).

After the emission data was collected, it was sorted following a defined protocol. The data from the first block was sorted into 3 x 20 s, 3 x 1 min, 3 x 2 min, 2 x 5 min, 3 x 10 min, whereas the data of the second and third blocks was sorted into 10 min frames. Additionally, before each block there was a low dose CT performed to allow the attenuation correction of the emission data (see *Principles of PET*.) in the subsequent block (120 kV, 40 mAs). An overview of the protocol of the measurement process of the PET-CT is shown in *Figure 9.* The subjects were allowed to move during the breaks, resulting in potential artifacts. These artifacts may have been a result of an incomplete repositioning of the subjects after the break and hence a spatial mismatch between CT and PET. Therefore, we corrected for motion, which was possible with the data acquired by the low dose CT.

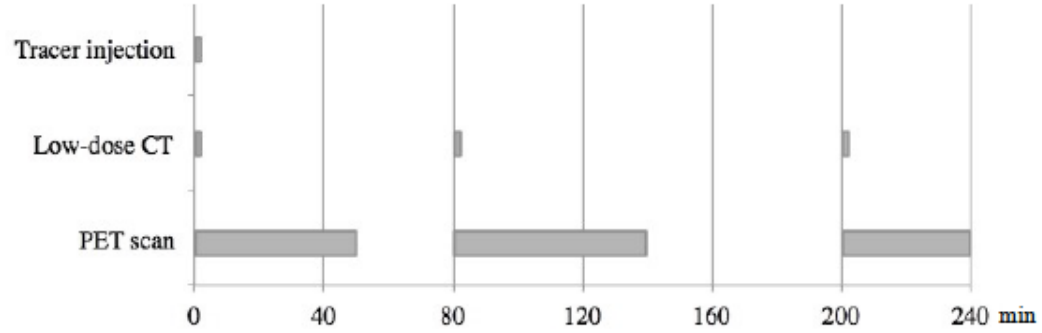

Figure 9. PET-CT protocol. Retrieved with permission from Zacharias (2018).

Processing of PET data.

For our analyses we processed the raw data obtained from PET. Preprocessing included corrections for time, motion, filtering and smoothing of the images. After reconstructing the PET images, they were coregistered to the structural T1-weighted MRI images of the subjects. For this, the sum of early PET frames (during the perfusion phase) was used. Using the 8th version of Statistical Parametric Mapping (SPM8), the individual MRI was stereotactically normalized into the Montreal Neurological Institute (MNI) template (*SPM*, 2017). Furthermore, the PET images were stereotactically normalized by applying the same transformation parameters to the PET frames. The distributions of grey matter (GM), white matter (WM) and cerebro-spinal fluid (CSF) were calculated for every individual brain image to allow individual normalization of each image.

SPM8 was used to compare the reference groups and perform voxel-wise correlations via the factor *group* (LR, HR, AD subjects). Rominger et al. (2012) observed an altered age-related loss of DR2/3 availability in AD compared to healthy controls and thus we added age as a covariate as regressor of no interest. (Rominger et al., 2012). Furthermore, smoking status was included as a covariate as regressor of no interest based on several recent PET studies in which an association of tobacco consumption with lowered striatal DR availabilities was observed (Albrecht et al., 2013; Fehr et al., 2008; Wiers et al., 2017). Rex toolbox was used for extracting the BP_{ND}, measured by ^{18}F-fallypride PET, from our regions of interest (ROIs), based on our hypotheses (*MATLAB 8.0 and Statistics Toolbox 8.1*, 2012).

Regions of interest.

The regions of interest were defined on the basis of the work of Martinez et al. (2003) and Mawlawi et al. (2001). Referring to my hypotheses elaborated above, my focus was the striatum including its subregions. As described in Mawlawi et al. (2001), on the one hand there is a functional subdivision into a limbic, an associative and a sensorimotor part of the striatum or on the other hand an anatomic subdivision. The anatomic classification means a subdivision into a ventral and dorsal striatum with their respective subunits (see *Figure 10, Table 1, Figure 11*). Thus, the ROIs we explored were the LS, AS and SMS on the left and the right cerebral hemispheres. Based on these regions of interest (ROIs), time activity curves (TACs) were generated.

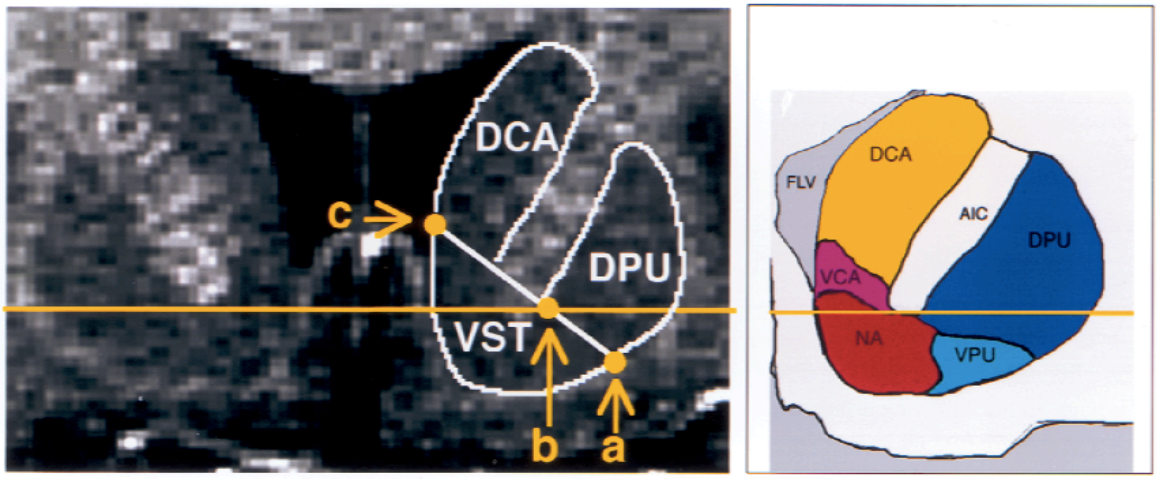

Figure 10. Subregions of the striatum according to Mawlawi et al. 2001.

Coronal slice of a magnetic resonance image in a human subject. VST= ventral striatum, DCA=dorsal caudate, DPU = dorsal putamen. The horizontal solid line identifies the transaxial anterior commissure–posterior commissure plane. See text for boundary criteria (a, b, c). FLV= frontal horn lateral ventricle; AIC= anterior internal capsule; VPU= ventral putamen; VCA= ventral caudate, NA= nucleus accumbens.

Table 1. Regions of interest (ROIs).

Striatum		
Functional Subdivision	Anatomic Subdivision	
Limbic striatum (LS)	Ventral striatum (VS)	
		Ventral caudate (VC)
		Ventral putamen (VP)
		Nucleus accumbens (NA)
Associative striatum (AS)	Dorsal striatum (DS)	
		precomissural dorsal putamen (preDP)
		precommissural dorsal caudate (preDC)
		postcommissural caudate (postC)
Sensorimotor striatum (SMS)	Dorsal striatum (DS)	
		postcommisural putamen (postP)

Notes. Table adapted from Martinez et al. (2003)

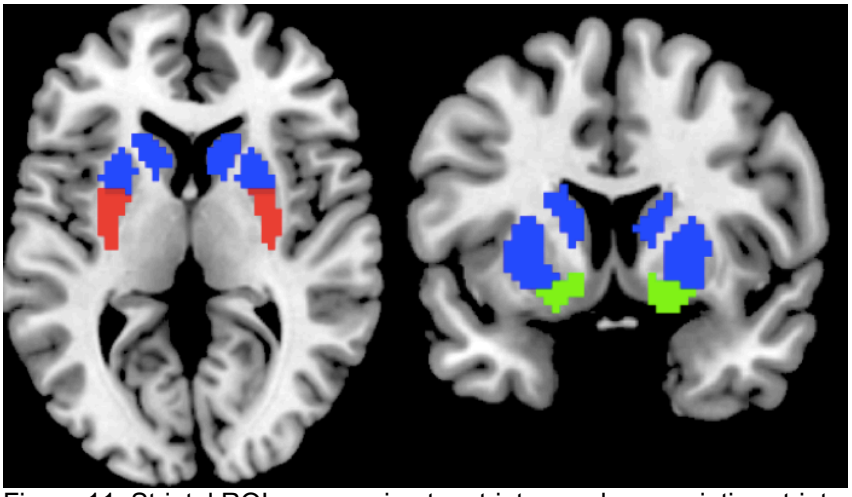

Figure 11. Striatal ROIs: sensorimotor striatum red-, associative striatum blue- and limbic striatum green-colored retrieved with permission from Sebold et al. (2019).

Non-displaceable binding potential.

The primary outcome parameter was the non-displaceable binding potential ($BP_{ND)}$. It is a combined measure of the density of the available receptors and the affinity of the radiotracer to these specific receptors. BP_{ND} is the ratio at equilibrium of a specifically bound to non-specifically bound (non-displaceable) radiotracer in tissue (Innis et al., 2007). This parameter for the comparison of receptor densities is normally calculated using reference tissue methods and comparing receptor-rich and receptor-free regions as we did. Precisely, the binding potential equals the volume of the total radiotracer uptake in tissue (V_T) minus the distribution volume of the non-displaceable volume (V_{ND}). V_{ND} is defined as the sum of free readiotracer in tissue plus non-specific binding in tissue. The unit of BP_{ND} is ml/cm^3 and its equation is the following:

$$BP_{ND} = \frac{V_T - V_{ND}}{V_{ND}} = \frac{V_T}{V_{ND}} - 1$$

Simplified reference tissue method.

To estimate the non-displaceable distribution volume, a reference tissue with a negligible density of the targeted receptors is needed. This method, the two-step simplified reference tissue method (SRTM), was used in this study for modelling on the level of voxels supplying parametric maps of the BP_{ND} (Buchert and Thiele, 2008; Gunn et al., 1997; Wu and Carson, 2002). Potential reference tissues are, for example, the cerebellum, white matter and the visual cortex, as they are all regions with nearly no

DR2/3 (Hakan et al., 1996; Ishibashi et al., 2013; Narendran et al., 2011). We agreed to perform our analyses with a white matter reference region, particularly the bilateral superior longitudinal fasciculus (SLF) as defined by the white-matter tractography atlas provided by the Laboratory of Brain Anatomical MRI of Johns Hopkins University (Hua et al. 2008). This decision was, among other factors, due to the work of Ishibashi et al. (2013) who concluded that the SLF may offer statistical facilities in studies using ^{18}F-fallypride PET. As AD is associated with cerebellar atrophy, we decided not to use the cerebellum as reference region (Beck et al. 2012). Further, time-radioactivity curves (TACs) were then generated on the basis of our specific striatal ROIs described above.

Statistics

Statistical analyses were performed with IBM SPSS Statistics Version 24.0 (IBM Corp., Armonk, NY) for mac OS and carried out according to the description in Field (2009). Beforehand, we tested our data visually for normal distribution via Q-Q-plots. The Q-Q-plots revealed a non-normal distribution of most of our variables and therefore we used non-parametric tests for our further analyses. Due to the sample size of this study, neither the Shapiro-Wilk nor the Kolmogorov-Smirnov tests were the appropriate tests for determining normal distribution.

For descriptive statistics we reported the mean, standard deviation (SD), minimum (min), maximum (max), median and interquartile range (IQR) of the questionnaire scores. The median and IQR were chosen for a better representation of the non-normally distributed questionnaire data. The IQR means the difference between the 25th and the 75th percentiles, being equal to the 1st quartile subtracted from the 3rd quartile. For demographic variables we used only mean, SD, min and max to describe our sample.

The Kruskal-Wallis (K-W) test was used as the non-parametric alternative for the one-way independent ANOVA (univariate analysis of variance (ANOVA)). The K-W test was performed to compare the DR availability represented by the BP_{ND} of ^{18}F-fallypride in our chosen ROIs (see above) (Kruskal and Wallis, 1952). The grouping variable were our three reference groups (LR, HR, AD).

Age as well as the smoking status were included as covariates in our statistical analyses, because both factors have been associated with a reduced striatal DR2/3 availability (Albrecht et al., 2013; Fehr et al., 2008; Rominger et al., 2012; Wiers et al., 2017).

Furthermore, we performed three Mann-Whitney tests as non-parametric post-hoc tests for the pairwise comparisons (Mann and Whitney, 1947). We compared the LR and HR, the LR and AD as well as the HR and AD groups for the respective ROIs which had reached significance in the K-W test. A Bonferroni correction was applied and so all the effects of the post-hoc tests are reported at a two-sided .0167 level of significance.

Furthermore, the DR availability in our respective ROIs were correlated with clinical data. As clinical scales we used the ADS and the OCDS scores, as well as the OCDS subscales "*thoughts"* and "*impulse to act*". Spearman's rho was applied as a non-parametric correlation coefficient.

Results

Description of the sample

Low-risk and high-risk subjects.

We successfully recruited 33 LR subjects. 8 were recruited by our research team and 25 subjects were included from our partner project (DFG FOR 16/17, Project 2). 19 LR controls completed the entire study and participated in the PET-CT. The average age in this subsample was 45.2 years (min 30.8, max 60.8, standard deviation 8.7 years) and there were 3 women and 16 men in this subgroup.

Additionally, we recruited 32 HR controls, from which 19 subjects completed the whole study including the PET-CT. The average age in this group was 42.9 years (min 26.8, max 57.6, standard deviation 9.1 years) and there were 2 women and 17 men in this subgroup. *Figure 12* gives an overview of the sample, its exact size and the recruitment process and of the study in general. The descriptive statistics are displayed in *Table 2.*

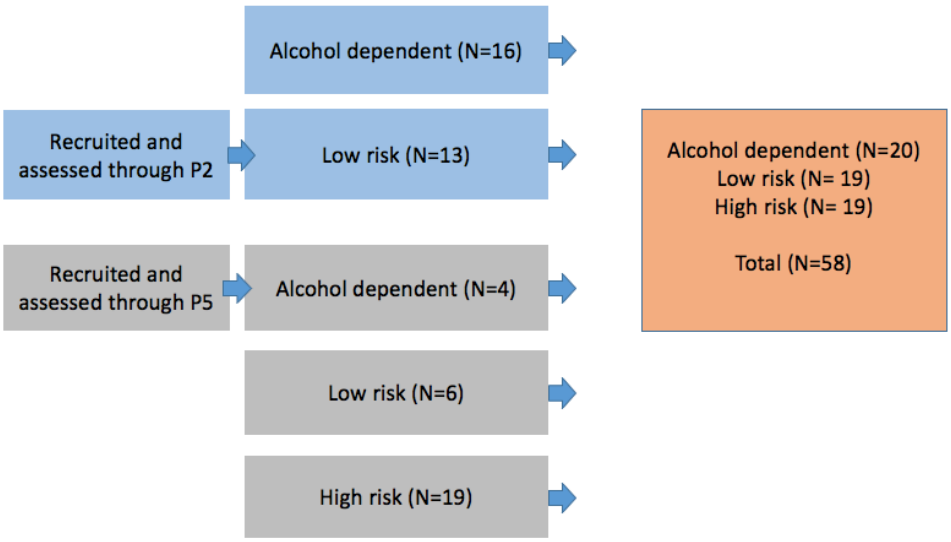

Figure 12. Number of participants, recruitment and process.

Alcohol-dependent patients.

31 patients with a diagnosed AD participated in our study. P2 recruited 27 and our research team recruited 4 patients. 19 of them completed the whole study including the PET-CT. The mean age in this group was 45.4 years (min 29.4, max 58.3, standard deviation 8.4 years) and there were 3 women and 17 men in this group.

The mean abstinence duration among the patients was 36.5 days (min 9, max 96, median 29, standard deviation 20.1 days). The measurement of one patient was very delayed (after 96 days) due to technical difficulties and an electricity failure at the PET scanner. Without inclusion of this participant, the mean abstinence rate would be 30.2 days (standard deviation 15.2 days). For the detailed drinking variables of the AD subjects, see *Table 2.*

Table 2. Sample characteristics.

	Group			*p* Values for Test Statistic		
Variable (available data for LR/HR/AD)	LR *(n=19)*	HR *(n=19)*	AD *(n=20)*	LR vs. HR	LR vs. AD	HR vs. AD
Gender	3 female, 17 male	2 female, 17 male	3 female, 16 male	1.0[d]	1.0[d]	1.0[d]
Handedness	19 right handed	19 right handed	19 right, 1 left handed	-	1.0[d]	1.0[d]
Smokers, %	53	89	84	.03[e]	.08[e]	1.0[e]
	Mean (SD) / Median (IQR)*					
Demographic Variables						
Education, years (19/17/20)	14.6 (3.1)	17.5 (5.4)	15.1 (3.3)	.87[b]	.69[b]	.16[b]
Age, years (19/19/20)	45.2 (8.7)	42.9 (9.1)	45.4 (8.4)	.27[c]	.91[c]	.21[c]
Duration of abstinence, days (20)	-	-	36.4 (20.1)	-	-	-
Clinical characteristics						
AUD identification test (6/19/0)	4.5 (2.25)*	12.0 (5.0)*	-	<.001[a,b]	-	-
Severity of AD (19/14/19)	2.1 (5.2)*	5.2 (5.1)*	17.0 (8.0)*	.02[a,b]	<.001[a,b]	<.001[a,b]
Craving (19/18/16)	2.0 (3.0)*	8.0 (5.0)*	11.0 (12.0)*	<.001[a,b]	<.001[a,b]	.03[a,b]
Craving "thoughts" (19/18/16)	0 (0)*	0.5 (3.0)*	6.0 (6.5)*	.01[a,b]	<.001[a,b]	<.001[a,b]
Craving "impulse" (19/18/16)	2.0 (2.0)*	6.0 (3.0)*	6.6 (7.2)*	<.001[a,b]	<.001[a,b]	.45[b]
Age at first drink (14/0/16)	14.7 (2.1)	-	14.3 (2.0)	-	.62[b]	-
Age of first AD diagnosis (16)	-	-	32.3 (11.2)	-	-	-
Years since AD diagnosis (15)	-	-	13.9 (11.0)	-	-	-

*Notes: [a] significant difference, [b] p value of Wilcoxon rank sum test with continuity correction, [c] p value of Welch Two sample t-test, [d] p value of Chi-square test, [e] Pearson's Chi squared. *Nonparametric test statistics are displayed as median and interquartile ranges instead of mean and standard deviation. Clinical variables are determined as follows: AUD Identification: Alcohol Use Disorder Identification Test (Saunders et al., 1993); severity of alcohol dependence: Alcohol Dependence Scale* (Skinner and Allen, 1982; Skinner and Horn, 1984)*; craving: Obsessive-Compulsive Drinking Scale, subscale "thoughts", subscale "impulse to act (Anton et al., 1995)"; age first drink: the first consumption of one alcoholic beverage; age of first AD diagnosis and years since AD diagnosis: the onset / duration of the alcohol dependence according to the DSM-IV classification (American Psychiatric Association, 2000). Handedness was assessed via the Edinborough Handedness Scale (Oldfield, 1971); smoking was assessed via the Fragerström Nicotine Dependence Scale (Heatherton et al., 1991). Part of this table was published in Sebold et al. (2019).*

Dopamine Receptor Availability

Group comparison.

