Aus der Klinik für Psychiatrie und Psychologie
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DISSERTATION

Extrastriatal $D_{2/3}$ Receptor Availability and Executive Functioning in Alcohol Use Disorder

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List of Abbreviations

^{18}F	^{18}fluorine
^{18}F-FDG	^{18}fluorine-fluorodeoxy-glucose
3D	three-dimensional
5-HT	serotonin
ACC	anterior cingulate cortex
ACD	annihilation coincidence detection
ADH	alcohol dehydrogenase
ADQ	alcohol and drug questionnaire
ADS	alcohol dependence scale
ALDH	aldehyde dehydrogenase
ALL	Automated Anatomic Labelling
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	univariate analysis of variance
AUD	alcohol use disorder
AUDIT	alcohol use disorder identification test
BA	Brodmann area
BAC	blood alcohol concentration
BMI	body mass index
BP	binding potential
BP_{ND}	non-displaceable binding potential
BPS	biopsychosocial model
cAMP	cyclic adenosine monophosphate
CIDI	WHO composite international diagnostic interview
Cl^-	chloride
CNS	central nervous system
CS	conditioned stimulus
CSF	cerebro-spinal fluid
d/r/sgACC	dorsal/rostral/subgenual anterior cingulate cortex
$D_{1/2/3/4/5}R$	dopamine type 1/2/3/4/5 receptor
DA	dopamine
dACC	dorsal ACC
DALYs	disability-adjusted life years
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
e^-	electron
e^+	positron
ED	effective dose
EF	executive functioning
EOI	effect of interest
F	fluorine
FAL	(^{18}F)-Fallypride
FASD	fetal alcohol spectrum disorders
FHA	family history of addiction

fMRI	functional magnetic resonance imaging
FU	follow-up assessment
GABA	γ-aminobutyric acid
GM	grey matter
$G_{s/i}$ protein	guanosine triphosphate–binding (stimulating/ inhibiting) protein
H	hypothesis
HED	heavy episodic drinking
HR	high-risk
i.v.	intra venous
ICD-10	International Classification of Diseases and Related Health Problems, 10th Revision
IPSP	inhibitory postsynaptic potential
K-S test	Kolmogorov-Smirnov Test
K-W test	Kruskal-Wallis Test
K^+	kalium / potassium
keV	kilo electronvolt
LOP	line of projection
LR	low-risk
LTD	long-term depression
LTP	long-term potentiation
MBq	megabecquerel
MDA	amphetamines
MDEFT	modified driven equilibrium Fourier transform
MDMA	methamphetamines
MFB	medial forebrain bundle
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
Na^+	natrium/sodium
NAc	nucleus accumbens
NMDA	N-methyl-d-aspartate
O	oxygen
OCDS	obsessive compulsive drinking scale
P2	Project 2
P5	Project 5
PET/CT	positron emission tomography/computed tomography
PFC	prefrontal cortex
PIF	prolactin inhibiting factor
RAC	^{11}C-Raclopride
rACC	rostral ACC
r_s	Spearman's rho
SD	standard deviation
SLF	superior longitudinal fasciculus
SNc	substantia nigra pars compacta
SPECT	single photon emission computed tomography
SRTM	simplified reference tissue model
SUD	substance use disorder
TAC	time-radioactivity curves
TE	echo time
TMT	trail-making test
TR	repetition time
US	unconditioned stimulus
V_{ND}	distribution volume of the non-displaceable compartment

V_T	distribution volume of total ligand uptake in tissue	WKS	Wernicke-Korsakoff syndrome
VTA	ventral tegmental area	WM	white matter
WAIS	Wechsler Adult Intelligence Scale	ZNR	Digit Span Test
		ZST	Digit Symbol Coding/Test
WFU	Wake Forest University	β^+	beta plus
WHO	World Health Organisation		

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I. Abstract

I.1. Abstract (English)

Introduction: Alcohol use disorder (AUD) is a chronically relapsing disorder and characterized by dysfunctional learning or in other words the continued intake of alcohol despite negative consequences. Cognitive processes of decision-making, information-updating and set-shifting are associated with the prefrontal cortex (PFC) that is involved in these so called *executive functions* (EF). The dopaminergic (DA) neurotransmitter system plays a significant role in the modulation of reward learning and can be disrupted in AUD. $D_{2/3}$ receptor ($D_{2/3}R$) availability in the striatum has been positively associated with performance on laboratory tests that reflect and rely on EF. Also, striatal $D_{2/3}R$ availability is typically lower in detoxified AUD patients than in healthy controls. It has been shown that chronic alcohol consumption can have an effect on extrastriatal $D_{2/3}R$ availability. This is especially of interest in areas connected to prefrontal integrity and EF. However, only a few studies have examined extrastriatal $D_{2/3}R$ availability in AUD and its relation to performance in laboratory measures of EF. We aim to determine this relationship with a focus on group differences between low risk controls (LR), high risk subjects (HR) with a score > 8 in the Alcohol Use Disorder Identification Test (AUDIT) and AUD patients.

Methods: We included 58 subjects (19 LR, 19 HR and 20 AUD) who were equally assessed in EF performance. Extrastriatal $D_{2/3}R$ availability was examined with (^{18}F)-Fallypride (FAL) positron emission tomography/computed tomography (PET/CT) and magnetic resonance imaging (MRI) and brought in relation to performance on common laboratory measures of EF.