(H1) There is a significant reduction in the BP_{ND} of ^{18}F-fallypride – measuring striatal DR2/3 availability – in AD compared to LR.

(H2) Striatal DR2/3 availability in HR lies intermediately between that of AD and LR with significant reductions in striatal DR2/3 availability in HR compared to LR controls (H2a) and significantly higher striatal DR2/3 availability in HR compared to AD patients (H2b).

Primarily, a Kruskal-Wallis (K-W) test was performed throughout our whole sample (N=58) to test for differences with regard to the mean DR availability (BP_{ND} of ^{18}F-fallypride) extracted from each striatal ROI.

The K-W test showed a significant group difference of the BP_{ND} in the SMS in the right (H(2)=13.46, *p*=.001) and in the left brain hemisphere (H(2)=6.07 *p*=.047), as well as in the AS in the right brain hemisphere (H(2)=9.67, *p*=.005). Additionally, there was a trend towards significance in the left AS (H(2)= 5.09, *p*=.077). There were no significant group differences in the left and right LS. For an overview of the group comparison of the BP_{ND} see *Table 3.* The mean DR2/3 availability (BP_{ND} of ^{18}F-fallypride) is shown for our three respective study groups in the AS, SMS and LS in both hemispheres in *Figure 13, Figure 14, Figure 15* respectively.

Further, post-hoc group comparisons were conducted between the three subgroups for pairwise comparison using Mann-Whitney tests. A Bonferroni correction was applied and thus all effects are reported at a .0167 level of significance ($p < .05/3$).

LR subjects showed a significantly higher BP_{ND} of ^{18}F-fallypride than AD subjects in the SMS (U=58, *z*=-3.709, *p*<.001, r=-0.594) as well as in the AS on the right brain hemisphere (U=100, *z*=-2.782, *p*=.011, r=-0.405). Additionally, there was a trend towards significance in the SMS in the left hemisphere (U=113.5, *z*=-2.15, *p*=.030, r=-0.347) For an overview see *Table 4*. Thus, H1 could be confirmed for the SMS and AS in the right brain hemisphere.

There were no significant differences of the BP_{ND} of ^{18}F-fallypride in the respective brain regions of LR compared to HR subjects (see *Table 5*). As there were no significant differences shown for the BP_{ND} of ^{18}F-fallypride of LR compared to HR subjects, H2a was rejected in behalf of the null hypothesis.

HR subjects showed a significantly lower BP_{ND} of 18F-fallypride than AD subjects in the AS in the right brain hemisphere (U=91, *z*=-2.782, *p*=.005, r=-0.445). Additionally, there were trends towards significance shown in SMS in the right (U=120, *z*=-1.967, r=-0.315, *p*=.049) and in the left hemispheres (U=116, *z*=-2.079, *p*=.038, r=-0.333). For an overview see *Table 6.* As there were significant differences shown for the BP_{ND} of ^{18}F-fallypride in HR compared to AD subjects in the AS, H2b could be confirmed.

Table 3. Group comparison of the mean BP_{ND} in the respective ROIs (N=58)

DA availability

Regions of Interest	Group									Group Comparison	
	LR *(n=19)*			HR *(n=19)*			AD *(n=20)*			Kruskal-Wallis test	
	Mean	Mean Ranks	Median (IQR)	Mean	Mean Ranks	Median (IQR)	Mean	Mean Ranks	Median (IQR)	H	*p*
Sensorimotor Striatum											
left	28.10	33.76	28.06 (4.44)	27.73	33.16	27.37 (3.51)	25.85	21.98	25.7 (4.45)	6.07	**.047**
right	32.35	39.18	32.40 (3.86)	30.40	30.45	30.78 (4.46)	28.07	19.40	28.49 (3.88)	13.46	**.001**
Associative Striatum											
left	21.67	30.00	20.68 (4.73)	22.16	35.5	22.37 (4.11)	19.88	23.33	20.12 (4.37)	5.09	.077
right	26.58	33.58	26.13 (8.39)	26.63	35.37	26.90 (4.94)	23.03	20.05	23.03 (6.27)	9.67	**.005**
Limbic Striatum											
left	24.93	34.32	25.21 (4.09)	24.14	29.21	24.18 (3.57)	22.52	25.20	23.61 (3.66)	2.84	.241
right	23.43	31.61	23.63 (3.58)	22.99	29.68	23.14 (4.52)	21.90	27.33	22.04 (3.07)	0.629	.729

Notes: The mean BP_{ND} was extracted for each striatal ROI using SPM8 and analysed via the K-W test in SPSS throughout the whole sample. Significant p values are marked and reported for p<.05

Table 4. BP_{ND} of LR compared to AD subjects in the respective ROIs.

Regions of Interest	Groups				Post-hoc Test		
	LR *(n=19)*		AUD *(n=20)*		Mann-Whitney Test 1		
	Mean Ranks	Sum of Ranks	Mean Ranks	Sum of Ranks	U	Z	*p* value
Sensorimotor Striatum							
left	24.03	455.5	16.18	323.5	113.5	-2.15	.030
right	26.95	512	13.4	268	58	-3.709	**<.001**
Associative Striatum							
right	24.74	470	15.5	310	100	-2.529	**.011**

Table 5. BP_{ND} of LR compared to HR subjects in the respective ROIs.

Regions of Interest	Groups				Post-hoc Test		
	LR (*n=19*)		HR (*n=19*)		Mann-Whitney Test 2		
	Mean Ranks	Sum of Ranks	Mean Ranks	Sum of Ranks	U	Z	*p* value
Sensorimotor Striatum							
left	19.74	375	19.26	366	176	-.131	.895
right	22.24	422	16.79	318	128	-1.504	.129
Associative Striatum							
right	18.84	358	20.16	383	168	-.365	.715

Table 6. BP_{ND} of HR compared to AD subjects in the respective ROIs.

Regions of Interest	Groups				Post-hoc Test		
	HR *(n=19)*		AUD (*n=20*)		Mann-Whitney Test 3		
	Mean Ranks	Sum of Ranks	Mean Ranks	Sum of Ranks	U	Z	*p* value
Sensorimotor Striatum							
left	23.89	454	16.3	326	116	-2.079	.038
right	23.68	450	16.5	330	120	-1.967	.049
Associative Striatum							
right	25.21	479	15.05	301	91	-2.782	**.005**

Notes: Table 4, Table 5 and Table 6 show the mean BP_{ND} extracted for the respective striatal ROIs using SPM8 and analysed via Mann-Whitney tests in SPSS for pairwise comparisons. Significant p values are marked and reported at p < .0167.

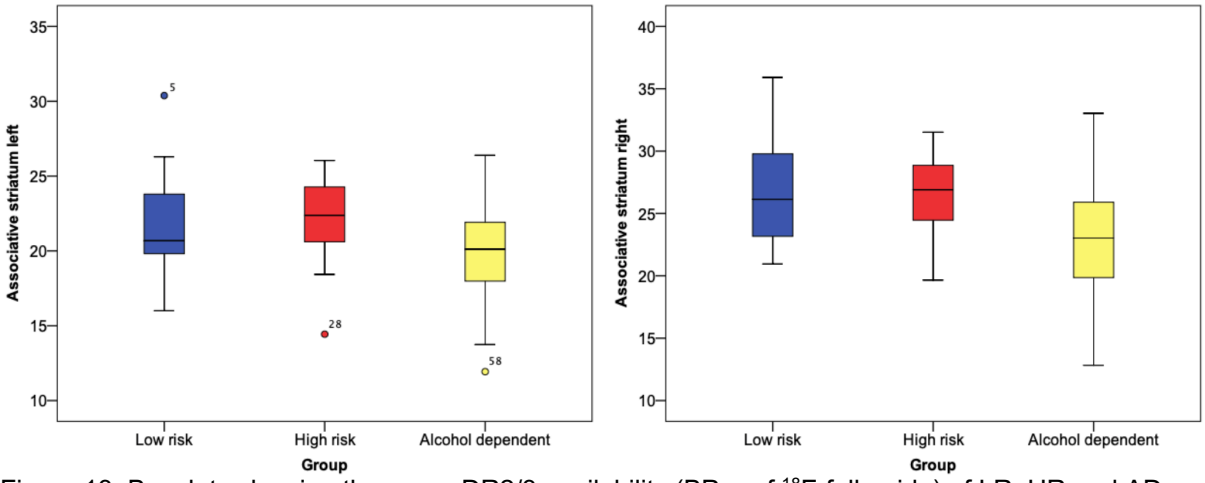

Figure 13. Boxplots showing the mean DR2/3 availability (BP_{ND} of ^{18}F-fallypride) of LR, HR and AD subjects in the left and right hemispheres of the AS, respectively.

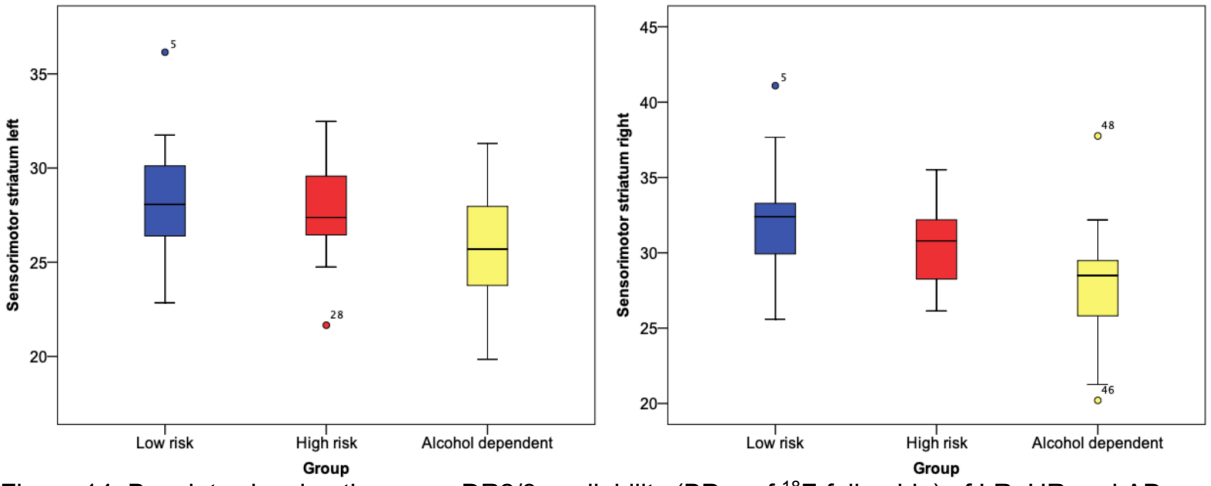

Figure 14. Boxplots showing the mean DR2/3 availability (BP_{ND} of ^{18}F-fallypride) of LR, HR and AD subjects in the left and right hemispheres of the SMS, respectively.

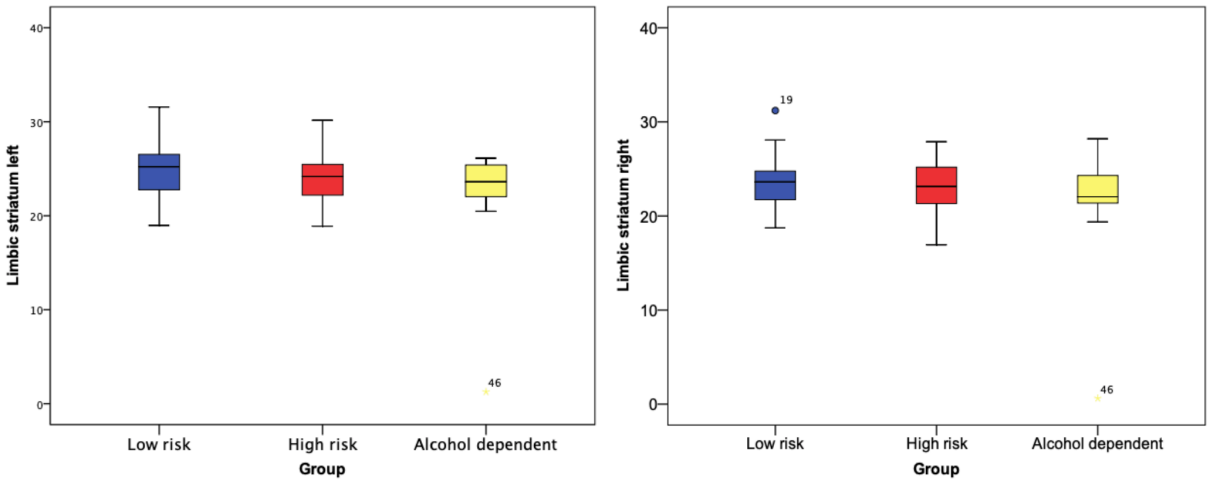

Figure 15. Boxplots showing the mean DR2/3 availability (BP_{ND} of ^{18}F-fallypride) of LR, HR and AD subjects in the left and right hemispheres of the LS, respectively.

Correlation with Clinical Scales

(H3) Higher scores of the ADS and the OCDS correlate negatively with the striatal binding potential of ^{18}F-fallypride.

Dopamine receptor availability and the Alcohol Dependence Scale.

We performed a bivariate correlation between the BP_{ND} in the respective striatal ROIs and the sum of the ADS score throughout the whole sample (n=51, see *Table 7*). The results show a negative correlation between the sum of the ADS score and the BP_{ND} in the SMS in the right hemisphere (r_s= -0.332, *p*=.017) and in the AS in the right hemisphere (r_s= -0.390, *p*=.005). There was no significant correlation in the LS (see *Table 8*). *Figure 16* and *Figure 17* show the visualized correlation of the BP_{ND} in the SMS and the AS with the sum of the ADS score, respectively.

Table 7. Correlations of BP_{ND} and ADS-Score (N=51).

Regions of Interest	Correlation	
	r_s[1]	p value[2]
Sensorimotor Striatum		
left	-0.207	.145
right	-0.332	**.017**
Associative Striatum		
left	-0.248	.079
right	-0.39	**.005**
Limbic Striatum		
left	-0.105	.462
right	-0.067	.639

Notes: [1] r_s= Spearman's correlation coefficient; [2] significance (2-tailed). Significant p values are marked and reported at p<.05.

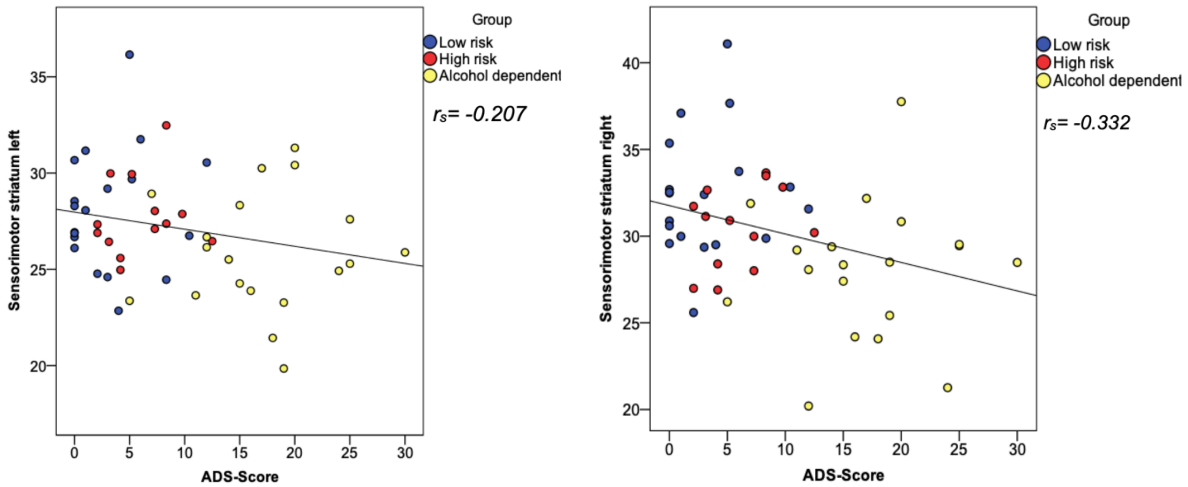

Figure 16. Scatterplot of the correlation of the BP_{ND} in the SMS in the left and right brain hemispheres with the sum of the ADS score, respectively.

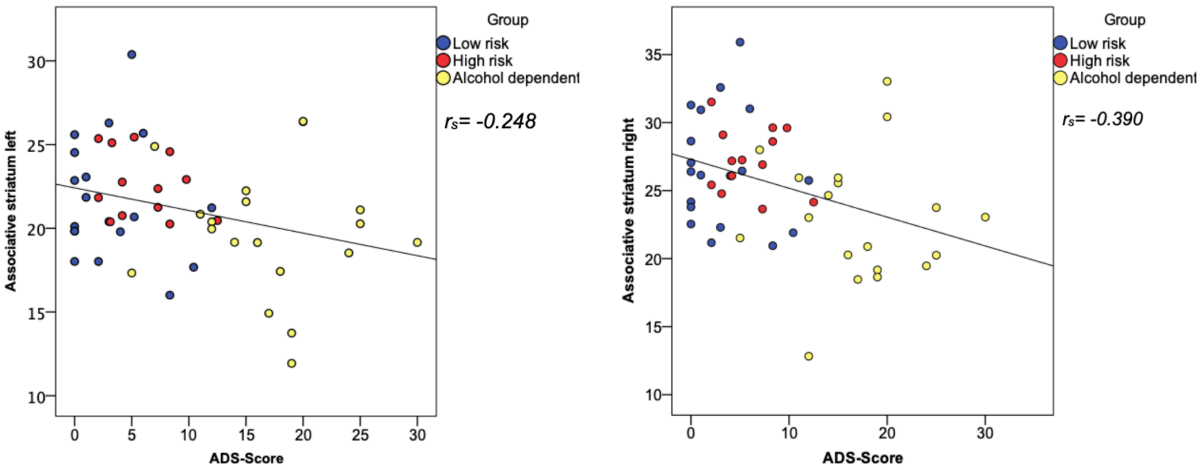

Figure 17. Scatterplot of the correlation of the BP_{ND} in the AS in the left and right brain hemispheres with the sum of the ADS score, respectively.

Dopamine receptor availability and Obsessive-Compulsive-Drinking-Scale.

We performed a bivariate correlation between the BP_{ND} in the respective striatal ROIs and the sum of the OCDS score throughout the whole sample (for n=53, see *Table 8*).

The results show a negative correlation between the sum of the OCDS-Score and the BP_{ND} in the SMS on the right hemisphere (r_s= -0.315, *p*=.022). There was no significant correlation either in the AS or in the LS.

For the OCDS subscale *"thoughts",* representing obsessions in relation to alcohol consumption, there was a significant correlation between the SMS in the left (r_s=-0.317, *p*=.021) and the right brain hemispheres (r_s=-0.424, *p*=.002) as well as in the AS in the right hemisphere (r_s= -0.305, *p*=.026). Additionally, there was a trend towards significance between the AS in the left brain hemisphere (r_s=-0.241, *p*=.083) and this subscale. There was no significant correlation between the LS and the OCDS subscale "*thoughts"*. *Figure 19* shows the correlation of the BP_{ND} in the SMS and the AS in the right hemisphere with the sum of the OCDS subscale "thoughts", respectively.

The OCDS score *"impulse to act",* representing compulsions in relation to alcohol consumption, showed a trend towards significance in the SMS in the right hemisphere (r_s=-0.234, *p*=.083).

Table 8. Correlations of BP_{ND} and OCDS (N=53).

Regions of Interest	OCDS					
	Total Score		Subscale "thoughts"		Subscale "impulse to act"	
	r_s[1]	p value[2]	r_s[1]	p value[2]	r_s[1]	p value[2]
Sensorimotor Striatum						
left	-0.176	.207	-0.317	**.021**	-0.059	.668
right	-0.315	**.022**	-0.424	**.002**	-0.234	.083
Associative Striatum						
left	-0.131	.349	-0.241	.082	-0.043	.755
right	-0.189	.175	-0.305	**.026**	-0.133	.330
Limbic Striatum						
left	-0.082	.560	-0.112	.424	-0.019	.887
right	-0.102	.469	-0.122	.383	-0.053	.696

Notes: [1] r_s= Spearman's correlation coefficient; [2] significance (2-tailed). Significant p values are marked and reported at p<.05.

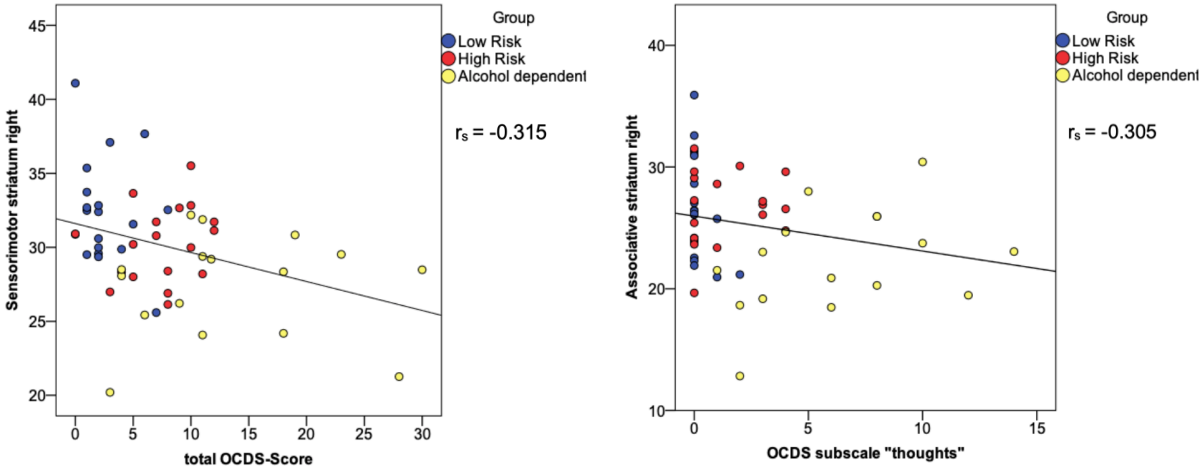

Figure 18. Scatterplot of the correlation of the BP_{ND} in the SMS in the right hemisphere with the sum of the total OCDS score as well as of the AS in the right hemisphere with the OCDS subscale "thoughts", respectively.

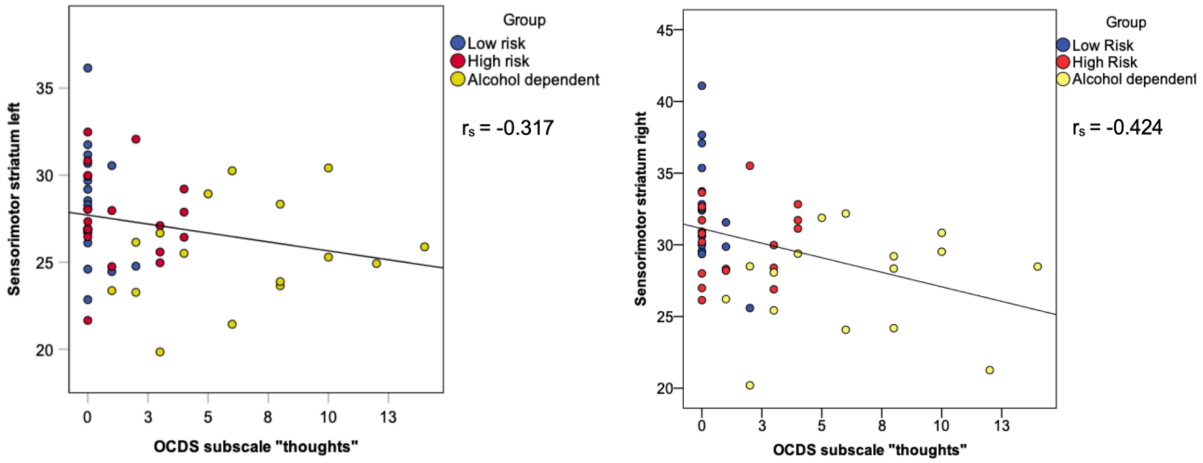

Figure 19. Scatterplot of the correlation of the BP_{ND} in SMS in the left and right hemispheres with the OCDS subscale "thoughts", respectively.

Discussion

In the present work, we investigated striatal DR2/3 availability in recently abstinent alcoholics (AD), subjects with riskful alcohol consumption (HR), and subjects with a low-risk pattern of alcohol consumption (LR). Quantification of DR2/3 availability was performed via ^{18}F-fallypride PET. We analyzed the BP_{ND} of ^{18}F-fallypride in subregions of the striatum including the SMS, the AS and the LS of both cerebral hemispheres in each subject. To link our physiological observations with clinical data, we performed the OCDS and the ADS questionnaires, which assess craving symptoms and the severity of alcohol dependence, respectively.

We observed differences in striatal DR2/3 availability (BP_{ND} of ^{18}F-fallypride) between our three reference groups. Specifically, we found significant reductions of the DR availability in the SMS and in the AS comparing the AD patients with LR controls as well as with HR controls. In contrast, we did not observe significant differences between the LR and the HR groups.

Across groups, the dorsostriatal DR2/3 availability was inversely correlated with the severity of alcohol dependence as well as with craving symptoms, respectively. Contrary to our expectations, we did not observe significant group differences in the ventral striatum.

Group comparison of dopamine receptor availability

Low-risk individuals compared to alcohol-dependent patients.

In acordance with our first hypothesis (H1), we observed a significantly lower DR2/3 availability in the striatum of the AD patients compared to the LR controls. Specifically, alterations of the BP_{ND} of ^{18}F-fallypride were shown in the SMS as well as in the AS.

These findings are supported by a recent meta-analysis by Kamp et al. (2018) and several recent PET studies, which have reported associations between low densities of striatal DA receptors and alcohol dependence specifically in AD patients compared to healthy controls (Heinz et al., 2005, 2004b; Kamp et al., 2018; Martinez et al., 2005; Ravan et al., 2014; Volkow et al., 2007, 2002b, 1996). In most of these PET studies a decreased DR availability in the dorsal and in the ventral parts of the

striatum was observed, whereas we found decreases in the dorsostriatal brain areas but no significant differences in the ventral striatum.

One potential reason may be the low spatial resolution of *in vivo* imaging methods in PET studies in the past and the resulting difficulties in differntiating the ventral from the dorsal striatum (Tupala et al., 2001).

Moreover, as there are indices of a potential recovery of striatal DR2/3 availabilities during prolonged abstinence in AD patients, differences in the abstinence duration in comparison to other PET studies may eventually contribute to the divergent results between our study and the works of Heinz et al. and Rominger et al. (Heinz et al., 2005, 2004b; Rominger et al., 2012). In the studies of Heinz et al. in 2004 and 2005, a lowered DR2 availability was shown in the bilateral putamen as well as in the ventral striatum of AD subjects compared to healthy controls (Heinz et al., 2004b, 2005). In the sample of the AD patients in their study, the mean abstinence duration was 2 to 4 weeks, while our patient group had a mean abstinence duration of about five weeks at the moment of the PET scan (36.4 days, SD: 20.1, see *Table 2)*. Thus, the comparable longer abstinence duration in our AD subsample might be one contributing factor to the absence of ventrostriatal DR impairments, as there might have been a partial recovery of DR during the prolonged abstinence.

Another contributing factor to our divergent results from other comparable PET studies may be the fact that we included age and the smoking status as covariates in our statistical analyses as both factors have been associated with reduced striatal DR2/3 availabilities (Albrecht et al., 2013; Fehr et al., 2008; Rominger et al., 2012; Wiers et al., 2017). In many other studies, such as Martinez et al. (2005) these factors have not been included as nuisance regressors. Moreover, our sample of 58 study subjects was relatively large for a PET study. This may have increased the power of our study and thus allowed the identification of effects in the dorsal striatum, which is formed by the AS and SMS.

Koob and Volkow (2010) argue that ventrostriatal and dorsostriatal alterations in AD subjects seem to be different neuroadaptational steps in the transition towards alcohol dependence. More precisely, they argue that these steps include impairments of the ventral and the dorsal striatum, the orbitofrontal, prefrontal cortex, as well as the cingulate gyrus and later the extended amygdala (Koob and Volkow, 2010).

This shift from ventrostriatal to dorsostriatal neuroadaptational processes might proceed via ascending spiral connections in the midbrain as Haber et al. (2000) and

Ikemoto et al. (2007) have shown in preclinical studies. Along with these findings, Vollstädt-Klein et al. (2010) observed lower activations in the ventral striatum and prefrontal cortical brain areas and higher fMRI activations in the dorsal striatum of high-risk drinkers compared to people with a moderate alcohol consumption during the presentation of alcohol related cues. Furthermore, Corbit et al. (2012) observed an association of a shift from dorsomedial towards dorsolateral striatal adaptational processes in ethanol accustomed rats with automatized drinking patterns because in the animals returned a more flexible behavior pattern after this brain region was inactivated.

Given that we did not observe further ventrostriatal impairments of the DR2/3 availability and considering the above-mentioned preclinical and clinical studies, one may speculate that the AD subjects in our sample shifted from ventrostriatal to dorsostriatal adaptational processes and hence to a more automatized and compulsive drinking pattern. Our sample may thus represent a group of AD individuals progressed more towards the development of AD than individuals in earlier studies showing alterations in the ventral striatum. This assumption is supported by the fact that craving symptoms correlated significantly with the BP_{ND} in the SMS and AS in our sample. In contrast, in the study of Martinez et al. (2005), who observed a decreased DR2 availability in the LS, AS and SMS in AD subjects compared to healthy controls, the BP_{ND} in these brain areas did not correlate with craving symptoms. Taken together one could argue that in our sample the dorsostriatal alterations may be the neurobiological correlate of a compulsive drinking pattern.

These considerations are in line with the observation of Koob and Volkow (2010) that the dorsal striatum is linked to associative learning processes and habit learning as well as action initiation and might thus play an important role in automatized behavior patterns (Koob and Volkow, 2010). Many studies regarding other SUD have observed particularly dorsostriatal but no ventrostriatal dopaminergic impairments as we did in alcohol-addicted subjects. For example, Kim et al. (2011) showed a reduced DR2 availability in the bilateral dorsal caudate and the right putamen in internet-addicted subjects compared to healthy controls. Furthermore, Lee et al. (2009) did not find group differences with regard to DR2/3 availability in the ventral striatum in methamphetamine-dependent subjects compared to healthy controls, but they did find significant alterations in the dorsal striatum. The dorsal striatum has also been in the focus of studies investigating the acute effects of substance abuse on the

dopaminergic system. Volkow et al. (2006) were able to show that cocaine-associated cues seem to induce DA release in the bilateral DS, which correlated significantly with the increase of craving scores (Volkow et al., 2006b). This work was based on preclinical studies of Ito et al. (2000, 2002), who were able to show an acute increase of DA in the dorsal but not in the ventral striatum when drug-associated conditioned stimuli were presented to cocaine-seeking rats. (Ito et al., 2002).

These findings show the importance of the dorsal striatum in the development and maintenance of addiction. Thus, our study contributes to a growing body of evidence demonstrating the importance of particularly dorsal striatal impairments for the understanding of alcohol dependence. As we were able to show reduced DR2/3 availabilities in a particularly large sample of AD patients compared to LR as well as HR subjects, this emphasizes the presumably important role of the dorsal striatum. More studies with a focus on the dorsal striatum are needed to explore more closely its role in addiction.

DR availability in high-risk individuals compared to low-risk controls.

In contrast to our hypothesis (H2a), there were no significant alterations in the DR2/3 availability of the LR controls compared to the HR controls. However, in accordance with our hypothesis (H2b), HR subjects showed significantly higher DR2/3 availability in the AS than AD patients. Hence, LR and HR subjects both possessed a significantly higher DR2/3 availability compared to AD subjects, but there were no significant differences observed when comparing LR and HR controls. Thus, we have to reject our hypothesis that the DR2/3 availabilities of HR lie intermediately between those of AD and LR subjects.

Despite the fact that the HR subgroup practiced a significantly riskier drinking pattern than the LR group (AUDIT score of 9 or higher), the postsynaptic DR2/3 availability was not significantly impaired compared to that of the LR subjects. Interestingly, the HR subjects had not yet shifted towards manifest alcohol dependence despite their riskful alcohol drinking patterns. Moreover, their striatal DR2/3 availabilities did not lie intermediately between those of LR and AD subjects, as there were no significant reductions of the BP_{ND} of the HR compared to that of the LR group. Further, the HR individuals seemed to have striatal DR2/3 densities comparable with those of the LR subjects. One explanatory approach for the

relatively high striatal DR2/3 availabilities in the HR subgroup may be that the study participants of the HR group may not have had such a strong predisposition for alcoholism or perhaps they even possessed protective factors that prevented them from developing an alcohol dependence.

These predisposing factors may be, for example, genetic influences as it is known that about 50% of the vulnerabilities related to AD are associated with genetic factors (Prescott and Kendler, 1999; Schuckit, 2009). Several potential genetic influences on the development of AD-involved genes are associated with the DR expression (Berggren et al., 2006; Comings and Blum, 2000; Schellekens et al., 2012). Nevertheless, these associations seem to be polygenetic and many other transmitter systems or enzymes besides the dopaminergic system are involved as well (Tawa et al., 2016). Additionally, several epigenetic mechanisms such as DR2 promotor methylation may be involved with the DR2/3 availability in AD subjects (Bidwell et al., 2018). Thus, the HR subjects may have possessed DR2/3 availabilities comparable to those of the LR group and higher than those of the AD subjects due to potential contributing genetic and epigenetic influences.

Further, the relatively high densities of DR2/3 availabilities in the HR group may have been a protective factor for not developing an AD despite their risky drinking patterns. This hypothesis is supported by Volkow et al. (2006), who observed higher levels of DR2 in nonalcoholic subjects of families with many alcohol dependent family members. The authors argue that high levels of DR2 availability may be a protective factor against AD (Volkow et al., 2006a). This may indicate that the relatively high striatal DR2/3 availabilities in the HR subjects of our sample may have been a factor that might have protected them from developing an AD. On the other hand, this may indicate that the lower DR2/3 availabilities in AD patients were a result of their stronger predisposition for AD. Of course, we do not know how extensive the genetic influences are or to what extent the DR availability may be epigenetically or behaviorally influenced.

Moreover, we have to consider the numerous other potential predispositions for alcohol dependence which exist apart from the dopamine recepotor availability, such as variants in the metabolism enzyme genes alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), which were identified as the most significant single nucleotide polymorphisms (SNPs) in AD in the meta-analysis of genome-wide association studies (GWAS) by Tawa et al. (2016). So, there may have been

numerous non-dopaminergic genetic alterations in our HR group compared to the LR sample, which may be potential explanations for their risky drinking pattern.

Further, we can argue that the HR subjects in our sample may have had certain behavioral predipositions which shifted them to a risky alcohol consumption pattern. These behavioral predisposing factors for riskful alcohol consumption may be, for example, dysfunctional learning processes such as habitual choices instead of goal-directed choices (Everitt et al., 2008; Voon et al., 2015). Accordingly, HR individuals in our sample might have possessed other learning strategies than LR individuals, leading to a more habituated alcohol consumption.

Individuals at high risk compared to alcohol-dependent patients

As mentioned above, in accordance with our hypothesis (H2b), HR subjects showed significantly higher striatal DR2/3 availability than AD patients. Interestingly, when comparing AD with HR subjects, we only observed alterations in the AS, whereas when comparing AD patients with LR controls we found differences in the AS and the SMS.

The SMS plays a critical role in fine motor control and locomotion, whereas the AS seems to be relevant for stimulus response learning and associative functions (Joel and Weiner, 2000; Martinez et al., 2003). The lowered DR2/3 availability in the AS of AD patients compared to HR and LR subjects might indicate differences in the learning behavior and associative functions of the subgroups. It has been shown that AD is associated with a shift to more habitual, automatized decision making and more disorganized learning mechanisms (Garbusow et al., 2014b; Sebold et al., 2014, 2017). Additionally, in AD subjects compared to LR there seems to be a stronger effect of the determination of instrumental behavior such as alcohol-seeking and consumption through Pavlovian conditioned cues (Garbusow et al., 2014a).