Results: Differences in extrastriatal non-displaceable binding potential (BP_{ND}) for FAL were observed in several regions of interest (ROIs) (bilateral dorsal anterior cingulate cortex (ACC), bilateral rostral ACC, left dorsolateral prefrontal cortex (PFC) and left ventrolateral PFC). Patients presented significantly less BP_{ND} in a number of these areas when compared to HR. EF tests amongst the groups differed in a Digit Span Backwards task. AUD showed a significantly better performance when compared to LR. Performance in the Trail-Making Test Part B (TMT-B) showed a trend to be decreased amongst LR compared to HR and AUD. Extrastriatal BP_{ND} in several ROIs was negatively correlated with TMT-B performance.

Conclusion: Alcohol consumption has an impact on extrastriatal $D_{2/3}R$ availability and can influence DA transmission in subregions of the ACC and the PFC. This study is adding

evidence to a growing body of work with heterogeneous results of responses to chronic alcohol consumption and its impact on EF performance.

I.2. Abstract (German)

Hintergrund/Einleitung: Alkoholabhängigkeit (AUD) ist eine chronische Erkrankung, die im Krankheitsverlauf durch Rückfälle gekennzeichnet ist. Dies wird zum Teil auf dysfunktionales Lernverhalten zurückgeführt, da der Konsum trotz negativer Konsequenzen weitergeführt wird und keine Verhaltensanpassung erfolgt. Dafür wichtige Prozesse wie Entscheidungsfindung, mentale Flexibilität und Anpassungsfähigkeit werden mit dem präfrontalen Kortex (PFC) assoziiert und unter dem Begriff *Exekutiv Funktionen* (EF) zusammengefasst. Diese können bei AUD geschwächt sein. Der Neurotransmitter Dopamin (DA) spielt eine Rolle bei der Modulation von Lernvorgängen im Gehirn, vor allem im Belohnungslernen. Das DA-System kann bei chronischem Alkohol Konsum stark verändert sein. So korreliert beispielsweise eine höhere $D_{2/3}$-Rezeptorverfügbarkeit ($D_{2/3}R$) im Striatum mit besserem Abschneiden in Tests, die EF testen. Die $D_{2/3}$-Rezeptorverfügbarkeit im Striatum ist bei AUD typischerweise erniedrigt. Es lässt sich also vermuten, dass auch extrastriatale DA-vermittelte Veränderungen bei chronischem Alkoholkonsum eine Rolle spielen. Diese Veränderungen können in Bereichen wie dem PFC auftreten, die für EF wichtig sind. Jedoch haben nur wenige Studien die extrastriatale $D_{2/3}$-Rezeptorverfügbarkeit in AUD untersucht. Auch die Verbindung zum Abschneiden in EF Tests ist bisher nicht eingehend untersucht worden. In dieser Studie wollen wir eine Assoziation von $D_{2/3}$-Rezeptorverfügbarkeit und dem Abschneiden in EF Tests überprüfen. Dies geschieht mit einem Fokus auf die Unterscheidung in Kontrollen (LR), Hochrisiko-Konsumenten (HR) mit einem Score > 8 im Alcohol Use Disorder Identification Test (AUDIT) und alkoholabhängige Patienten (AUD).

Methoden: Es wurden 58 Subjekte eingeschlossen (19 LR, 19 HR, 20 AUD). Alle Teilnehmenden durchliefen eine Abfolge von neuropsychologischen Tests. Die extrastriatale $D_{2/3}$-Rezeptorverfügbarkeit wurde mittels (^{18}F)-Fallypride (FAL) Positronen-Emissions-Tomographie/Computertomographie (PET/CT) und Magnetresonanztomographie (MRI) gemessen und mit den experimentellen Messergebnissen in Verbindung gesetzt.

Ergebnisse: Das extrastriatale non-displaceable binding potential (BP_{ND}) unterscheidet sich im Gruppenvergleich in mehreren ROIs (bilateraler dorsaler anteriorer zingulärer Kortex (ACC) und rostraler ACC; linker dorsolateraler PFC und linker ventrolateraler PFC). Bei

Patienten zeigte sich ein signifikant geringeres BP_{ND} im Vergleich zu HR. In den EF Tests wurde ein Unterschied im Abschneiden im Digit Span Backwards Test gefunden, wobei Patienten signifikant besser abschnitten als LR. Auch beim Trail-Making Test Part B (TMT-B) konnte ein entsprechender Trend beobachtet werden. Das BP_{ND} war in mehreren Regionen negativ mit dem Abschneiden im TMT-B korreliert.

Schlussfolgerung: Alkoholkonsum kann einen Einfluss auf die extrastriatale Verfügbarkeit von DA Rezeptoren haben und dopaminerge Signalübermittlung vor allem in den Unterregionen des ACC sowie des PFC beeinflussen. Diese Arbeit fügt weitere Erkenntnisse zu den heterogenen Ergebnissen in diesem Feld hinzu, auch bezüglich der Beziehung zu EF.

II. Introduction

For the remainder of this work ethanol will be referred to as alcohol, and alcoholism and alcohol addiction/dependence are the functional equivalent of alcohol use disorder as described in the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM V). Executive functions and executive functioning will also be used as synonyms. All tables and figures were produced by the author of the present work. References to the original, if based on such, are named in the description of the table/figure.

II.1. Alcohol Use Disorder

II.1.1. Definition, Dimensional and Categorical Approach

Ethanol, a widely consumed and abused substance, causes multiple alcohol-related problems (ARP) in social, mental and physical health (Lange, Manz, Rommel, Schienkiewitz, & Mensink, 2016; World Health Organisation, 2014). Worldwide, problematic alcohol consumption ranks amongst the top five risk factors for the development of diseases, disabilities and deaths for both sexes (Lim et al., 2012; World Health Organisation, 2014).