These learning mechanisms reflect a shift to habitual behavior patterns which seem to be associated with habitual and compulsive drug use whose potential neurobiological correlate may be the dorsostriatal neurocircuitry (Everitt et al., 2008; Everitt and Robbins, 2005; Taylor et al., 2013; Yin et al., 2004).

Thus, we may assume that these impairments of the DR2/3 in the AS of AD compared to HR subjects indicate a shift to a habitual compulsive drug use, which is in line with the aforementioned observations of Koob and Volkow, suggesting a shift

from ventral to dorsal striatal impariments with progressive alcohol misuse (Koob and Volkow, 2010).

The differences between HR and AD subjects might also correspond with genetic and epigenetic alterations, indicating that HR individuals are categorially different from AD individuals. Further, HR subjects may have had the above-mentioned resilient factors that protect them from developing a manifest AD. Thus, our results suggest a categorial difference between these two subgroups. Despite this categorial difference, we did not find any significant alterations between the LR and the HR group. This is not automatically a contradiction to the idea of alcohol consumption and risky acohol use as a spectrum, as one potential explanation may be that LR and HR subjects lie closer together on the spectrum, and in a larger sample it might be possible to find HR subjects with an intermediate impairment of DR2/3 availabilities between manifest AD and LR controls (Saitz, 2005). Another potential explanation might be that there were different subgroups within the HR subjects and that some of the individuals were more resilient than others.

Furthermore, lowered striatal DR availability may also have an impact on the subjective experience of alcohol intake. Volkow et al. (2005) observed that individuals with higher striatal DR2/3 availability reported the effects of intravenous ethanol intake as aversive in contrast to subjects with lower levels, who experienced the effect positively (Volkow et al., 2002a, 1999). Yoder et al. (2005) support these findings. In their study, the subjective response to an intravenous alcohol dose was higher in healthy controls with lower striatal D2/3 receptor levels than in individuals with higher striatal receptor levels (Yoder et al., 2005). This may explain through which mechanism DR2/3 availability can be a predisposing factor for developing an AD, but it could also be seen as a reinforcing factor once the dopaminergic system has adapted to AD through a compensatory downregulation of striatal DR2/3 availabilities.

Volkow et al. (2002b) observed a persisting striatal D2/3 receptor reduction in alcoholics after four months of abstinence, whereas Rominger et al. (2012) report a higher striatal level in 4 out of 17 AD subjects who remained abstinent for 1 year (Rominger et al., 2012; Volkow et al., 2002b). These contradictory results indicate that longitudinal studies are necessary to explore the DR availability before the development of AD and during the disease as well as in withdrawal and after a long time of abstinence, as we do not know whether the AD subjects had a lower DR2/3

availability due to genetic or epigenetic influences before developing the disease or if they developed the dopaminergic impairment as a neurobiological compensatory reaction because of the DA excess in chronic alcohol abuse.

Correlation with Clinical Scales

Dopamine receptor availability and Alcohol Dependence Scale.

Furthermore, we observed correlations of the DR2/3 availability with clinical scales throughout the whole sample. Confirming our hypothesis (H3), the severity of alcohol abuse – measured with the ADS score – correlated significantly with the DR2/3 availability in the SMS as well as with the AS. However, the correlation of the ADS-Score with the DR2/3 availability in the LS did not reach significance.

With this additional analysis we were able to validate our findings of a reduced dorsostriatal DR2/3 availability in AD subjects compared to LR and HR controls. This emphasizes the association of reduced striatal DA receptors with clinical symptoms. This may be interpreted on the one hand as a compensatory receptor downregulation due to chronic alcohol intake or on the other hand as a predisposing factor caused by genetic or epigenetic influences.

Koob and Volkow (2010) discuss that both the dorsal and the ventral striatum seem to play an important role in the transition towards AD and thus support our findings in the dorsal striatum and their association with the severity of alcohol misuse. They see dorsostriatal dopaminergic impairments as one important step in the circuitry of addiction (Koob and Volkow, 2010). Along with Everitt et al. (2008) we can interpret the correlation between the severity of alcohol dependence and lowered dorsostriatal DR2/3 availability as a gradual loss of control over the drinking behavior and its possible neurobiological correlate (Everitt et al., 2008). Nevertheless, genetic and epigenetic factors may also contribute to the lowered DR availabilitiy and its correlation with the severity of alcohol dependence.

Dopamine receptor availability and Obsessive Compulsive Drinking Scale.

In accordance with our hypothesis (H3), we observed a significant inverse correlation of the total OCDS score with DR2/3 availability in the SMS. Specifically, the more thoughts the study participants had about alcohol consumption when they were

not drinking and the more difficulty they had controlling their drinking behavior, the lower was their availability of DR2/3 in the SMS. In contrast, the DR2/3 availability of the LS was not significantly correlated with the OCDS score.

This finding is in accordance with several recent PET studies which investigated craving symptoms and striatal DR availability as well as DA turnover in AD (Heinz et al., 2005, 2004b; Kumakura et al., 2013).

Furthermore, the investigation of the two subscales of the OCDS led to interesting results. The subscale “impulse to act”, representing compulsions and thus the ability to control drinking behavior, correlated significantly only with the DR2/3 availability in the SMS. Compulsions and self-perception of uncontrolled drinking behavior are thus associated with a substriatal region, which plays a critical role for fine motor control and locomotion (Joel and Weiner, 2000; Martinez et al., 2003). This is supported by the fact that there are several studies indicating that joystick training for approaching and avoiding alcohol cues, e.g. pictures of alcoholic beverages, may have an impact on the clinical outcome of AD subjects (Sharbanee et al., 2014; Wiers et al., 2015, 2011, 2010). Thus, we were able to observe an association of compulsions with the reduced striatal DR2/3 availabilities in the SMS, which emphasizes a potential role of the motor system in controlling drinking behavior. It may be worth exploring striatal DR2/3 availabilities together with an approach and avoidance task in AD.

On the other hand, the subscale “thoughts”, representing obsessions and thus thoughts associated with alcohol consumption, correlated significantly with the DR2/3 availability in the SMS as well as in the AS in the whole sample. As the AS seems to be relevant for stimulus response learning and associative functions, its coherence with recurring thoughts about alcohol consumption when not drinking seems logical (Joel and Weiner, 2000; Martinez et al., 2003).

These results nevertheless suggest that craving symptoms and the subjective loss of control over drinking behavior may be related to dorsostriatal DR dysfunctions, which has been shown in several studies with regard to SUD (Heinz et al., 2005; Vanderschuren et al., 2005; Volkow et al., 2006b). For example, Heinz et al. (2005) observed a negative correlation between craving symptoms and the DR2/3 availability in the bilateral putamen and the right caudate in AD subjects. Moreover, Volkow et al. (2006b) found reduced DR2 availabilities in the dorsal but not in the ventral striatum of cocaine-addicted subjects who were exposed to cocaine cues. These impaired DR availabilities also correlated with self-reports of craving. Furthermore, Vanderschuren

et al. (2005) have shown that a blockade of DR in the dorsal striatum inhibits cocaine seeking behavior in rats.

All in all, with this work we were able to show a potential link between the severity of craving symptoms and the dorsostriatal dysfunction. More precisely, we were able to show differential results for substriatal regions. Craving symptoms have been associated with relapse prediction in many previous studies (Addolorato et al., 2005; Paliwal et al., 2008; Schneekloth et al., 2012). It would be very interesting to investigate craving symptoms, dorsostriatal DR2/3 availability and their potential impact on relapse probabilities in our sample. Therefore, the future analysis of our clinical follow-ups will be very important.

Limitations

Measuring the BP_{ND} of ^{18}F-fallypride is a well-established method to assess the DR2/3 availability in striatal brain regions (Mukherjee et al., 2002). However, it is not possible to exclude potential confounders in the data acquisition. As Laruelle et al. (1997) have shown for other radiotracers, free endogenous DA may influence the DR2/3 availability via PET. Nevertheless, as we did not expose our study participants during the data acquisition to any direct positive stimuli which might have caused a DA release, such as pictures of alcoholic beverages, we suggest that this confounder is insignificant.

Another important point which has to be considered when interpreting and comparing our results is the selection of ROIs. The ROIs of Martinez et al. (2003) and Mawlawi et al. (2001) that we have chose in our study follow a functional approach; dividing the striatum into the SMS, the AS and the LS. Other studies, for instance, use the Talairach space, which divides the striatum into the putamen, caudate and nucleus accumbens (Heinz et al., 2005; Lancaster et al., 2000; Talairach and Tournoux, 1988). These differences play a role in the comparability of the studies.

Although we tried to match our respective subgroups carefully, we did not differentiate between men and women when evaluating their drinking behavior. We categorized the study participants into the reference groups using the same criteria for both sexes. This can be criticized, as the female body has on average more fat and less water and also metabolizes alcohol differently (American Psychiatric Association, 2013). Thus, the same amount of ethanol causes a higher blood alcohol level in

females. However, we did not include many women in our reference groups (3 women in the LR, 2 in the HR and 3 in the AD group), which is why we refrained from a further differentiation.

Additionally, we cannot rule out that the individuals in the LR group may have underreported their drinking behavior. LR as well as HR subjects were practicing an active drinking pattern and it is very difficult to objectify the exact amount and the habits of their alcohol consumption.

Moreover, a potential confounder could have been the variability of days of abstinence in our AD group (min 9, max 96, mean 36.5, SD 20.1) at the time of the baseline data acquisition. Furthermore, long time spans between clinical assessments and the ^{18}F-fallypride data acquisition were partly inevitable as problems with tracer synthesis and limited time slots for using the PET scan were not predictable. Nevertheless, other samples in comparable PET studies had similar abstinence rates in their patient groups (Spreckelmeyer et al., 2011).

Furthermore, when interpreting the correlations of the DR2/3 availabilities with the clinical scales, the effect sizes have to be taken into account. For example, the correlation coefficient (Spearman's R) when correlating the ADS score with the dorsostriatal DR2/3 availability varied from -0.332 to -0.390 in the respective ROIs, so the effect sizes should be interpreted as small to medium. Additionally, the correlation coefficient (Spearman's R) when correlating the OCDS score with the striatal DR2/3 availability varied from -0.305 to -0.424, with consequently small to medium effect sizes. Thus, our conclusions about these clinical correlations are limited to a certain extent. Moreover, these significant correlations only show coherences and associations, but do not enable us to deduce causalities.

Perspectives

In our study, we were able to observe significant reductions in the BP_{ND} of ^{18}F-fallypride – measuring striatal DR2/3 availability – in the SMS and AS of AD patients compared to the LR and HR controls.

It will be very interesting to investigate whether our findings allow a potential association with the prediction of relapse and long-term clinical outcome of AD patients. Especially craving symptoms seem to be associated with a higher relapse probability (Heinz et al., 2010; Paliwal et al., 2008; Potgieter et al., 1999; Schneekloth et al., 2012). The correlation of the measured DR2/3 availability with clinical craving

symptoms in our data might potentially allow assumptions about relapse prediction. As we also collected clinical follow-up data for the study participants over a period of 12 months, we might potentially soon have new insights of the associations of dorsotriatal dysfunctions with craving symptoms and relapse probabilities.

Moreover, as mentioned above, Rominger et al. (2012) observed that the BP_{ND} of ^{18}F-fallypride may increase in some AD patients during abstinence or pronounced reduction of alcohol consumption. This potential recovery of the DR2/3 availability is an interesting phenomenon and might be worth investigating further. A design of a ^{18}F-fallypride PET study in subjects before developing an AD, then during the diagnosed AD with an active drinking pattern and after a short and a longer time while abstinent would be the best option to further investigate whether the dopaminergic alterations are predisposing factors or resulting adaptive processes.

Furthermore, it might be very interesting to investigate different levels of alcohol consumption in a larger sample in a PET study. We did not find any difference between the DR2/3 availabilities of LR compared to HR subjects, but maybe in a larger sample of HR subjects it might be possible to observe DR2/3 availabilities lying intermediately between those of the LR and AD subjects.

Additionally, we plan to investigate the MRS data we collected and the. potential associations of different transmitter concentrations with striatal DR2/3 availability. Specifically, prefrontal glutamate concentration with its potential modulating impact on the striatal dopaminergic neurotransmitter system may be of great interest (Carlsson et al., 1999). Through this we may be able to find associations of this blunted striatal dopaminergic neurotransmission with the prefrontal glutamate concentration.

Another interesting research domain is the investigation of extrastriatal DR2/3 availabilities and their clinical coherences. Rominger et al. (2012) were able to show that changes in dopaminergic functions in AD may not be limited to striatal regions, and may for instance extend to frontal or other extrastriatal. We also acquired ^{18}F-fallypride PET data of extrastriatal regions such as the prefrontal cortex or the anterior cingulate cortex, leading to contradictory results (Zacharias, 2018).

Moreover, it may be interesting to investigate potential genetic and epigenetic factors determining DR2/3 availability in our study participants and especially in AD subjects in association with the striatal DR2/3 availabilities. Due to the large number of potentially involved genes and epigenetic factors, this sample is unfortunately not large enough for analyses. Nevertheless, it may be very interesting to combine genetic

and epigenetic investigations with PET data acquisition in a larger sample of AD subjects to further the understanding of the extent of genetic predisposition on impaired dopaminergic neurotransmission and its impact on the development of AD.

Thus, due to the complexity of psychiatric disorders such as AD, a multidimensional approach considering genetic, behavioral and neurochemical factors seems necessary to be able to gain more knowledge about the development and maintenance of this SUD.

Our finding of lowered DR2/3 availability in the dorsal striatum of AD subjects compared to LR and HR controls contributes to a better understanding of the pathophysiology of the disease but does not provide a comprehensive account of it. Further research will be necessary to investigate the dorsal striatum in order to explore a potential association with relapse prediction.

Potentially, the observed alterations in DR2/3 availability may also serve as a starting point for the development of innovative interventions. Vanderschuren et al. (2005) were able to show in their preclinical study that an infusion of the DR antagonist alpha-flupenthixol into the dorsal striatum inhibited cocaine-seeking behavior in rats. This study emphasizes the impact of the dorsal striatum and allows an experimental outlook on a potential clinical application.

Moreover, identifying predisposing factors for developing an AD by assessing the striatal DR2/3 availability might be one option to potentially help to prevent alcohol misuse. This could then contribute to an increased understanding of AD and help to optimize specific treatment options and therapy plans for the patients.

References

Addolorato, G., Leggio, L., Abenavoli, L., Gasbarrini, G., 2005. Neurobiochemical and clinical aspects of craving in alcohol addiction: A review. Addict. Behav. 30, 1209–1224. https://doi.org/10.1016/j.addbeh.2004.12.011

Albrecht, D.S., Kareken, D.A., Yoder, K.K., 2013. Effects of smoking on D_2/D_3 striatal receptor availability in alcoholics and social drinkers. Brain Imaging Behav. 7, 326–334. https://doi.org/10.1007/s11682-013-9233-4

American Psychiatric Association, 2013. Diagnostic and statistical manual of mental disorders (5th ed.). Washington, DC.

American Psychiatric Association, 2000. Diagnostic and statistical manual of mental disorders (4th ed., text rev.). Washington, DC.

Andrews, J.A., Tildesley, E., Hops, H., Li, F., 2002. The influence of peers on young adult substance use. Health Psychol. 21, 349–357. https://doi.org/10.1037/0278-6133.21.4.349

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. Alcohol. Clin. Exp. Res. 19, 92–99.

Antonini, A., Leenders, K.L., Spiegel, R., Meier, D., Vontobel, P., Weigell-Weber, M., Sanchez-Pernaute, R., Yébenez, D., G, J., Boesiger, P., Weindl, A., Maguire, R.P., 1996. Striatal glucose metabolism and dopamine D2 receptor binding in asymptomatic gene carriers and patients with Huntington's disease. Brain 119, 2085–2095. https://doi.org/10.1093/brain/119.6.2085

Ashok, A.H., Marques, T.R., Jauhar, S., Nour, M.M., Goodwin, G.M., Young, A.H., Howes, O.D., 2017. The dopamine hypothesis of bipolar affective disorder: the state of the art and implications for treatment. Mol. Psychiatry 22, 666–679. https://doi.org/10.1038/mp.2017.16

Babor, T.F., Higgins-Biddle, J.C., Saunders, J.B., Monteiro, M.G., 2001. AUDIT The Alcohol Use Disorders Identification Test: Guidelines for Use in Primary Care, WHO/MSD/MSB/01.6a. World Health Organization, Department of Mental Health and Substance Dependence.

Bachteler, D., Spanagel, R., 2005. Glutamatergic compounds: a perspective, in: Karl, F. (Ed.), Drugs for Relapse Prevention of Alcoholism, Milestones in Drig Therapy MDT. Birkhäuser, Basel, pp. 205–216.

Ballard, M.E., Mandelkern, M.A., Monterosso, J.R., Hsu, E., Robertson, C.L., Ishibashi, K., Dean, A.C., London, E.D., 2015. Low Dopamine D2/D3 Receptor Availability is Associated with Steep Discounting of Delayed Rewards in Methamphetamine Dependence. Int. J. Neuropsychopharmacol. 18. https://doi.org/10.1093/ijnp/pyu119

Barbor, T.F., de la Fuente, J.R., Saunders, J., Grant, M., 1989. AUDIT The Alcohol Use Disorders Identification Test: Guidelines for Use in Primary Health Care., WHO/MNH/DAT 89.4. World Health Organization, Department of Mental Health and Substance Dependence, Geneva.

Batra, A., Müller, C.A., Mann, K., Heinz, A., 2016. Alcohol Dependence and Harmful Use of Alcohol. Dtsch. Ärztebl. Int. 113, 301–310. https://doi.org/10.3238/arztebl.2016.0301

Beaulieu, J.-M., Gainetdinov, R.R., 2011. The Physiology, Signaling, and Pharmacology of Dopamine Receptors. Pharmacol. Rev. 63, 182–217. https://doi.org/10.1124/pr.110.002642

Beck, A., Schlagenhauf, F., Wüstenberg, T., Hein, J., Kienast, T., Kahnt, T.,

Schmack, K., Hägele, C., Knutson, B., Heinz, A., Wrase, J., 2009. Ventral striatal activation during reward anticipation correlates with impulsivity in alcoholics. Biol. Psychiatry 66, 734–742. https://doi.org/10.1016/j.biopsych.2009.04.035
Benjamin, D., Grant, E.R., Pohorecky, L.A., 1993. Naltrexone reverses ethanol-induced dopamine release in the nucleus accumbens in awake, freely moving rats. Brain Res. 621, 137–140.
Berggren, U., Fahlke, C., Aronsson, E., Karanti, A., Eriksson, M., Blennow, K., Thelle, D., Zetterberg, H., Balldin, J., 2006. The taqI DRD2 A1 allele is associated with alcohol-dependence although its effect size is small. Alcohol Alcohol. Oxf. Oxfs. 41, 479–485. https://doi.org/10.1093/alcalc/agl043
Bidwell, L.C., Karoly, H.C., Thayer, R.E., Claus, E.D., Bryan, A.D., Weiland, B.J., YorkWilliams, S., Hutchison, K.E., 2018. DRD2 promoter methylation and measures of alcohol reward: functional activation of reward circuits and clinical severity. Addict. Biol. [Epub ahead of print]. https://doi.org/10.1111/adb.12614
Biederman, J., 2005. Attention-deficit/hyperactivity disorder: a selective overview. Biol. Psychiatry 57, 1215–1220. https://doi.org/10.1016/j.biopsych.2004.10.020
Boileau, I., Assaad, J.-M., Pihl, R.O., Benkelfat, C., Leyton, M., Diksic, M., Tremblay, R.E., Dagher, A., 2003. Alcohol promotes dopamine release in the human nucleus accumbens. Synap. N. Y. N 49, 226–231. https://doi.org/10.1002/syn.10226
Bonnet, U., Lensing, M., Specka, M., Scherbaum, N., 2011. Comparison of two oral symptom-triggered pharmacological inpatient treatments of acute alcohol withdrawal: clomethiazole vs. clonazepam. Alcohol Alcohol. Oxf. Oxfs. 46, 68–73. https://doi.org/10.1093/alcalc/agq081
Boothby, L.A., Doering, P.L., 2005. Acamprosate for the treatment of alcohol dependence. Clin. Ther. 27, 695–714. https://doi.org/10.1016/j.clinthera.2005.06.015
Brody, A.L., Mandelkern, M.A., London, E.D., Childress, A.R., Lee, G.S., Bota, R.G., Ho, M.L., Saxena, S., Baxter, L.R., Madsen, D., Jarvik, M.E., 2002. Brain Metabolic Changes During Cigarette Craving. Arch. Gen. Psychiatry 59, 1162–1172. https://doi.org/10.1001/archpsyc.59.12.1162
Brownell, G.L., 1999. A History of Positron Imaging.pdf.
Brust, J.C.M., 2014. Chapter 8 - Acute withdrawal: diagnosis and treatment, in: Edith V. Sullivan and Adolf Pfefferbaum (Ed.), Handbook of Clinical Neurology. Elsevier, pp. 123–131. https://doi.org/10.1016/B978-0-444-62619-6.00008-2
Buchert, R., Thiele, F., 2008. The simplified reference tissue model for SPECT/PET brain receptor studies - Interpretation of its parameters. Nuklearmedizin 47, 167–174. https://doi.org/10.3413/nukmed-0153
Bush, K., Kivlahan, D.R., McDonell, M.B., Fihn, S.D., Bradley, K.A., 1998. The AUDIT alcohol consumption questions (AUDIT-C): an effective brief screening test for problem drinking. Ambulatory Care Quality Improvement Project (ACQUIP). Alcohol Use Disorders Identification Test. Arch. Intern. Med. 158, 1789–1795.
Carelli, R.M., Ijames, S.G., Crumling, A.J., 2000. Evidence That Separate Neural Circuits in the Nucleus Accumbens Encode Cocaine Versus “Natural” (Water and Food) Reward. J. Neurosci. 20, 4255–4266.
Carlson, N.R., 1998. Psychology of Behavior, 11th edition. ed. MA, Boston.
Carlsson, A., Waters, N., Carlsson, M.L., 1999. Neurotransmitter interactions in schizophrenia--therapeutic implications. Biol. Psychiatry 46, 1388–1395.

Carroll, J.F., 1978. Viewpoints on abstinence and controlled use. Am. J. Drug Alcohol Abuse 5, 327–333.
Chamberlain, S.R., Robbins, T.W., Winder-Rhodes, S., Müller, U., Sahakian, B.J., Blackwell, A.D., Barnett, J.H., 2011. Translational approaches to frontostriatal dysfunction in attention-deficit/hyperactivity disorder using a computerized neuropsychological battery. Biol. Psychiatry 69, 1192–1203. https://doi.org/10.1016/j.biopsych.2010.08.019
Chan, R.C.K., Shum, D., Toulopoulou, T., Chen, E.Y.H., 2008. Assessment of executive functions: review of instruments and identification of critical issues. Arch. Clin. Neuropsychol. Off. J. Natl. Acad. Neuropsychol. 23, 201–216. https://doi.org/10.1016/j.acn.2007.08.010
Chastain, G., 2006. Alcohol, neurotransmitter systems, and behavior. J. Gen. Psychol. 133, 329–335. https://doi.org/10.3200/GENP.133.4.329-335
Comings, D.E., Blum, K., 2000. Reward deficiency syndrome: genetic aspects of behavioral disorders. Prog. Brain Res. 126, 325–341. https://doi.org/10.1016/S0079-6123(00)26022-6
Conigrave, K.M., Hall, W.D., Saunders, J.B., 1995. The AUDIT questionnaire: choosing a cut-off score. Addiction 90, 1349–1356. https://doi.org/10.1046/j.1360-0443.1995.901013496.x
Corbit, L.H., Nie, H., Janak, P.H., 2012. Habitual Alcohol Seeking: Time Course and the Contribution of Subregions of the Dorsal Striatum. Biol. Psychiatry, New Insights into the Consumption of Rewarding Substances 72, 389–395. https://doi.org/10.1016/j.biopsych.2012.02.024
Corral, M., Holguín, S.R., Cadaveira, F., 2003. Neuropsychological characteristics of young children from high-density alcoholism families: a three-year follow-up. J. Stud. Alcohol 64, 195–199. https://doi.org/10.15288/jsa.2003.64.195
Cowen, M.S., Lawrence, A.J., 1999. The role of opioid-dopamine interactions in the induction and maintenance of ethanol consumption. Prog. Neuropsychopharmacol. Biol. Psychiatry 23, 1171–1212. https://doi.org/10.1016/S0278-5846(99)00060-3
Del Arco, A., Segovia, G., Mora, F., 2008. Blockade of NMDA receptors in the prefrontal cortex increases dopamine and acetylcholine release in the nucleus accumbens and motor activity. Psychopharmacology (Berl.) 201, 325–338. https://doi.org/10.1007/s00213-008-1288-3
Del Campo, N., Chamberlain, S.R., Sahakian, B.J., Robbins, T.W., 2011. The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. Biol. Psychiatry 69, e145-157. https://doi.org/10.1016/j.biopsych.2011.02.036
Deserno, L., Beck, A., Huys, Q.J.M., Lorenz, R.C., Buchert, R., Buchholz, H.-G., Plotkin, M., Kumakara, Y., Cumming, P., Heinze, H.-J., Grace, A.A., Rapp, M.A., Schlagenhauf, F., Heinz, A., 2015. Chronic alcohol intake abolishes the relationship between dopamine synthesis capacity and learning signals in the ventral striatum. Eur. J. Neurosci. 41, 477–486. https://doi.org/10.1111/ejn.12802
Di Chiara, G., 1997. Alcohol and dopamine. Alcohol Health Res. World 21, 108–114.
Di Chiara, G., Bassareo, V., 2007. Reward system and addiction: what dopamine does and doesn't do. Curr. Opin. Pharmacol., Neurosciences 7, 69–76. https://doi.org/10.1016/j.coph.2006.11.003
Di Chiara, G., Imperato, A., 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. U. S. A. 85, 5274–5278.

Doyon, W.M., Thomas, A.M., Ostroumov, A., Dong, Y., Dani, J.A., 2013. Potential Substrates for Nicotine and Alcohol Interactions: a Focus on the Mesocorticolimbic Dopamine System. Biochem. Pharmacol. 86, 1181–1193. https://doi.org/10.1016/j.bcp.2013.07.007
Drummond, D.C., 2001. Theories of drug craving, ancient and modern. Addiction 96, 33–46. https://doi.org/10.1080/09652140020016941
Drummond, D.C., Litten, R.Z., Lowman, C., Hunt, W.A., 2000. Craving research: future directions. Addiction 95, S247–S255.
Effertz, T., Mann, K., 2013. The burden and cost of disorders of the brain in Europe with the inclusion of harmful alcohol use and nicotine addiction - 1-s2.0-S0924977X12001927-main.pdf European Neuropsychopharmacology Volume 23, Issue 7, Pages 742–748.
EMA (European Medicines Agency). Annual report 2010 (No. EMA/306870/2011.), 2011.
Erritzoe, D., Tziortzi, A., Bargiela, D., Colasanti, A., Searle, G.E., Gunn, R.N., Beaver, J.D., Waldman, A., Nutt, D.J., Bani, M., Merlo-Pich, E., Rabiner, E.A., Lingford-Hughes, A., 2014. In Vivo Imaging of Cerebral Dopamine D3 Receptors in Alcoholism. Neuropsychopharmacology 39, 1703–1712. https://doi.org/10.1038/npp.2014.18
Everitt, B.J., Belin, D., Economidou, D., Pelloux, Y., Dalley, J.W., Robbins, T.W., 2008. Review. Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 363, 3125–3135. https://doi.org/10.1098/rstb.2008.0089
Everitt, B.J., Dickinson, A., Robbins, T.W., 2001. The neuropsychological basis of addictive behaviour. Brain Res. Rev., The Brain in Health and Disease - from Molecules to Man. Swiss National Foundation Symposium NRP 38 36, 129–138. https://doi.org/10.1016/S0165-0173(01)00088-1
Everitt, B.J., Robbins, T.W., 2005. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat. Neurosci. 8, 1481–1489. https://doi.org/10.1038/nn1579
Fagerström, K.O., 1978. Measuring degree of physical dependence to tobacco smoking with reference to individualization of treatment. Addict. Behav. 3, 235–241.
Fagerstrom, K.O., Schneider, N.G., 1989. Measuring nicotine dependence: a review of the Fagerstrom Tolerance Questionnaire. J. Behav. Med. 12, 159–182.
Farde, L., Gustavsson, J.P., Jönsson, E., 1997. D2 dopamine receptors and personality traits. Nature 385, 590–590. https://doi.org/10.1038/385590a0
Fehr, C., Yakushev, I., Hohmann, N., Buchholz, H.-G., Landvogt, C., Deckers, H., Eberhardt, A., Kläger, M., Smolka, M.N., Scheurich, A., Dielentheis, T., Schmidt, L.G., Rösch, F., Bartenstein, P., Gründer, G., Schreckenberger, M., 2008. Association of Low Striatal Dopamine D 2 Receptor Availability With Nicotine Dependence Similar to That Seen With Other Drugs of Abuse. Am. J. Psychiatry 165, 507–514. https://doi.org/10.1176/appi.ajp.2007.07020352
Ferguson, S.G., Shiffman, S., 2008. The relevance and treatment of cue-induced cravings in tobacco dependence.pdf. Journal of Substance Abuse Treatment 36, 235–243.
Field, A.P., 2009. Discovering statistics using SPSS: (and sex and drugs and rock “n” roll)., 3rd edition. ed. Thousand Oaks, Calif.: SAGE Publications., Los Angeles.
Floresco, S.B., Todd, C.L., Grace, A.A., 2001. Glutamatergic afferents from the hippocampus to the nucleus accumbens regulate activity of ventral tegmental

area dopamine neurons. J. Neurosci. Off. J. Soc. Neurosci. 21, 4915–4922.
Fotros, A., Casey, K.F., Larcher, K., Verhaeghe, J.A., Cox, S.M., Gravel, P., Reader, A.J., Dagher, A., Benkelfat, C., Leyton, M., 2013. Cocaine Cue-Induced Dopamine Release in Amygdala and Hippocampus: A High-Resolution PET [18F]Fallypride Study in Cocaine Dependent Participants. Neuropsychopharmacology 38, 1780–1788. https://doi.org/10.1038/npp.2013.77
Fowler, J.S., Wolf, A.P., 1973. Short-Lived Positron Emitter Labeled Radiotracers - Present Status. Chemistry Department, Brookhaven National Laboratory, Upton, NY.
Gaertner, B., Freyer-Adam, J., Meyer, C., John, U., 2015. Gaertner B, Freyer-Adam J, Meyer C, John U: Alkohol – Zahlen und Fakten zum Konsum. Pabst, Lengerich.
Gallagher, B.M., Ansari, A., Atkins, H., Casella, V., Christman, D.R., Fowler, J.S., Ido, T., MacGregor, R.R., Som, P., Wan, C.N., Wolf, A.P., Kuhl, D.E., Reivich, M., 1977. Radiopharmaceuticals XXVII. 18F-labeled 2-deoxy-2-fluoro-d-glucose as a radiopharmaceutical for measuring regional myocardial glucose metabolism in vivo: tissue distribution and imaging studies in animals. J. Nucl. Med. Off. Publ. Soc. Nucl. Med. 18, 990–996.
Garbusow, M., Schad, D.J., Sommer, C., Jünger, E., Sebold, M., Friedel, E., Wendt, J., Kathmann, N., Schlagenhauf, F., Zimmermann, U.S., Heinz, A., Huys, Q.J.M., Rapp, M.A., 2014a. Pavlovian-to-Instrumental Transfer in Alcohol Dependence: A Pilot Study. Neuropsychobiology 70, 111–121. https://doi.org/10.1159/000363507
Garbusow, M., Sebold, M., Beck, A., Heinz, A., 2014b. Too Difficult to Stop: Mechanisms Facilitating Relapse in Alcohol Dependence. Neuropsychobiology 70, 103–110. https://doi.org/10.1159/000362838
Gleich, T., Deserno, L., Lorenz, R.C., Boehme, R., Pankow, A., Buchert, R., Kühn, S., Heinz, A., Schlagenhauf, F., Gallinat, J., 2015. Prefrontal and Striatal Glutamate Differently Relate to Striatal Dopamine: Potential Regulatory Mechanisms of Striatal Presynaptic Dopamine Function? J. Neurosci. Off. J. Soc. Neurosci. 35, 9615–9621. https://doi.org/10.1523/JNEUROSCI.0329-15.2015
Goldman, D., Oroszi, G., Ducci, F., 2005. The genetics of addictions: uncovering the genes. Nat. Rev. Genet. 6, 521–532. https://doi.org/10.1038/nrg1635
Goldstein, R.Z., Volkow, N.D., 2011. Dysfunction of the prefrontal cortex in addiction: neuroimaging findings and clinical implications. Nat. Rev. Neurosci. 12, 652–669. https://doi.org/10.1038/nrn3119
Grant, S., London, E.D., Newlin, D.B., Villemagne, V.L., Liu, X., Contoreggi, C., Phillips, R.L., Kimes, A.S., Margolin, A., 1996. Activation of memory circuits during cue-elicited cocaine craving. Proc. Natl. Acad. Sci. U. S. A. 93, 12040–12045.
Grobin, A.C., Matthews, D.B., Devaud, L.L., Morrow, A.L., 1998. The role of GABA(A) receptors in the acute and chronic effects of ethanol. Psychopharmacology (Berl.) 139, 2–19.
Grüsser, S.M., Wrase, J., Klein, S., Hermann, D., Smolka, M.N., Ruf, M., Weber-Fahr, W., Flor, H., Mann, K., Braus, D.F., Heinz, A., 2004. Cue-induced activation of the striatum and medial prefrontal cortex is associated with subsequent relapse in abstinent alcoholics. Psychopharmacology (Berl.) 175, 296–302. https://doi.org/10.1007/s00213-004-1828-4
Guardia, J., Catafau, A.M., Batlle, F., Martín, J.C., Segura, L., Gonzalvo, B., Prat, G.,

Carrió, I., Casas, M., 2000. Striatal dopaminergic D(2) receptor density measured by [(123)I]iodobenzamide SPECT in the prediction of treatment outcome of alcohol-dependent patients. Am. J. Psychiatry 157, 127–129. https://doi.org/10.1176/ajp.157.1.127
Gunn, R.N., Lammertsma, A.A., Hume, S.P., Cunningham, V.J., 1997. Parametric imaging of ligand-receptor binding in PET using a simplified reference region model. NeuroImage 6, 279–287. https://doi.org/10.1006/nimg.1997.0303
Haber, S.N., Fudge, J.L., McFarland, N.R., 2000. Striatonigrostriatal Pathways in Primates Form an Ascending Spiral from the Shell to the Dorsolateral Striatum. J. Neurosci. 20, 2369–2382. https://doi.org/10.1523/JNEUROSCI.20-06-02369.2000
Hakan, H., Halldin, C., Dijkstra, D., Wilkström, H., Wise, L.D., Pugsley, T.A., Sokoloff, P., Farde, L., Pauli, S., Sedvall, G., 1996. Autoradiographic localisation of D3 - dopamine receptors in the human brain using the selective D3 - dopamine receptor agonist (+)-[3 H]PD 128907. Psychopharmacology 128, 240–247.
Hamacher, K., Coenen, H.H., Stöcklin, G., 1986. Efficient stereospecific synthesis of no-carrier-added 2-[18F]-fluoro-2-deoxy-d-glucose using aminopolyether supported nucleophilic substitution. J Nucl Med 27, 235–238.
Heatherton, T.F., Kozlowski, L.T., Frecker, R.C., Fagerstrom, K.-O., 1991. The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. Br. J. Addict. 86, 1119–1127.
Heinz, A., 2002. Dopaminergic dysfunction in alcoholism and schizophrenia – psychopathological and behavioral correlates. Eur. Psychiatry 17, 9–16. https://doi.org/10.1016/S0924-9338(02)00628-4
Heinz, A., Beck, A., Mir, J., Grüsser, S.M., Grace, A.A., Wrase, J., 2010. Alcohol Craving and Relapse Prediction: Imaging Studies, in: Kuhn, C.M., Koob, G.F. (Eds.), Advances in the Neuroscience of Addiction, Frontiers in Neuroscience. CRC Press/Taylor & Francis, Boca Raton (FL).
Heinz, A., Beck, A., Wrase, J., Mohr, J., Obermayer, K., Gallinat, J., Puls, I., 2009. Neurotransmitter Systems in Alcohol Dependence. Pharmacopsychiatry 42, S95–S101. https://doi.org/10.1055/s-0029-1214395
Heinz, A., Dettling, M., Kuhn, S., Dufeu, P., Gräf, K.J., Kürten, I., Rommelspacher, H., Schmidt, I.G., 1995. Blunted growth hormone response is associated with early relapse in alcohol-dependent patients. Alcohol. Clin. Exp. Res. 19, 62–65.
Heinz, A., Dufeu, P., Kuhn, S., Dettling, M., Gräf, K., Kürten, I., Rommelspacher, H., Schmidt, L.G., 1996. Psychopathological and behavioral correlates of dopaminergic sensitivity in alcohol-dependent patients. Arch. Gen. Psychiatry 53, 1123–1128.
Heinz, A., Friedel, E., Müller, D.J., Puls, I., Wrase, J., 2007. Genetic Research with Intermediate Phenotypes: Phenocopies, Perspectives and Pitfalls. Addiction 102, 1696–1697. https://doi.org/10.1111/j.1360-0443.2007.01994.x
Heinz, A., Goldman, D., Gallinat, J., Schumann, G., Puls, I., 2004a. Pharmacogenetic insights to monoaminergic dysfunction in alcohol dependence. Psychopharmacology (Berl.) 174, 561–570. https://doi.org/10.1007/s00213-004-1903-x
Heinz, A., Goldman, D., Jones, D.W., Palmour, R., Hommer, D., Gorey, J.G., Lee, K.S., Linnoila, M., Weinberger, D.R., 2000. Genotype Influences In Vivo Dopamine Transporter Availability in Human Striatum. Neuropsychopharmacology 22, 133–139. https://doi.org/10.1016/S0893-