Alcohol use disorder (AUD) is the most common of all substance use disorders (SUD) and represents the most pronounced and severe form of alcohol misuse. It is a chronically relapsing disorder characterized by the compulsion to seek and take the drug, the loss of control in limiting the intake, the emergence of negative emotional states as well as dysfunctional learning – or in other words, by the continued intake of alcohol despite negative consequences. Since 2013, the Diagnostic and Statistical Manual of Mental Disorders (DSM V) includes not only a categorical but also a dimensional approach within the AUD diagnosis – encompassing not only the dependence on, but also the abuse, of alcohol. AUD can thus be defined as a *mild* (meeting 2 or 3 criteria), *moderate* (meeting 4-5 symptoms) or *severe* disorder (meeting six or more criteria) at the time of diagnosis (American Psychiatric Association, 2013). This approach and change does not distinguish between the clear term *dependence* and the less defined term *abuse* and implies that these two belong to the same disease entity (Batra et al., 2016; Heinz & Friedel, 2014). If seen on a continuum, they could be preceded by abstinence, low-risk (LR) consumption and high-risk (HR) consumption. The DSM V lists a total of 11 criteria that cover four characteristic symptom classes (see table 1).

Table 1. DSM V Criteria for Alcohol Use Disorder Diagnosis

I. Impaired control

1. Alcohol is often taken in larger amounts or over a longer period than was intended
2. There is a persistent desire or unsuccessful efforts to cut down or control alcohol use
3. A great deal of time is spent in activities necessary to obtain alcohol, use alcohol, or recover from its effects
4. Craving, or a strong desire or urge to use alcohol

II. Social impairment

5. Recurrent alcohol use resulting in a failure to fulfil major role obligations at work, school, or home
6. Continued alcohol use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of alcohol
7. Important social, occupational, or recreational activities are given up or reduced because of alcohol use

III. Risky use

8. Recurrent alcohol use in situations in which it is physically hazardous.
9. Alcohol use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by alcohol

IV. Pharmacological criteria

10. Tolerance, as defined by either of the following:
    a. A need for markedly increased amounts of alcohol to achieve intoxication or desired effect.
    b. A markedly diminished effect with continued use of the same amount of alcohol

11. Withdrawal, as manifested by either of the following:
    a. The characteristic withdrawal syndrome for alcohol
    b. Alcohol (or a closely related substance, such as a benzodiazepine) is taken to relieve or avoid withdrawal symptoms

Meeting 2-3 criteria = mild; 4-5 criteria = moderate; ≥ 6 criteria = severe disorder.

Retrieved from the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition; American Psychiatric Association (2013)

As discussed in Helzer et al. (2006), there is evidence that all SUDs could be arrayed along a continuum concerning their intensity. This is of high interest not only in theory but also important in the monitoring and prevention of disorder progression and concerning treatment efforts that can thus address the full range of a disorder. A variety of tools and questionnaires such as the alcohol use disorder identification test (AUDIT), alcohol and drug questionnaire (ADQ) and CAGE (acronym for four screening questions concerning the "cut down" of consumption/ "annoyed" about consumption/ "guilt" feelings/ "eye opener" in the morning) are in regular use for identification and quantification of AUD. Not only is there a dimensional approach to the disorder itself but likewise in alcohol consumption patterns concerning frequency, quantity and motivation. Therefore, not only the disorder as such but also the specific patterns of no alcohol intake, LR, HR consumption and the different levels of AUD can be arrayed along the continuum. Identifying subjects with a HR consumption pattern and preventing progression to disorder diagnosis is the main goal of the tools mentioned above.

On the other hand, a categorical approach that helps to define if a subject is suffering or not suffering from a disease or disorder is necessary, not only for social acceptance but especially for decision-making in clinical contexts. This is illustrated by the following statement: "Clinicians who must decide whether to treat or not treat a patient, to hospitalize or not, to treat a patient with drug or with psychotherapy, to use this drug or that drug, [...] must inevitably use a

categorical approach to diagnosis" (Kraemer, Noda, & O'Hara, 2004). Therefore, it is essential to link dimensional and categorical approach with one another.

II.1.1.2. High-risk Drinking Patterns

Treatment-seeking patients with AUD diagnosis represent only a small fraction of people who abuse alcohol, but many more individuals consume alcohol to a harmful extent without fulfilling enough criteria to be classified as AUD patients (World Health Organisation, 2014). In Germany, approximately 13.1 % of the female and 18.5% of the male population aged 18 to 79 years consume a potentially harmful daily amount of alcohol (Lange et al., 2016). They have been described as HR consumers and are often unaware of their problematic alcohol intake and its possible consequences. Various definitions have been established to describe cut-offs and subclasses of HR consumption. For instance, the intake of more than 10 g of pure ethanol per day for women and more than 20 g per day for men (Lange et al., 2016) or the *risk* (hazardous use) or *presence* (harmful use) of physical or psychological harm (World Health Organisation, 2016). The *Deutsche Hauptstelle für Suchtfragen* classifies the following subgroups of daily alcohol consumption (in the last 30 days) as shown in table 2.

Table 2. Classification of daily Alcohol Consumption according to Deutsche Hauptstelle für Suchtfragen.

	women	*men*
Low risk (LR) consumption:	<12g	<24g
At risk intake:	12-40g	24-60g
Dangerous intake:	40-80g	60-120g
Very high intake:	>80g	>120g

Retrieved from Seitz, Lesch, Spanagel, Beutel, & Redecker (2013).