133X(99)00099-8
Heinz, A., Mann, K., Weinberger, D.R., Goldman, D., 2001. Serotonergic dysfunction, negative mood states, and response to alcohol. Alcohol. Clin. Exp. Res. 25, 487–495.
Heinz, A., Ragan, P., Jones, D.W., Hommer, D., Williams, W., Knable, M.B., Gorey, J.G., Doty, L., Geyer, C., Lee, K.S., Coppola, R., Weinberger, D.R., Linnoila, M., 1998. Reduced central serotonin transporters in alcoholism. Am. J. Psychiatry 155, 1544–1549. https://doi.org/10.1176/ajp.155.11.1544
Heinz, A., Siessmeier, T., Wrase, J., Buchholz, H.G., Gründer, G., Kumakura, Y., Cumming, P., Schreckenberger, M., Smolka, M.N., Rösch, F., Mann, K., Bartenstein, P., 2005. Correlation of Alcohol Craving With Striatal Dopamine Synthesis Capacity and D2/3 Receptor Availability: A Combined [18F]DOPA and [18F]DMFP PET Study in Detoxified Alcoholic Patients. Am. J. Psychiatry 162, 1515–1520. https://doi.org/10.1176/appi.ajp.162.8.1515
Heinz, A., Siessmeier, T., Wrase, J., Hermann, D., Klein, S., Grüsser-Sinopoli, S.M., Flor, H., Braus, D.F., Buchholz, H.G., Gründer, G., Schreckenberger, M., Smolka, M.N., Rösch, F., Mann, K., Bartenstein, P., 2004b. Correlation Between Dopamine D2 Receptors in the Ventral Striatum and Central Processing of Alcohol Cues and Craving. Am. J. Psychiatry 161, 1783–1789. https://doi.org/10.1176/ajp.161.10.1783
Herz, A., 1997. Endogenous opioid systems and alcohol addiction. Psychopharmacology (Berl.) 129, 99–111.
Hyman, S.E., 2005. Addiction: A Disease of Learning and Memory. Am. J. Psychiatry 162, 1414–1422. https://doi.org/10.1176/appi.ajp.162.8.1414
Ido, T., Wan, C.-N., Casella, V., Fowler, J.S., Wolf, A.P., Reivich, M., Kuhl, D.E., 1978. Labeled 2-deoxy-D-glucose analogs. 18F-labeled 2-deoxy-2-fluoro-D-glucose, 2-deoxy-2-fluoro-D-mannose and 14C-2-deoxy-2-fluoro-D-glucose. J. Label. Compd. Radiopharm. 14, 175–183. https://doi.org/10.1002/jlcr.2580140204
Ido, T., Wan, C.N., Fowler, J.S., Wolf, A.P., 1977. Fluorination with F2. A convenient synthesis of 2-deoxy-2-fluoro-d-glucose. J Org Chem 42, 2341–2342.
Ikemoto, S., 2010. Brain reward circuitry beyond the mesolimbic dopamine system: A neurobiological theory. Neurosci. Biobehav. Rev. 35, 129–150. https://doi.org/10.1016/j.neubiorev.2010.02.001
Ikemoto, S., 2007. Dopamine reward circuitry: Two projection systems from the ventral midbrain to the nucleus accumbens–olfactory tubercle complex. Brain Res. Rev. 56, 27–78. https://doi.org/10.1016/j.brainresrev.2007.05.004
Innis, R.B., Cunningham, V.J., Delforge, J., Fujita, M., Gjedde, A., Gunn, R.N., Holden, J., Houle, S., Huang, S.-C., Ichise, M., Iida, H., Ito, H., Kimura, Y., Koeppe, R.A., Knudsen, G.M., Knuuti, J., Lammertsma, A.A., Laruelle, M., Logan, J., Maguire, R.P., Mintun, M.A., Morris, E.D., Parsey, R., Price, J.C., Slifstein, M., Sossi, V., Suhara, T., Votaw, J.R., Wong, D.F., Carson, R.E., 2007. Consensus Nomenclature for in vivo Imaging of Reversibly Binding Radioligands. J. Cereb. Blood Flow Metab. 27, 1533–1539. https://doi.org/10.1038/sj.jcbfm.9600493
Ishibashi, K., Robertson, C.L., Mandelkern, M.A., Morgan, A.T., London, E.D., 2013. The Simplified Reference Tissue Model with 18F-fallypride PET: Choice of Reference Region. Mol. Imaging 12.
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. J. Neurosci. 22, 6247–6253.

Jacobi, F., Mack, S., Gerschler, A., Scholl, L., Höfler, M., Siegert, J., Bürkner, A., Preiss, S., Spitzer, K., Busch, M., Hapke, U., Gaebel, W., Maier, W., Wagner, M., Zielasek, J., Wittchen, H.-U., 2013. The design and methods of the mental health module in the German Health Interview and Examination Survey for Adults (DEGS1-MH). Int. J. Methods Psychiatr. Res. 22, 83–99. https://doi.org/10.1002/mpr.1387

Joel, D., Weiner, I., 2000. The connections of the dopaminergic system with the striatum in rats and primates: an analysis with respect to the functional and compartmental organization of the striatum. Neuroscience 96, 451–474.

Kalivas, P.W., Volkow, N., Seamans, J., 2005. Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission. Neuron 45, 647–650. https://doi.org/10.1016/j.neuron.2005.02.005

Kamp, F., Proebstl, L., Penzel, N., Adorjan, K., Ilankovic, A., Pogarell, O., Koller, G., Soyka, M., Falkai, P., Koutsouleris, N., Kambeitz, J., 2018. Effects of sedative drug use on the dopamine system: a systematic review and meta-analysis of in vivo neuroimaging studies. Neuropsychopharmacology 1. https://doi.org/10.1038/s41386-018-0191-9

Kim, S.H., Baik, S.-H., Park, C.S., Kim, S.J., Choi, S.W., Kim, S.E., 2011. Reduced striatal dopamine D2 receptors in people with Internet addiction. Neuroreport 22, 407–411. https://doi.org/10.1097/WNR.0b013e328346e16e

Köhler, C., Hall, H., Ögren, S.-O., Gawell, L., 1985. Specific in vitro and in vivo binding of 3H-raclopride a potent substituted benzamide drug with high affinity for dopamine D-2 receptors in the rat brain. Biochem. Pharmacol. 34, 2251–2259. https://doi.org/10.1016/0006-2952(85)90778-6

Koob, G.F., 2014. Chapter 3 - Neurocircuitry of alcohol addiction: synthesis from animal models, in: Edith V. Sullivan and Adolf Pfefferbaum (Ed.), Handbook of Clinical Neurology. Elsevier, pp. 33–54. https://doi.org/10.1016/B978-0-444-62619-6.00003-3

Koob, G.F., Volkow, N.D., 2010. Neurocircuitry of addiction. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 35, 217–238. https://doi.org/10.1038/npp.2009.110

Kozlowski, L.T., Wilkinson, D.A., 1987. Use and Misuse of the Concept of Craving by Alcohol, Tobacco, and Drug Researchers. Br. J. Addict. 82, 31–36.

Kruskal, W., Wallis, W., 1952. Use of ranks in one criterion variance analysis. Journal of the American Statistical Association 47, 583–621.

Krystal, J.H., Staley, J., Mason, G., Petrakis, I.L., Kaufman, J., Harris, R.A., Gelernter, J., Lappalainen, J., 2006. Gamma-aminobutyric acid type A receptors and alcoholism: intoxication, dependence, vulnerability, and treatment. Arch. Gen. Psychiatry 63, 957–968. https://doi.org/10.1001/archpsyc.63.9.957

Kumakura, Y., Gjedde, A., Caprioli, D., Kienast, T., Beck, A., Plotkin, M., Schlagenhauf, F., Vernaleken, I., Gründer, G., Bartenstein, P., Heinz, A., Cumming, P., 2013. Increased Turnover of Dopamine in Caudate Nucleus of Detoxified Alcoholic Patients. PLoS ONE 8. https://doi.org/10.1371/journal.pone.0073903

Lancaster, J.L., Woldorff, M.G., Parsons, L.M., Liotti, M., Freitas, C.S., Rainey, L., Kochunov, P.V., Nickerson, D., Mikiten, S.A., Fox, P.T., 2000. Automated Talairach atlas labels for functional brain mapping. Hum. Brain Mapp. 10, 120–131.

Lange, K., Carson, R., 1984. EM reconstruction algorithms for emission and transmission tomography. J. Comput. Assist. Tomogr. 8, 306–316.

Laruelle, M., Abi-Dargham, A., Dyck, C.H. van, Gil, R., D'Souza, C.D., Erdos, J., McCance, E., Rosenblatt, W., Fingado, C., Zoghbi, S.S., Baldwin, R.M., Seibyl, J.P., Krystal, J.H., Charney, D.S., Innis, R.B., 1996. Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. Proc. Natl. Acad. Sci. 93, 9235–9240.

Laruelle, M., D'Souza, C.D., Baldwin, R.M., Abi-Dargham, A., Kanes, S.J., Fingado, C.L., Seibyl, J.P., Zoghbi, S.S., Bowers, M.B., Jatlow, P., Charney, D.S., Innis, R.B., 1997. Imaging D2 Receptor Occupancy by Endogenous Dopamine in Humans. Neuropsychopharmacology 17, 162–174. https://doi.org/10.1016/S0893-133X(97)00043-2

Laruelle, M., Slifstein, M., Huang, Y., 2003. Relationships between radiotracer properties and image quality in molecular imaging of the brain with positron emission tomography. Mol. Imaging Biol. MIB Off. Publ. Acad. Mol. Imaging 5, 363–375.

Lee, B., London, E.D., Poldrack, R.A., Farahi, J., Nacca, A., Monterosso, J.R., Mumford, J.A., Bokarius, A.V., Dahlbom, M., Mukherjee, J., Bilder, R.M., Brody, A.L., Mandelkern, M.A., 2009. Striatal Dopamine D2/D3 Receptor Availability is Reduced in Methamphetamine Dependence and is Linked to Impulsivity. J. Neurosci. Off. J. Soc. Neurosci. 29, 14734. https://doi.org/10.1523/JNEUROSCI.3765-09.2009

Lim, S.S., Vos, T., Flaxman, A.D., Danaei, G., Shibuya, K., Adair-Rohani, H., Amann, M., Anderson, H.R., Andrews, K.G., Aryee, M., Atkinson, C., Bacchus, L.J., Bahalim, A.N., Balakrishnan, K., Balmes, J., Barker-Collo, S., Baxter, A., Bell, M.L., Blore, J.D., Blyth, F., Bonner, C., Borges, G., Bourne, R., Boussinesq, M., Brauer, M., Brooks, P., Bruce, N.G., Brunekreef, B., Bryan-Hancock, C., Bucello, C., Buchbinder, R., Bull, F., Burnett, R.T., Byers, T.E., Calabria, B., Carapetis, J., Carnahan, E., Chafe, Z., Charlson, F., Chen, H., Chen, J.S., Cheng, A.T.-A., Child, J.C., Cohen, A., Colson, K.E., Cowie, B.C., Darby, S., Darling, S., Davis, A., Degenhardt, L., Dentener, F., Des Jarlais, D.C., Devries, K., Dherani, M., Ding, E.L., Dorsey, E.R., Driscoll, T., Edmond, K., Ali, S.E., Engell, R.E., Erwin, P.J., Fahimi, S., Falder, G., Farzadfar, F., Ferrari, A., Finucane, M.M., Flaxman, S., Fowkes, F.G.R., Freedman, G., Freeman, M.K., Gakidou, E., Ghosh, S., Giovannucci, E., Gmel, G., Graham, K., Grainger, R., Grant, B., Gunnell, D., Gutierrez, H.R., Hall, W., Hoek, H.W., Hogan, A., Hosgood, H.D., Hoy, D., Hu, H., Hubbell, B.J., Hutchings, S.J., Ibeanusi, S.E., Jacklyn, G.L., Jasrasaria, R., Jonas, J.B., Kan, H., Kanis, J.A., Kassebaum, N., Kawakami, N., Khang, Y.-H., Khatibzadeh, S., Khoo, J.-P., Kok, C., Laden, F., Lalloo, R., Lan, Q., Lathlean, T., Leasher, J.L., Leigh, J., Li, Y., Lin, J.K., Lipshultz, S.E., London, S., Lozano, R., Lu, Y., Mak, J., Malekzadeh, R., Mallinger, L., Marcenes, W., March, L., Marks, R., Martin, R., McGale, P., McGrath, J., Mehta, S., Mensah, G.A., Merriman, T.R., Micha, R., Michaud, C., Mishra, V., Hanafiah, K.M., Mokdad, A.A., Morawska, L., Mozaff arian, D., Murphy, T., Naghavi, M., Neal, B., Nelson, P.K., Nolla, J.M., Norman, R., Olives, C., Omer, S.B., Orchard, J., Osborne, R., Ostro, B., Page, A., Pandey, K.D., Parry, C.D.H., Passmore, E., Patra, J., Pearce, N., Pelizzari, P.M., Petzold, M., Phillips, M.R., Pope, D., Pope III, C.A., Powles, J., Rao, M., Razavi, H., Rehfuess, E.A., Rehm, J.T., Ritz, B., Rivara, F.P., Roberts, T., Robinson, C., Rodriguez-Portales, J.A., Romieu, I., Room, R., Rosenfeld, L.C., Roy, A., Rushton, L., Salomon, J.A., Sampson, U., Sanchez-Riera, L., Sanman, E., Sapkota, A., Seedat, S., Shi, P., Shield, K., Shivakoti, R., Singh,

G.M., Sleet, D.A., Smith, E., Smith, K.R., Stapelberg, N.J.C., Steenland, K., Stöckl, H., Stovner, L.J., Straif, K., Straney, L., Thurston, G.D., Tran, J.H., Van Dingenen, R., van Donkelaar, A., Veerman, J.L., Vijayakumar, L., Weintraub, R., Weissman, M.M., White, R.A., Whiteford, H., Wiersma, S.T., Wilkinson, J.D., Williams, H.C., Williams, W., Wilson, N., Woolf, A.D., Yip, P., Zielinski, J.M., Lopez, A.D., Murray, C.J.L., Ezzati, M., 2012. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380, 2224–2260. https://doi.org/10.1016/S0140-6736(12)61766-8

Malenka, R., Nestler, E., Hyman, S., 2009a. Reinforcement and Addictive Disorders, in: Molecular Neuropharmacology: A Foundation for Clinical Neuroscience (2nd Ed.). McGraw-Hill, New York, NY, p. Chapter 15, p 372.

Malenka, R., Nestler, E., Hyman, S., 2009b. Neural and Neuroendocrine Control of the Internal Milieu, in: Molecular Neuropharmacology: A Foundation for Clinical Neuroscience (2nd Ed.). McGraw-Hill, New York, NY, p. Chapter 10, p 249.

Malenka, R., Nestler, E., Hyman, S., 2009c. Widely Projecting Systems: Monoamines, Acetylcholine, and Orexin, in: Molecular Neuropharmacology: A Foundation for Clinical Neuroscience (2nd Ed.). McGraw-Hill, New York, NY, p. Chapter 6, pp. 147–148, 154–157.

Mann, H., Whitney, D., 1947. On a test of whether one of two random variables is stochastically larger than the other. Annals of Mathematical Statistics 18, 50–60.

Mann, K., Batra, A., Hoch, E., Reymann, G., Lorenz, G., Petersen, K.U. (Eds.), 2016. S3-Leitlinie: Screening, Diagnose und Behandlung alkoholbezogener Störungen.

Martinez, D., Gil, R., Slifstein, M., Hwang, D.-R., Huang, Y., Perez, A., Kegeles, L., Talbot, P., Evans, S., Krystal, J., Laruelle, M., Abi-Dargham, A., 2005. Alcohol dependence is associated with blunted dopamine transmission in the ventral striatum. Biol. Psychiatry 58, 779–786. https://doi.org/10.1016/j.biopsych.2005.04.044

Martinez, D., Slifstein, M., Broft, A., Mawlawi, O., Hwang, D.-R., Huang, Y., Cooper, T., Kegeles, L., Zarahn, E., Abi-Dargham, A., Haber, S.N., Laruelle, M., 2003. Imaging human mesolimbic dopamine transmission with positron emission tomography. Part II: amphetamine-induced dopamine release in the functional subdivisions of the striatum. J. Cereb. Blood Flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab. 23, 285–300. https://doi.org/10.1097/01.WCB.0000048520.34839.1A

MATLAB 8.0 and Statistics Toolbox 8.1, 2012. . The MathWorks, Inc., Natick, Massachusetts, United States.