However, not only the quantity of alcohol consumption plays a role in the definition of HR drinking. Also drinking patterns and physical, psychological and social consequences and components need to be included. As mentioned before, a well-known and widely used tool for their identification is the AUDIT (for more detailed information see III.2.1). This questionnaire goes further than the sole definition by quantity and addresses different distinctions of problematic alcohol intake with regard to categorical and dimensional approaches. The questions are aimed at the following subgroups shown in table 3.

Table 3. Aspects of High Risk Drinking Behaviour as assessed with the AUDIT Questionnaire

hazardous use:	frequency of drinking, typical quantity, frequency of heavy drinking
harmful use:	guilt, blackouts, alcohol-related injuries, other people's concern about drinking
dependence symptoms:	impaired control, increased salience, morning drinking

Retrieved from Barbor, Higgins-Biddle, Saunders, & Monteiro (2001)

People involved in *binge drinking* or *heavy episodic drinking* (HED) are part of a special subgroup and present a more clearly defined drinking pattern that is classified as being a high-risk behaviour of alcohol consumption. It is defined as the intake of 60 g or more of pure ethanol (an equivalent of 5 or more standard drinks) on a single occasion at least once a month (World Health Organisation, 2014). However, obviously it needs to be seen in relation to different aspects such as blood alcohol concentration (BAC), gender, age, BMI and other individual factors. In this context, the hazardous and harmful as well as problematic use are summarized under the term of HR consumption. This comprises not only the potential harm to the consuming subject but also the possible effects on other individuals, such as children, partner, co-workers or strangers in contact with the subject.

II.1.2. Epidemiology

AUD is a common psychiatric disorder with devastating consequences (medical, social, financial) for affected individuals and their relatives. In 2012, 3.4 % of the German adult population (4.8 % of the male and 2.0 % of the female population, about 1.8 million people) met the DSM IV or ICD 10 criteria for an AUD diagnosis (Pabst, Kraus, Matos & Piontek, 2013). An additional 3.1 % (4.7 % of men and 1.5 % of women) met the criteria for harmful alcohol abuse (Pabst et al., 2013). The prevalence of problematic alcohol consumption as identified by the AUDIT (including HED) is very common. The estimated prevalence in Germany varies between 21.1 % (amongst men: 32.4 % and women: 8.9 %) according to Kraus, Pabst, Piontek, & Müller (2010) and 41.5% for men and 25.6% for women (Hapke, v. der Lippe, & Gaertner, 2013). German hospitals listed alcohol-related disorders as the main diagnose in the year 2016 with 322 608 treatment cases (Statistisches Bundesamt, 2017). 5.1 % of the global burden of disease and

injury is attributable to alcohol, as measured in disability-adjusted life years (DALYs) and about 3.3 million deaths (5.9 % of all global deaths) were attributable to alcohol consumption in the year 2012 (World Health Organisation, 2015). Furthermore, individuals with AUD have a large number of psychiatric co-morbidities such as depression and suicidal behaviour, bipolar disorders, anxiety disorders, insomnia or other SUD. There is also a causal relationship to the development of many somatic diseases, injuries and deaths (World Health Organisation, 2015). HR alcohol consumption and AUD are partly responsible for more than 200 conditions such as non-communicable diseases like cancers (especially in the gastro-intestinal tract), liver dysfunction, coronary heart disease, neurological diseases, fetal alcohol spectrum disorders (FASD) and infectious diseases (Lange et al., 2016; World Health Organisation, 2015).

Alcohol consumption and AUD are also causing high direct and indirect costs to society. In Germany the total costs added up to 24.398 billion euros in the year 2002 – taking into account treatments of AUD and somatic diseases associated with alcohol consumption, loss of working hours and mortality (Küfner, 2010).

Relapse rates amongst AUD patients lie at about 85 % when no further treatment (psychotherapeutic and pharmacological) after the initial detoxification is arranged (Boothby & Doering, 2005; Walter et al., 2015), emphasizing the chronic condition of the disorder and the life-long struggle for the patients.

II.1.3. Aetiology

AUD is a complex disease; the aetiology and development are unclear in their details. Most likely, different components interact in form of a bio-psycho-social model (BPS). Amongst others, factors such as alcohol-related neurological changes, individual dispositions (e.g. genetic/biological, psychological) as well as environmental and cultural aspects seem to have an effect on problematic alcohol-related behaviour and, its most pronounced form, AUD. There is little doubt that learning mechanisms, especially in reinforcement learning, play a central role in AUD development. Repeated alcohol consumption despite negative consequences or absence of positive consequences may be due to an impaired flexibility in changing and adapting to environmental changes (e.g. reward contingencies (Park et al., 2010)). This learning deficit is of great clinical relevance during disorder development, therapy as well as for the psychosocial outcome (von der Goltz & Kiefer, 2009). Qualities, unique to mankind, that verify and adapt behaviour in relation to changing environmental circumstances and outcomes fall under so called

executive functioning (EF) (for more detailed information see II.3.) that may be impaired in AUD patients (Ratti, Bo, Giardini, & Soragna, 2002). It is hard to distinguish if this is a condition prior to the consumption or a consequence of repeated alcohol intake.

> [...] to understand the effects of alcoholism, it is important to consider the influence of a wide range of variables on a particular behaviour or set of behaviours. The underpinnings of alcohol-induced brain defects are multivariate; to date, the available literature does not support the assertion that any one variable can consistently and completely account for these impairments. Instead, the identification of the most salient variables is a primary focus of current research. In the search for answers, we recommend an integrative approach that recognizes the interconnectivity of the different functional systems to account for the heterogeneity of outcome variables associated with alcoholism-related impairments and recovery of functions. (Oscar-Berman et al., 2014)

II.1.3.1. Diathesis-Stress Model and Risk Factors

With today's understanding of the development of psychiatric disorders or diseases, the most suited approach towards AUD (and other psychopathologies) may be the diathesis-stress model, a psychological theory trying to explain behaviour as an outcome of individual vulnerability (diathesis) and external factors (stress, negative events) (Hankin & Abela, 2005). It serves to explore how individual predispositions (e.g. genetic and biological traits, family history, early life experiences, cognitive factors, personality traits) interact with stressors from the environment (e.g. socio-cultural environment, long-term negative conditions, discrete stressful life events) to enhance the development of disorders. Using this approach, it is also possible to describe risk and protective factors, moderators and mediators that work on a dynamic and reciprocal basis. It is even possible to make predictions about individuals who are at a higher risk of developing a disorder than others (Gazelle & Ladd, 2003; Hankin & Abela, 2005).

A large number of twin and adoption studies have been performed in recent decades and were able to show a strong heritability of AUD, estimated at around 50 % (Kendler, 1997). For example, a subject can be highly vulnerable to a certain disorder (e.g. for AUD because of a positive family history of AUD) but does not develop the disorder until exposed to certain stressors. Another individual might not develop the disorder despite being exposed to the same stressors. This can indicate less vulnerability (e.g. no family history) to a particular disorder, differing susceptibility, more risk-resilience or protective factors amongst the individuals. These resilience factors can also be both genetic and environmental. For example, functional

polymorphisms in the alcohol dehydrogenases (ADH) and aldehyde dehydrogenases (ALDH) are common amongst Chinese and other East Asian individuals and increase their sensitivity to alcohol. Consumption of alcohol leads to adverse effects such as flush symptoms, nausea and vomiting that prevent individuals from the intake (Thomasson et al., 1991). Protective environmental factors can include for example less alcohol availability, intact family structures with support from family members and friends, better coping strategies, more resources or less exposure to stress (Enoch, 2006). With growing scientific evidence about the multifactorial origins and favourable conditions of AUD development, a number of important risk factors can be identified (see figure 1).

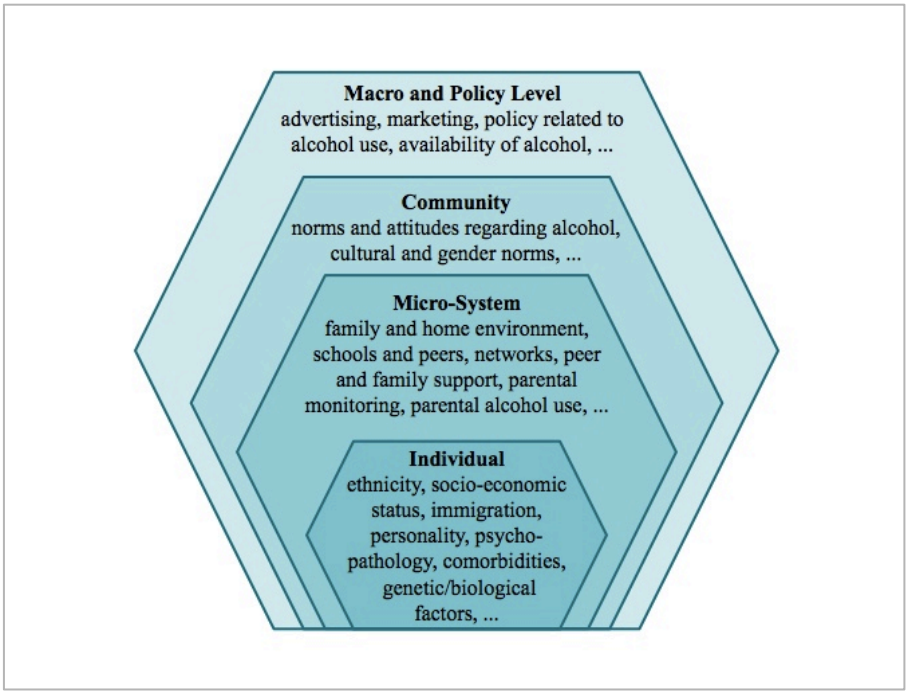

Figure 1. Social, cultural and individual Risk Factors of AUD Development

Adapted from Sudhinaraset et al. (2016)

Individual risk and predisposition may be due to negative early life experiences and include factors such as the socioeconomic status, personality traits (e.g. impulsivity), psychopathology and comorbidities (e.g. depression, anxiety or bipolar disorder). Other possible risk factors are differences in cognitive and EF abilities, male gender and other biological factors (e.g. genetic vulnerability, positive family history) as well as the alteration of neurological circuits due to alcohol consumption itself. Environmental factors play an important role on different levels (macro, micro and community) and contain aspects such as social acceptance and availability of alcohol, cultural drinking practice, peer group pressure and influence, parental lifestyle or dysfunctional family structures and exposure to stress or traumatic life events.