Mawlawi, O., Martinez, D., Slifstein, M., Broft, A., Chatterjee, R., Hwang, D.-R., Huang, Y., Simpson, N., Ngo, K., Van Heertum, R., Laruelle, M., 2001. Imaging Human Mesolimbic Dopamine Transmission with Positron Emission Tomography: I. Accuracy and Precision of D2 Receptor Parameter Measurements in Ventral Striatum. J. Cereb. Blood Flow Metab. 21, 1034–1057. https://doi.org/10.1097/00004647-200109000-00002

McClernon, F.J., Hiott, F.B., Huettel, S.A., Rose, J.E., 2005. Abstinence-Induced Changes in Self-Report Craving Correlate with Event-Related fMRI Responses to Smoking Cues. Neuropsychopharmacology 30, 1940–1947. https://doi.org/10.1038/sj.npp.1300780

Miele, G.M., Carpenter, K.M., Smith Cockerham, M., Dietz Trautman, K., Blaine, J., Hasin, D.S., 2000. Concurrent and predictive validity of the Substance Dependence Severity Scale (SDSS). Drug Alcohol Depend. 59, 77–88.
Mikla, V.I., Mikla, V.V., 2014. 4 - Positron Emission Tomography, in: Medical Imaging Technology. Elsevier, Oxford, pp. 53–64. https://doi.org/10.1016/B978-0-12-417021-6.00004-6
Moal, M.L., Simon, H., 1991. Mesocorticolimbic dopaminergic network: functional and regulatory roles. Physiol. Rev. 71, 155–234.
Morales, A.A., Kohno, M., Robertson, C.L., Dean, A.C., Mandelkern, M.A., London, E.D., 2015. Gray-Matter Volume, Midbrain Dopamine D2/D3 Receptors and Drug Craving in Methamphetamine Users. Mol. Psychiatry 20, 764–771. https://doi.org/10.1038/mp.2015.47
Möykkynen, T., Korpi, E.R., 2012. Acute effects of ethanol on glutamate receptors. Basic Clin. Pharmacol. Toxicol. 111, 4–13. https://doi.org/10.1111/j.1742-7843.2012.00879.x
Mukherjee, J., Christian, B.T., Dunigan, K.A., Shi, B., Narayanan, T.K., Satter, M., Mantil, J., 2002. Brain imaging of 18F-fallypride in normal volunteers: blood analysis, distribution, test-retest studies, and preliminary assessment of sensitivity to aging effects on dopamine D-2/D-3 receptors. Synap. N. Y. N 46, 170–188. https://doi.org/10.1002/syn.10128
Mukherjee, J., Yang, Z.Y., Das, M.K., Brown, T., 1995. Fluorinated benzamide neuroleptics--III. Development of (S)-N-[(1-allyl-2-pyrrolidinyl)methyl]-5-(3-[18F]fluoropropyl)-2, 3-dimethoxybenzamide as an improved dopamine D-2 receptor tracer. Nucl. Med. Biol. 22, 283–296.
Mukherjee, J., Yang, Z.Y., Lew, R., Brown, T., Kronmal, S., Cooper, M.D., Seiden, L.S., 1997. Evaluation of d-amphetamine effects on the binding of dopamine D-2 receptor radioligand, 18F-fallypride in nonhuman primates using positron emission tomography. Synap. N. Y. N 27, 1–13. https://doi.org/10.1002/(SICI)1098-2396(199709)27:1<1::AID-SYN1>3.0.CO;2-9
Munro, C.A., McCaul, M.E., Oswald, L.M., Wong, D.F., Zhou, Y., Brasic, J., Kuwabara, H., Kumar, A., Alexander, M., Ye, W., Wand, G.S., 2006. Striatal Dopamine Release and Family History of Alcoholism. Alcohol. Clin. Exp. Res. 30, 1143–1151. https://doi.org/10.1111/j.1530-0277.2006.00130.x
Naber, D., Soble, M.G., Pickar, D., 1981. Ethanol increases opioid activity in plasma of normal volunteers. Pharmacopsychiatria 14, 160–161. https://doi.org/10.1055/s-2007-1019590
Narendran, R., Mason, N.S., Chen, C.-M., Himes, M., Keating, P., May, M.A., Rabiner, E.A., Laruelle, M., Mathis, C.A., Frankle, W.G., 2011. Evaluation of dopamine $D_2/_3$ specific binding in the cerebellum for the positron emission tomography radiotracer [^{11}C]FLB 457: implications for measuring cortical dopamine release. Synap. N. Y. N 65, 991–997. https://doi.org/10.1002/syn.20926
Nees, F., Tzschoppe, J., Patrick, C.J., Vollstädt-Klein, S., Steiner, S., Poustka, L., Banaschewski, T., Barker, G.J., Büchel, C., Conrod, P.J., Garavan, H., Heinz, A., Gallinat, J., Lathrop, M., Mann, K., Artiges, E., Paus, T., Poline, J.-B., Robbins, T.W., Rietschel, M., Smolka, M.N., Spanagel, R., Struve, M., Loth, E., Schumann, G., Flor, H., 2012. Determinants of Early Alcohol Use In Healthy Adolescents: The Differential Contribution of Neuroimaging and Psychological Factors. Neuropsychopharmacology 37, 986–995. https://doi.org/10.1038/npp.2011.282

NICE. Alcohol-use disorders: diagnosis, assessment and management of harmful drinking and alcohol dependence. (No. CG 115), 2011. National Institute for Health and Clinical Excellence.
Nishikawa, M., Diksic, M., Sakai, Y., Kumano, H., Charney, D., Palacios-Boix, J., Negrete, J., Gill, K., 2009. Alterations in Brain Serotonin Synthesis in Male Alcoholics Measured Using Positron Emission Tomography. Alcohol. Clin. Exp. Res. 33, 233–239. https://doi.org/10.1111/j.1530-0277.2008.00820.x
Okita, K., Ghahremani, D.G., Payer, D.E., Robertson, C.L., Dean, A.C., Mandelkern, M.A., London, E.D., 2016. Emotion dysregulation and amygdala dopamine D2-type receptor availability in methamphetamine users. Drug Alcohol Depend. 161, 163–170. https://doi.org/10.1016/j.drugalcdep.2016.01.029
Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9, 97–113.
Olds, J., 1977. Drives and Reinforcement. Raven, New York, NY.
Pabst, A., Kraus, L., Matos, E., Piontek, D., 2013. Substanzkonsum und substanzbezogene Störungen in Deutschland im Jahr 2012. In: DHS (eds), Jahrbuch Sucht 2013. Pabst, Lengerich.
Paliwal, P., Hyman, S.M., Sinha, R., 2008. Craving predicts time to cocaine relapse: Further validation of the Now and Brief versions of the cocaine craving questionnaire. Drug Alcohol Depend. 93, 252–259. https://doi.org/10.1016/j.drugalcdep.2007.10.002
Pavlov, I., 1927. Conditioned Reflexes. Oxford Univ. Press, Oxford.
Pfeifer, P., Tüscher, O., Buchholz, H.G., Gründer, G., Vernaleken, I., Paulzen, M., Zimmermann, U.S., Maus, S., Lieb, K., Eggermann, T., Fehr, C., Schreckenberger, M., 2016. Acute effect of intravenously applied alcohol in the human striatal and extrastriatal D2/D3 dopamine system. Addict. Biol. n/a-n/a. https://doi.org/10.1111/adb.12424
Pickens, R.W., Johanson, C.-E., 1992. Craving: consensus of status and agenda for future research. Drug Alcohol Depend. 30, 127–131. https://doi.org/10.1016/0376-8716(92)90017-7
Plass, D., Vos, T., Hornberg, C., Scheidt-Nave, C., Zeeb, H., Krämer, A., 2014. Trends in Disease Burden in Germany. Dtsch. Ärztebl. Int. 111, 629–638. https://doi.org/10.3238/arztebl.2014.0629
Plisson, C., Huiban, M., Pampols-Maso, S., Singleton, G., Hill, S.P., Passchier, J., 2012. Automated preparation of the dopamine D2/3 receptor agonist ligand [11C]-(+)-PHNO for human PET imaging studies. Appl. Radiat. Isot. Data Instrum. Methods Use Agric. Ind. Med. 70, 380–387. https://doi.org/10.1016/j.apradiso.2011.09.018
Politis, M., 2014. Neuroimaging in Parkinson disease: from research setting to clinical practice. Nat. Rev. Neurol. 10, 708–722. https://doi.org/10.1038/nrneurol.2014.205
Potgieter, A.S., Deckers, F., Geerlings, P., 1999. Craving and relapse measurement in alcoholism. Alcohol Alcohol. Oxf. Oxfs. 34, 254–260.
Prescott, C.A., Kendler, K.S., 1999. Genetic and environmental contributions to alcohol abuse and dependence in a population-based sample of male twins. Am. J. Psychiatry 156, 34–40. https://doi.org/10.1176/ajp.156.1.34
Racz, I., Schürmann, B., Karpushova, A., Reuter, M., Cichon, S., Montag, C., Fürst, R., Schütz, C., Franke, P.E., Strohmaier, J., Wienker, T.F., Terenius, L., Ösby, U., Gunnar, A., Maier, W., Bilkei-Gorzó, A., Nöthen, M., Zimmer, A., 2008. The Opioid Peptides Enkephalin and β-Endorphin in Alcohol Dependence. Biol. Psychiatry, Mechanisms of Addiction and Impaired Impulse Control 64, 989–

997. https://doi.org/10.1016/j.biopsych.2008.05.008
Ravan, S., Martinez, D., Slifstein, M., Abi-Dargham, A., 2014. Molecular imaging in alcohol dependence. Handb. Clin. Neurol. 125, 293–311. https://doi.org/10.1016/B978-0-444-62619-6.00018-5
Rennie, M.J., 1999. An introduction to the use of tracers in nutrition and metabolism. Proc. Nutr. Soc. 58, 935–944.
Repo, E., 1999. Dopamine transporter and D2-receptor density in late-onset alcoholism. - PubMed - NCBI [WWW Document]. URL https://www.ncbi.nlm.nih.gov/pubmed/?term=repo+1999+epidepride (accessed 11.20.18).
Repo, E., Kuikka, J.T., Bergström, K.A., Karhu, J., Hiltunen, J., Tiihonen, J., 1999. Dopamine transporter and D2-receptor density in late-onset alcoholism. Psychopharmacology (Berl.) 147, 314–318.
Rice, M.E., Patel, J.C., Cragg, S.J., 2011. Dopamine release in the basal ganglia. Neuroscience 198, 112–137. https://doi.org/10.1016/j.neuroscience.2011.08.066
Robertson, C.L., Ishibashi, K., Chudzynski, J., Mooney, L.J., Rawson, R.A., Dolezal, B.A., Cooper, C.B., Brown, A.K., Mandelkern, M.A., London, E.D., 2016. Effect of Exercise Training on Striatal Dopamine D2/D3 Receptors in Methamphetamine Users during Behavioral Treatment. Neuropsychopharmacology 41, 1629–1636. https://doi.org/10.1038/npp.2015.331
Robinson, T.E., Berridge, K.C., 1993. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res. Brain Res. Rev. 18, 247–291.
Romanelli, R., Williams, J., Neve, K., 2009. Chapter 6: Dopamine receptor signalling: intracellular pathways to behavior., in: The Dopamine Receptors. Springer, pp. 137–174.
Rominger, A., Cumming, P., Xiong, G., Koller, G., Böning, G., Wulff, M., Zwergal, A., Förster, S., Reilhac, A., Munk, O., Soyka, M., Wängler, B., Bartenstein, P., la Fougère, C., Pogarell, O., 2012. [18F]Fallypride PET measurement of striatal and extrastriatal dopamine D 2/3 receptor availability in recently abstinent alcoholics. Addict. Biol. 17, 490–503. https://doi.org/10.1111/j.1369-1600.2011.00355.x
Rose, R.J., Dick, D.M., Viken, R.J., Pulkkinen, L., Kaprio, J., 2001. Drinking or abstaining at age 14? A genetic epidemiological study. Alcohol. Clin. Exp. Res. 25, 1594–1604.
Saitz, R., 2005. Unhealthy Alcohol Use. N. Engl. J. Med. 352, 596–607. https://doi.org/10.1056/NEJMcp042262
Saitz, R., Mayo-Smith, M.F., Roberts, M.S., Redmond, H.A., Bernard, D.R., Calkins, D.R., 1994. Individualized treatment for alcohol withdrawal. A randomized double-blind controlled trial. JAMA 272, 519–523.
Sass, H., Soyka, M., Mann, K., Zieglgänsberger, W., 1996. Relapse prevention by acamprosate. Results from a placebo-controlled study on alcohol dependence. Arch. Gen. Psychiatry 53, 673–680.
Saunders, J.B., Aasland, O.G., Babor, T.F., De La Fuente, J.R., Grant, M., 1993. Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption-II. Addiction 88, 791–804. https://doi.org/10.1111/j.1360-0443.1993.tb02093.x
Schellekens, A.F.A., Franke, B., Ellenbroek, B., Cools, A., Jong, C.A.J. de, Buitelaar, J.K., Verkes, R.-J., 2012. Reduced Dopamine Receptor Sensitivity as an

Intermediate Phenotype in Alcohol Dependence and the Role of the COMT Val158Met and DRD2 Taq1A Genotypes. Arch. Gen. Psychiatry 69, 339–348. https://doi.org/10.1001/archgenpsychiatry.2011.1335
Schneekloth, T.D., Biernacka, J.M., Hall-Flavin, D.K., Karpyak, V.M., Frye, M.A., Loukianova, L.L., Stevens, S.R., Drews, M.S., Geske, J.R., Mrazek, D.A., 2012. Alcohol Craving as a Predictor of Relapse. Am. J. Addict. 21, S20–S26. https://doi.org/10.1111/j.1521-0391.2012.00297.x
Schuckit, M.A., 2016. Alcohol and Alcoholism, in: Harrison's Principles of Internal Medicine. McGraw-Hill, New York, NY.
Schuckit, M.A., 2009. An overview of genetic influences in alcoholism. J. Subst. Abuse Treat. 36, S5-14.
Schultz, W., 2015. Neuronal Reward and Decision Signals: From Theories to Data. Physiol. Rev. 95, 853–951. https://doi.org/10.1152/physrev.00023.2014
Schultz, W., Dayan, P., Montague, P.R., 1997. A neural substrate of prediction and reward. Science 275, 1593–1599.
Schumann, G., 2007. Okey Lecture 2006: identifying the neurobiological mechanisms of addictive behaviour*. Addiction 102, 1689–1695. https://doi.org/10.1111/j.1360-0443.2007.01942.x
Searle, G., Beaver, J.D., Comley, R.A., Bani, M., Tziortzi, A., Slifstein, M., Mugnaini, M., Griffante, C., Wilson, A.A., Merlo-Pich, E., Houle, S., Gunn, R., Rabiner, E.A., Laruelle, M., 2010. Imaging dopamine D3 receptors in the human brain with positron emission tomography, [11C]PHNO, and a selective D3 receptor antagonist. Biol. Psychiatry 68, 392–399. https://doi.org/10.1016/j.biopsych.2010.04.038
Sebold, M., Deserno, L., Nebe, Stephan, Nebe, Stefan, Schad, D.J., Garbusow, M., Hägele, C., Keller, J., Jünger, E., Kathmann, N., Smolka, M.N., Smolka, M., Rapp, M.A., Schlagenhauf, F., Heinz, A., Huys, Q.J.M., 2014. Model-based and model-free decisions in alcohol dependence. Neuropsychobiology 70, 122–131. https://doi.org/10.1159/000362840
Sebold, M., Nebe, S., Garbusow, M., Guggenmos, M., Schad, D.J., Beck, A., Kuitunen-Paul, S., Sommer, C., Frank, R., Neu, P., Zimmermann, U.S., Rapp, M.A., Smolka, M.N., Huys, Q.J.M., Schlagenhauf, F., Heinz, A., 2017. When Habits Are Dangerous: Alcohol Expectancies and Habitual Decision Making Predict Relapse in Alcohol Dependence. Biol. Psychiatry, Learning Theory, Neuroplasticity, and Addiction 82, 847–856. https://doi.org/10.1016/j.biopsych.2017.04.019
Sebold, M., Spitta, G., Gleich, T., Dembler-Stamm, T., Butler, O., Zacharias, K., Aydin, S., Garbusow, M., Rapp, M., Schubert, F., Buchert, R., Gallinat, J., Heinz, A., 2019. Stressful life events are associated with striatal dopamine receptor availability in alcohol dependence. J. Neural Transm. https://doi.org/10.1007/s00702-019-01985-2
Seitz, H., Müller, S., 2011. Alkohol und Karzinogenese, in: Alkohol Und Tabak. Grundlagen Und Folgeerkrankungen. Thieme, Stuttgart und New York, pp. 242–254.
Sharbanee, J.M., Hu, L., Stritzke, W.G.K., Wiers, R.W., Rinck, M., MacLeod, C., 2014. The effect of approach/avoidance training on alcohol consumption is mediated by change in alcohol action tendency. PloS One 9, e85855. https://doi.org/10.1371/journal.pone.0085855
Sher, K.J., Grekin, E.R., Williams, N.A., 2005. The Development of Alcohol Use Disorders. Annu. Rev. Clin. Psychol. 1, 493–523. https://doi.org/10.1146/annurev.clinpsy.1.102803.144107