II.1.3.2. Learning and Alcohol Use Disorder

Many psychological disorders also have a certain time span of vulnerability, during which a subject is more likely to develop a disorder if exposed to negative stressors. Concerning the development of AUD, adolescence and young adulthood are critical timespans with high vulnerability, as the central nervous system (CNS) is widely trying out, establishing and maintaining mechanisms and behaviours. Also, the prefrontal dopaminergic system undergoes important modifications, mainly starting in adolescence and continuing in early adulthood (Yetnikoff, Reichard, Schwartz, Parsely, & Zahm, 2014). This may make this important neurotransmitter system especially vulnerable during this time. Many AUD patients report to have started drinking earlier than matched healthy controls and around 40% of alcoholics were already drinking heavily at the end of their adolescence (Enoch, 2006). When, if and how individuals start drinking is strongly influenced by environmental, individual and genetic factors mentioned above (Hägele, Friedel, Kienast, & Kiefer, 2014; Sudhinaraset, Wigglesworth, & Takeuchi, 2016).

It is common for individuals to have their first contacts and make positively rated social experiences with alcohol during adolescence. In terms of classical conditioning, this can lead to a positive connotation of the substance in the sense of a conditioned stimulus (CS). The activation of reward structures in the brain (positive reinforcement) related to the CS plays a central role. Later in AUD development, it is mainly the alleviation of unpleasant conditions such as craving and withdrawal (negative reinforcement) that enhances the addictive behaviour. Addictive behaviour can thus be seen as a learned behaviour.

On the neurobiological level, persistent alterations of behaviour and psychological functioning are thought to be mediated by reorganization, modification and strengthening of synaptic connections in specific neural circuits and occur as a consequence of experience and learning. Changes on structural, neuronal and molecular level (e.g. neurotransmitter imbalances, neuro-adaptive responses) caused by chronic application of addictive drugs, have been shown in preclinical studies (Nestler, 1997; Terry E. Robinson & Kolb, 2004). They show similarities to physiological mechanisms of learning, such as in long term potentiation (LTP) (von der Goltz & Kiefer, 2009). These changes and adaptations of brain circuits can persist long beyond detoxification especially in cases of severe disorders with an impact on leaning capacity, neuropsychological functioning and EF (Stavro et al., 2013). This is of high clinical relevance. During inpatient treatment and during further rehabilitation, most strategies ask patients to learn new information and mechanisms to change and control their behaviour, to interact with others

and make difficult causal connections between situations, feelings and actions – all that in order to avoid the learned pattern that alcohol-related cues are followed by consumption. In this case, as shown in several studies, higher levels of neuropsychological functioning are associated with higher rates of successful inpatient treatment (O'Leary, Donovan, Chaney & Walker, 1979). Also, the attendance of outpatient groups (Guthrie & Elliott, 1980) and employment success correlate positively with intact neuropsychological functioning (McCrady & Smith, 1986; Meek, Clark & Solana, 1989). AUD is to a certain extent connected to dysfunctional learning mechanisms (Garbusow et al., 2016; Sebold et al., 2014). For an extensive review of learning mechanisms and risk factors for AUD development see Hägele et al. (2014) as a more detailed inquiry would exceed the framework of this exploration. More detailed information on explicit pathways, reward learning and molecular effects can be found in section II.2.

II.2. Neurobiology of Alcohol Use Disorder

Ethanol does not have a specific binding site in the brain but is thought to interact with a number of ethanol receptive elements in cell membranes, such as ligand-gated ion channels. Acute intoxications with ethanol cause direct cellular damage that lasts for hours, whereas chronic intake leads to widespread neuroadaptive processes in the CNS such as remodelling of synapses, altered neuro-transmission and neuro-adaptation. These changes can last for much longer, up to a lifetime. The effect on the CNS includes specific changes in cell function, gene expression, epigenetic modification and affects multiple neurotransmitter systems (GABAergic, glutamatergic, serotoninergic, opioid and dopaminergic) and brain circuits (Heinz et al., 2009; Most, Ferguson, & Harris, 2014). These effects are also reflected by their opposing reaction during withdrawal. The sum of molecular and cellular changes ultimately results in altered behavioural responses. The exact mechanisms and their interaction are, however, very complex and not yet completely understood. The following section will give a brief overview of the most important transmitter systems and their involvement in the development and maintenance of AUD.

γ-aminobutyric acid

γ-aminobutyric acid (GABA) is the main inhibiting transmitter in the mammalian CNS. Thus, its main role is to control and diminish neuronal excitability via negative changes of the membrane potential via inflow of negative ions or out-flow of positive ions. GABA mainly binds

to two different types of receptors, an inhibitory ligand-gated ion-channel ($GABA_A$) and a metabotropic, G-protein-coupled receptor ($GABA_B$). Ethanol and other substrates such as benzodiazepines, barbiturates, anaesthetics and anticonvulsants act on allosteric binding sites as agonists and multiply the activity of $GABA_A$ receptors (Sieghart, 1995). The receptors allow the flow of chloride (Cl^-) across the membrane into the cell. This leads to hyperpolarisation, inhibitory postsynaptic potentials (IPSP), less excitability of the targeted neuron and thus weakens the stimulating signals of e.g. glutamate (Sieghart, 1995). The inhibitory effects include sedation, impaired cognitive functions, anxiolytic and anticonvulsive effects as well as muscle relaxation and motor inco-ordination. All these are enhanced by alcohol. Chronic alcohol intake can cause a down-regulation of $GABA_A$ receptors due to the excessive stimulation as shown in figure 2 (Most et al., 2014). During acute withdrawal from alcohol or other potentiating drugs, this results in a relative shortage of inhibiting GABA effects due to the missing reinforcement on $GABA_A$ receptors leading to dysbalance between stimulating and inhibiting transmission.