Singer, M., Batra, A., 2011. Alkohol und Tabak. Grundlagen und Folgeerkrankungen. Thieme, Stuttgart und New York.
Sinha, R., 2013. The clinical neurobiology of drug craving. Curr. Opin. Neurobiol., 23/4 Addiction 23, 649–654. https://doi.org/10.1016/j.conb.2013.05.001
Skinner, B.F., 1938. The Behavior of Organisms. Appleton-Century-Crofts, New York, NY.
Skinner, H.A., Allen, B.A., 1982. Alcohol Dependence Scale: measurement and validation. J. Abnorm. Psychol. 91, 199–209. https://doi.org/10.1037/0021-843X.91.3.199
Skinner, H.A., Horn, J.L., 1984. Alcohol Dependence Scale: Users Guide. Toronto, Canada: Addiction Research Foundation.
Slifstein, M., Hwang, D.-R., Huang, Y., Guo, N., Sudo, Y., Narendran, R., Talbot, P., Laruelle, M., 2004. In vivo affinity of [18F]fallypride for striatal and extrastriatal dopamine D2 receptors in nonhuman primates. Psychopharmacology (Berl.) 175, 274–286. https://doi.org/10.1007/s00213-004-1830-x
Slifstein, M., Kegeles, L.S., Xu, X., Thompson, J.L., Urban, N., Castrillon, J., Hackett, E., Bae, S.-A., Laruelle, M., Abi-Dargham, A., 2010a. Striatal and extrastriatal dopamine release measured with PET and [(18)F] fallypride. Synap. N. Y. N 64, 350–362. https://doi.org/10.1002/syn.20734
Slifstein, M., Kegeles, L.S., Xu, X., Thompson, J.L., Urban, N., Castrillon, J., Hackett, E., Bae, S.-A., Laruelle, M., Abi-Dargham, A., 2010b. Striatal and extrastriatal dopamine release measured with PET and [(18)F] fallypride. Synap. N. Y. N 64, 350–362. https://doi.org/10.1002/syn.20734
Spanagel, R., 2003. [The role of the glutamatergic system in alcohol addiction]. Fortschr. Neurol. Psychiatr. 71 Suppl 1, S33-35. https://doi.org/10.1055/s-2003-40503
Spanagel, R., Pendyala, G., Abarca, C., Zghoul, T., Sanchis-Segura, C., Magnone, M.C., Lascorz, J., Depner, M., Holzberg, D., Soyka, M., Schreiber, S., Matsuda, F., Lathrop, M., Schumann, G., Albrecht, U., 2005. The clock gene Per2 influences the glutamatergic system and modulates alcohol consumption. Nat. Med. 11, 35–42. https://doi.org/10.1038/nm1163
Spreckelmeyer, K.N., Paulzen, M., Raptis, M., Baltus, T., Schaffrath, S., Van Waesberghe, J., Zalewski, M.M., Rösch, F., Vernaleken, I., Schäfer, W.M., Gründer, G., 2011. Opiate-Induced Dopamine Release Is Modulated by Severity of Alcohol Dependence: An [18F]Fallypride Positron Emission Tomography Study. Biol. Psychiatry, New Biomarkers and Treatments for Addiction 70, 770–776. https://doi.org/10.1016/j.biopsych.2011.05.035
Statistical Parametric Mapping (SPM), 2017. . Wellcome Department of Imaging Neuroscience, Institute of Neurology, London, UK.
Substance Abuse and Mental Health Services Administration (SAMHSA). Results from the 2005 National Survey of Drug Use and Health: National Findings., 2006. . SAMHSA – Federal Government Agency, Rockville.
Sullivan, J.T., Sykora, K., Schneiderman, J., Naranjo, C.A., Sellers, E.M., 1989. Assessment of Alcohol Withdrawal: the revised clinical institute withdrawal assessment for alcohol scale (CIWA-Ar). Br. J. Addict. 84, 1353–1357. https://doi.org/10.1111/j.1360-0443.1989.tb00737.x
Surti, S., Kuhn, A., Werner, M.E., Perkins, A.E., Kolthammer, J., Karp, J.S., 2007. Performance of Philips Gemini TF PET/CT scanner with special consideration for its time-of-flight imaging capabilities. J. Nucl. Med. Off. Publ. Soc. Nucl. Med. 48, 471–480.
Talairach, J., Tournoux, P., 1988. Co-planar stereotaxic atlas of the human brain.

Thieme, New York, NY.
Tawa, E.A., Hall, S.D., Lohoff, F.W., 2016. Overview of the Genetics of Alcohol Use Disorder. Alcohol Alcohol 51, 507–514. https://doi.org/10.1093/alcalc/agw046
Taylor, S.B., Lewis, C.R., Olive, M.F., 2013. The neurocircuitry of illicit psychostimulant addiction: acute and chronic effects in humans. Subst. Abuse Rehabil. 4, 29–43. https://doi.org/10.2147/SAR.S39684
Tiffany, S.T., Warthen, M.W., Goedeker, K.C., 2009. The Functional Significance of Craving in Nicotine Dependence, in: Caggiula, A.R., Bevins, R.A. (Eds.), The Motivational Impact of Nicotine and Its Role in Tobacco Use. Springer US, New York, NY, pp. 171–197. https://doi.org/10.1007/978-0-387-78748-0_10
Tsai, G., Gastfriend, D.R., Coyle, J.T., 1995. The glutamatergic basis of human alcoholism. Am. J. Psychiatry 152, 332–340. https://doi.org/10.1176/ajp.152.3.332
Tupala, E., Hall, H., Bergström, K., Särkioja, T., Räsänen, P., Mantere, T., Callaway, J., Hiltunen, J., Tiihonen, J., 2001. Dopamine D(2)/D(3)-receptor and transporter densities in nucleus accumbens and amygdala of type 1 and 2 alcoholics. Mol. Psychiatry 6, 261–267. https://doi.org/10.1038/sj.mp.4000859
Urban, N.B.L., Kegeles, L.S., Slifstein, M., Xu, X., Martinez, D., Sakr, E., Castillo, F., Moadel, T., O'Malley, S.S., Krystal, J.H., Abi-Dargham, A., 2010. Sex differences in striatal dopamine release in young adults after oral alcohol challenge: a PET imaging study with [11C]raclopride. Biol. Psychiatry 68, 689–696. https://doi.org/10.1016/j.biopsych.2010.06.005
Usun, Y., Eybrard, S., Meyer, F., Louilot, A., 2013. Ketamine increases striatal dopamine release and hyperlocomotion in adult rats after postnatal functional blockade of the prefrontal cortex. Behav. Brain Res. 256, 229–237. https://doi.org/10.1016/j.bbr.2013.08.017
Vanderschuren, L.J.M.J., Di Ciano, P., Everitt, B.J., 2005. Involvement of the dorsal striatum in cue-controlled cocaine seeking. J. Neurosci. Off. J. Soc. Neurosci. 25, 8665–8670. https://doi.org/10.1523/JNEUROSCI.0925-05.2005
Vardi, Y., Shepp, L.A., Kaufman, L., 1985. A Statistical Model for Positron Emission Tomography. J. Am. Stat. Assoc. 80, 8–20. https://doi.org/10.1080/01621459.1985.10477119
Verhoeff, N.P., Kapucu, O., Sokole-Busemann, E., Van, E.R., Janssen, A.G., 1993. Estimation of dopamine D2 receptor binding potential in the striatum with iodine-123-IBZM SPECT: technical and interobserver variability. J. Nucl. Med. Off. Publ. Soc. Nucl. Med. 34, 2076–2084.
Victor, M., Adams, R.D., 1953. The effect of alcohol on the nervous system. Res. Publ. - Assoc. Res. Nerv. Ment. Dis. 32, 526–573.
Volkow, N.D., Fowler, J.S., 2000. Addiction, a disease of compulsion and drive: involvement of the orbitofrontal cortex. Cereb. Cortex N. Y. N 1991 10, 318–325.
Volkow, N.D., Wang, G.-J., Begleiter, H., Porjesz, B., Fowler, J.S., Telang, F., Wong, C., Ma, Y., Logan, J., Goldstein, R., Alexoff, D., Thanos, P.K., 2006a. High Levels of Dopamine D2 Receptors in Unaffected Members of Alcoholic Families: Possible Protective Factors. Arch. Gen. Psychiatry 63, 999–1008. https://doi.org/10.1001/archpsyc.63.9.999
Volkow, N.D., Wang, G.J., Fowler, J.S., Logan, J., Gatley, S.J., Gifford, A., Hitzemann, R., Ding, Y.S., Pappas, N., 1999. Prediction of reinforcing responses to psychostimulants in humans by brain dopamine D2 receptor levels. Am. J. Psychiatry 156, 1440–1443. https://doi.org/10.1176/ajp.156.9.1440

Volkow, N.D., Wang, G.-J., Fowler, J.S., Logan, J., Hitzemann, R., Ding, Y.-S., Pappas, N., Shea, C., Piscani, K., 1996. Decreases in Dopamine Receptors but not in Dopamine Transporters in Alcoholics. Alcohol. Clin. Exp. Res. 20, 1594–1598. https://doi.org/10.1111/j.1530-0277.1996.tb05936.x

Volkow, N.D., Wang, G.-J., Fowler, J.S., Thanos, P.P.K., Logan, J., Gatley, S.J., Gifford, A., Ding, Y.-S., Wong, C., Pappas, N., Thanos, P., 2002a. Brain DA D2 receptors predict reinforcing effects of stimulants in humans: replication study. Synap. N. Y. N 46, 79–82. https://doi.org/10.1002/syn.10137

Volkow, N.D., Wang, G.-J., Maynard, L., Fowler, J.S., Jayne, B., Telang, F., Logan, J., Ding, Y.-S., Gatley, S.J., Hitzemann, R., Wong, C., Pappas, N., 2002b. Effects of alcohol detoxification on dopamine D2 receptors in alcoholics: a preliminary study. Psychiatry Res. 116, 163–172.

Volkow, N.D., Wang, G.-J., Telang, F., Fowler, J.S., Logan, J., Childress, A.-R., Jayne, M., Ma, Y., Wong, C., 2006b. Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. J. Neurosci. Off. J. Soc. Neurosci. 26, 6583–6588. https://doi.org/10.1523/JNEUROSCI.1544-06.2006

Volkow, N.D., Wang, G.-J., Telang, F., Fowler, J.S., Logan, J., Jayne, M., Ma, Y., Pradhan, K., Wong, C., 2007. Profound decreases in dopamine release in striatum in detoxified alcoholics: possible orbitofrontal involvement. J. Neurosci. Off. J. Soc. Neurosci. 27, 12700–12706. https://doi.org/10.1523/JNEUROSCI.3371-07.2007

Volkow, N.D., Wiers, C.E., Shokri-Kojori, E., Tomasi, D., Wang, G.-J., Baler, R., 2017. Neurochemical and metabolic effects of acute and chronic alcohol in the human brain: Studies with positron emission tomography. Neuropharmacology. https://doi.org/10.1016/j.neuropharm.2017.01.012

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, 1741–1749. https://doi.org/10.1111/j.1360-0443.2010.03022.x

Voon, V., Derbyshire, K., Rück, C., Irvine, M.A., Worbe, Y., Enander, J., Schreiber, L.R.N., Gillan, C., Fineberg, N.A., Sahakian, B.J., Robbins, T.W., Harrison, N.A., Wood, J., Daw, N.D., Dayan, P., Grant, J.E., Bullmore, E.T., 2015. Disorders of compulsivity: a common bias towards learning habits. Mol. Psychiatry 20, 345–352. https://doi.org/10.1038/mp.2014.44

Wadsak, W., Mitterhauser, M., 2010. Basics and principles of radiopharmaceuticals for PET/CT. Eur. J. Radiol., PET/CT - An Imaging Challenge 73, 461–469. https://doi.org/10.1016/j.ejrad.2009.12.022

Walter, M., Dürsteler, K.M., Petitjean, S.A., Wiesbeck, G.A., Euler, S., Sollberger, D., Lang, U.E., Vogel, M., 2015. Psychosoziale Behandlungen bei Suchterkrankungen – Suchtspezifische Psychotherapieformen und ihre Wirksamkeit. Fortschritte Neurol. · Psychiatr. 83, 201–210. https://doi.org/10.1055/s-0034-1399338

Wiers, C.E., Cabrera, E.A., Tomasi, D., Wong, C.T., Demiral, Ş.B., Kim, S.W., Wang, G.-J., Volkow, N.D., 2017. Striatal Dopamine D2/D3 Receptor Availability Varies Across Smoking Status. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. https://doi.org/10.1038/npp.2017.131

Wiers, C.E., Stelzel, C., Gladwin, T.E., Park, S.Q., Pawelczack, S., Gawron, C.K., Stuke, H., Heinz, A., Wiers, R.W., Rinck, M., Lindenmeyer, J., Walter, H., Bermpohl, F., 2015. Effects of cognitive bias modification training on neural alcohol cue reactivity in alcohol dependence. Am. J. Psychiatry 172, 335–343. https://doi.org/10.1176/appi.ajp.2014.13111495

Wiers, R.W., Eberl, C., Rinck, M., Becker, E.S., Lindenmeyer, J., 2011. Retraining automatic action tendencies changes alcoholic patients' approach bias for alcohol and improves treatment outcome. Psychol. Sci. 22, 490–497. https://doi.org/10.1177/0956797611400615

Wiers, R.W., Rinck, M., Kordts, R., Houben, K., Strack, F., 2010. Retraining automatic action-tendencies to approach alcohol in hazardous drinkers. Addict. Abingdon Engl. 105, 279–287. https://doi.org/10.1111/j.1360-0443.2009.02775.x

Wiese, J.G., Shlipak, M.G., Browner, W.S., 2000. The alcohol hangover. Ann. Intern. Med. 132, 897–902.

Wise, R.A., 2004. Dopamine, learning and motivation. Nat. Rev. Neurosci. 5, 483–494. https://doi.org/10.1038/nrn1406

Wise, R.A., 1996. Neurobiology of addiction. Curr. Opin. Neurobiol. 6, 243–251. https://doi.org/10.1016/S0959-4388(96)80079-1

Wise, R.A., Rompre, P.P., 1989. Brain dopamine and reward. Annu. Rev. Psychol. 40, 191–225. https://doi.org/10.1146/annurev.ps.40.020189.001203

Wittchen, H.U., Pfister, H., 1997. DIA-X-Interviews: Manual fur Screening-Verfahren und Interview; Interviewheft Langsschnittuntersuchung (DIA-X-Lifetime); Erganzungsheft (DIA-X-Lifetime); Interviewheft Querschnittuntersuchung (DIA-X-12 Monate); Erganzungsheft (DIA-X-12Monate); PC-Programm zur Durchfuhrung des Interviews (Langsund Querschnittuntersuchung); Auswertungsprogramm. Swets & Zeitlinger, Frankfurt.

World Health Organisation, 2014. Global status report on alcohol and health.

World Health Organization, 2016. International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10)-WHO.

Wrase, J., Schlagenhauf, F., Kienast, T., Wüstenberg, T., Bermpohl, F., Kahnt, T., Beck, A., Ströhle, A., Juckel, G., Knutson, B., Heinz, A., 2007. Dysfunction of reward processing correlates with alcohol craving in detoxified alcoholics. NeuroImage 35, 787–794. https://doi.org/10.1016/j.neuroimage.2006.11.043

Wu, Y., Carson, R.E., 2002. Noise Reduction in the Simplified Reference Tissue Model for Neuroreceptor Functional Imaging. J. Cereb. Blood Flow Metab. 22, 1440–1452. https://doi.org/10.1097/01.WCB.0000033967.83623.34

Yin, H.H., Knowlton, B.J., Balleine, B.W., 2004. Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. Eur. J. Neurosci. 19, 181–189.

Yoder, K.K., Kareken, D.A., Seyoum, R.A., O'connor, S.J., Wang, C., Zheng, Q.-H., Mock, B., Morris, E.D., 2005. Dopamine D(2) receptor availability is associated with subjective responses to alcohol. Alcohol. Clin. Exp. Res. 29, 965–970.

Zacharias, K., 2018. Extrastriatal D2/3 Receptor Availability and Executive Functioning in Alcohol Use Disorder. (unpublished doctoral dissertation) Charité Universitätsmedizin Berlin.

Appendix

Curriculum Vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Affidavit

Eidesstattliche Versicherung.

„Ich, Gianna Spitta, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: *Striatal dopamine receptor 2 and 3 availability in Alcohol dependence* selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung (siehe „Uniform Requirements for Manuscripts (URM)“ des ICMJE -*www.**icmje**.org*) kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) entsprechen den URM (s.o) und werden von mir verantwortet.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem Betreuer, angegeben sind. Sämtliche Publikationen, die aus dieser Dissertation hervorgegangen sind und bei denen ich Autor bin, entsprechen den URM (s.o) und werden von mir verantwortet.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst. “

Datum Unterschrift

Anteilserklärung an etwaigen erfolgten Publikationen

Gianna Spitta hatte folgenden Anteil an den folgenden Publikationen:

Publikation: [M. Sebold, G. Spitta, T. Gleich, T. Dembler-Stamm, O. Butler, K. Zacharias, S. Aydin, M. Garbusow, M. Rapp, F. Schubert, R. Buchert, J. Gallinat, A. Heinz], [Stressful life events are associated with striatal dopamine receptor availability in alcohol dependence], [Journal of Neural Transmission], [2019]

Beitrag im Einzelnen (bitte detailliert ausführen): geteilte Erstautorenschaft mit Miriam Sebold, Rekrutierung von 4 Patientinnen und Patienten in der in der Charité Campus Mitte und im Jüdischen Krankenhaus Berlin, Rekrutierung der Mehrheit der „High risk“ und eines Teils der „Low risk“ Probanden über Ebay Kleinanzeigen, Durchführung der telefonischen Screenings zum Ein- und Ausschluss der Probandinnen und Probanden, Erhebung der Fragebögen, Durchführung der Alkohol- und Drogentests mit den Probandinnen und Probanden, Organisation und Koordinierung der PET Messungen an der Charité Virchow Klinikum, Organisation und Koordinierung der MRT Messungen und Betreuung der Probanden am MRT Scanner in der Physikalischen Bundesanstalt Berlin (PTB), Mitwirkung bei der deskriptiven Statistik und Erstellen der Tabelle 1, Erstellen von Abbildung 1.a, Mitwirken beim Verfassen des Artikels.

Unterschrift, Datum und Stempel des betreuenden Hochschullehrers

Unterschrift der Doktorandin

Acknowledgements

This research was supported by the Deutsche Forschungsgesellschaft (clinical trial number: NCT01679145, grants: HE 2597/14-1, HE 2597/15-1, HE 2597/14-2, HE 2597/15-2, GA 707/6-1, RA 1047/2-1, RA 1047/2-2) and carried out in cooperation of Charité Universitätsmedizin Berlin and Technische Universität Dresden.

I would like to express my gratitude to the team of the LeAD study, especially Prof. Dr. Felix Bermpohl and Prof. Dr. Jürgen Gallinat for providing me with the opportunity to join the study group and supervising my thesis the way they did; Dr. Tobias Gleich for all our productive meetings, his support and guidance with the data analysis and his constructive feedback during the writing process; Dr. Ralph Buchert, Tim Kodalle and David Paul Weber for acquiring and processing the PET data; Semiha Aydin and Dr. Florian Schubert for conducting the MR measurements and processing the data; my reliable and supportive coworker Kristin Zacharias and all the other dedicated colleagues from our partner project for example Dr. Miriam Sebold, Dr. Maria Garbusow, Lena Hasselberg, Robin Frank and Jean Wendt. Sincere thanks also to every single study participant who took part in our study and enabled us to complete this project.

Moreover, I am very grateful for the advice and encouragement of my family and my friends during the whole process, especially Helga Baumann-Spitta, Wolfram Spitta, Robert Spitta as well as Vera Thormeyer and Laura Hanke Vela. Finally, I want to thank Armin Ligdorf for his help, patience and support at all times.