Glutamate

Glutamate is the main excitatory transmitter in the CNS. Glutamatergic transmission uses both metabotropic (mGluR) and ionotropic (N-methyl-d-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)) receptors. This particular system plays a key role in LTP and long-term depression (LTD), molecular correlates of learning and memory effects that are mediated mainly by repeated NDMA receptor stimulation. All receptors for glutamate are inhibited under the influence of alcohol, although some subtypes are affected only when very high concentrations of ethanol are present. Acute actions of alcohol on the glutamatergic system are involved in tolerance, withdrawal, craving, relapse and dependence (Tsai, 1998). Ethanol works as a non-competitive antagonist on AMPA receptors but needs to show high concentrations to affect transmission, whereas NMDA receptors are very sensitive to ethanol's acute antagonizing effects (Most et al., 2014). Chronic intake however seems to enhance NMDA receptor expression (Qiang & Ticku, 2005) leading to an upregulation to counteract the inhibition of the glutamatergic system and the inhibiting effects of increased GABAergic transmission under alcohol influence (for acute and chronic changes in synaptic transmission also see figure 2). Typical symptoms during withdrawal such as anxiety, dysphoria and, in severe cases, convulsions, are caused by the dysbalance between excess of glutamate receptors and a lack of $GABA_A$ receptors (Most et al., 2014). To prevent severe conditions such as seizures, a temporary treatment with anticonvulsive medication (e.g. benzodiazepines) is sometimes needed.

Serotonin

The neurotransmitter serotonin (5-HT) is involved in mood regulaton, sleep, appetite, learning, memory and others important functions. 5-HT neurons originate from the raphe nuclei and project almost into the entire brain. Most receptor subtypes are metabotropic and use secondary messenger pathways, except for the 5-HT_3 receptor which is a ligand-gated ion channel. Alcohol enhances serotonergic transmission in part by increasing the potency of 5-HT_3 receptor activation (Sung, Engel, Allan, & Lovinger, 2000). Generally, an inverse relation between alcohol consumption and 5-HT transmission can be observed (Most et al., 2014). Studies showed a reduction in brainstem 5-HT transporters that were correlated with lifetime alcohol intake in male detoxified alcoholics (Heinz et al., 1998). Reduced 5-HT transporters have also been shown in major depressions and were also directly associated with high anxiety and depression, particularly during acute withdrawal (Heinz et al., 1998).

Opioid

Berridge and Robinson (1998) suggested that the opioid system is responsible for the direct mediation of the hedonic effects of alcohol consumption. Studies with rodents have shown increased μ-opiate receptors in the ventral striatum amongst animals that prefer alcohol (Cowen & Lawrence, 1999). Increased μ-opiate receptors have also been found in AUD patients during early abstinence and even showed a correlation between high numbers of receptors in the ventral striatum and medial PFC (mPFC) and craving for alcohol (Heinz, Reimold et al., 2005a). The opioid system clearly interacts with reinforcing mechanisms of alcohol, as the opioid antagonist naltrexone has proven to be effective in decreasing alcohol consumption and craving (clinically and experimentally) and can also be used to prevent relapses in some individuals after detoxification treatment (Anton, 2008). Increased opioid activity caused by alcohol consumption also appears to facilitate dopamine (DA) release in the ventral tegmental area (VTA) and nucleus accumbens (NAc) (Anton, 2008) emphasizing the interconnectivity of neurotransmitter systems.

Dopamine

In AUD, alterations in the dopaminergic system play a central role. Especially dysfunctions during early abstinence are thought to be partly responsible for the problems with unlearning of well-established responses to alcohol-associated cues and learning motivational responses to new stimuli (Heinz et al., 2009). The role of DA in learning, reward and different brain circuits will be discussed in detail in the section about the dopaminergic system below (section II.2.1.).

II.2.1. The Dopaminergic System

Dopamine (from 3,4-dihydroxyphenethylamine) is a catecholamine and an important neurotransmitter in the CNS. It also plays different other important roles in the human body, including influence on cardiac output, vasodilatation, renal blood flow and urine output. DA is synthesized as a precursor chemical (L-DOPA) in brain and kidneys. After decarboxylation, it can bind to different types of dopamine receptors. In the CNS, we have two main classes of receptors: D_1- and D_2-like receptors, all of them are G-protein-coupled receptors that transmit information via secondary messengers and molecular cascades. Depending on the type of receptor, DA can act as an inhibiting or stimulating transmitter on other neurons. When binding to D_1-like receptors ($D_{1/5}R$) DA provokes increased intracellular cAMP via G_s-proteins that can lead to inhibition (e.g. opening potassium (K^+) channels, leading to hyperpolarisation) or excitation (e.g. opening sodium (Na^+) channels leading to depolarisation) on the targeted neuron. The activation of D_2-like receptors ($D_{2/3/4}R$) generally leads to inhibition of the targeted neuron, and they also have a higher affinity to DA than D_1-like receptors (Seamans & Yang, 2004). DA cannot be seen as a clearly inhibiting or excitatory neurotransmitter but rather as a neuromodulator on synaptic transmission that potentiates or attenuates responses (Seamans & Yang, 2004).

Multiple diseases of the nervous system are connected to DA dysfunctions. For example, in Parkinson's disease, a massive loss of DA neurons in the substantia nigra pars compacta (SNc) leads to typical motor symptoms such as shaking, rigidity, postural instability as well as psychiatric problems including depressions, dementia and emotional problems. Parkinson's disease can be initially treated with the substitution of L-DOPA (Scatton, Javoy-Agid, Rouquier, Dubois, & Agid, 1983). There is also evidence that schizophrenia is related to altered levels of DA. Treatment options for the latter involve a reduction of DA transmission by using antipsychotic drugs that act as DA antagonists (Seeman, 1987).

Cell bodies of DA neurons are concentrated mainly in the VTA and the SNc. The latter is a component of the basal ganglia and the nigrostriatal pathway that is crucial for motor function and is affected in Parkinson's disease. DA neurons are also found in the hypothalamus, affecting the secretion of the hormone prolactin from the pituitary gland via the tubero-infundibular pathway. DA reaches the pituitary gland via the hypophyseal portal system and inhibits the secretion of prolactin which otherwise is secreted continuously. In this context, DA is also called prolactin-inhibiting factor (PIF). The dopaminergic neurons that lie in the VTA project via the medial forebrain bundle (MFB) into different parts of the brain, including limbic (amygdala,

NAc, septum, hippocampus) and cortical regions such as the PFC. This circuit is called mesocorticolimbic projection and is thought to be involved in reinforcement, reward learning and motivation (see below).

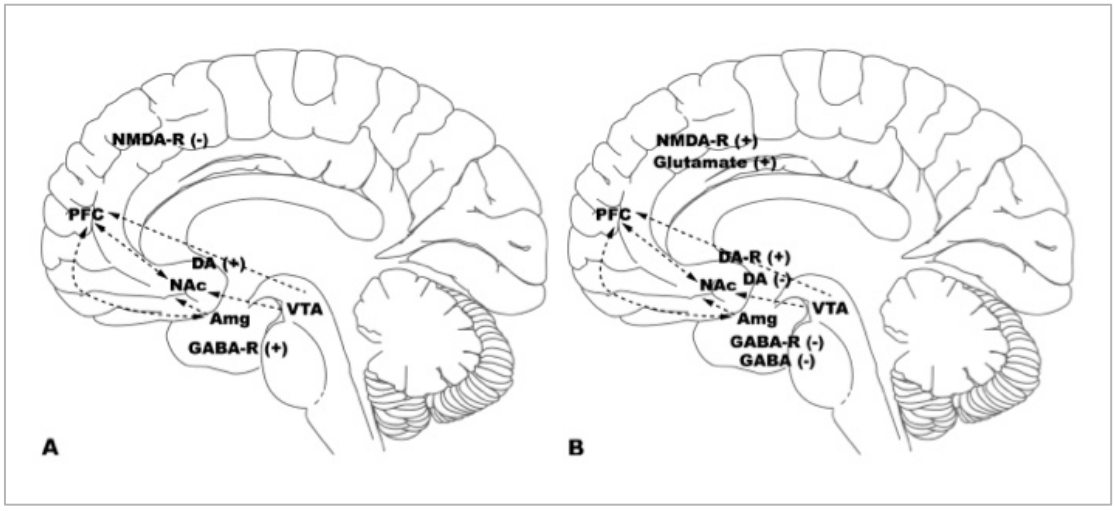

Figure 2. Synaptic Transmission and Neuroadaptations after (A) acute and (B) chronic Alcohol Exposure Retrieved and adapted from Most et al. (2014)

PFC = Prefrontal cortex; NMDA-R = N-methyl-d-aspartate receptor; DA = dopamine; DA-R = DA receptor; NAc = nucleus accumbens; Amg = amygdala; VTA = ventral tegmental area; GABA = γ-aminobutyric acid; GABA-R = GABA receptor

II.2.1.1. Dopamine and Reward – Mesocortical and Mesolimbic Circuits

As stated above, DA plays a role in different pathways in the brain. We will now focus on the mesocortical and mesolimbic pathways that are thought to be involved in the development of drug addiction and to play a major role in reward-motivated behaviour.

Acute alcohol exposure activates dopaminergic reward pathways, whereas chronic intake leads to hypodopaminergic states (see figure 2) that are associated with dysphoria and may lead to craving and relapse (Koob & Volkow, 2010). Changes in the motivation for drugs and natural rewards play a key role in addiction. As Robinson & Berridge (1993) suggested, DA mediates mainly a motivational response and is associated with the *craving* or *wanting* of drugs of abuse and does not directly mediate the hedonic effect of alcohol. It is nevertheless highly linked to reward learning processes. Rewards as such are needed for individual and gene survival and enhance behaviour and elementary processes such as drinking, eating and reproduction. As described by Mirenowicz & Schultz (1994), firing patterns of DA neurons can be either in a tonic mode with a low frequency or in a phasic mode with a higher frequency. The latter leads to a transient increased DA release, which is thought to signal the salience of a cue. Phasic bursts occur especially in early stages of reward learning when a reward is presented. Over time, a stimulus-action-reward mechanism is encoded. If the predicted reward fails to appear, activity is inhibited and if the reward appears sooner than thought, DA responses increase again. Thus, DA neurons in the midbrain seem to be coding the so called *prediction error* (PE), the degree to which a reward, or a certain cue that is associated with reward, is new and surprising (Schultz, Dayan & Montague, 1997).

The intake of alcohol (and other drugs of abuse) increases DA release in the midbrain via disinhibition of DA neurons, liberating them from GABA neuron inhibition, which enhances drug intake via positive reinforcement (Di Chiara, 2002). Studies in humans and animals have shown that drug-related reinforcement effects exceed reinforcement effects from natural reinforcers such as food (Di Chiara, 2002). Since DA neurons fire in response to salient stimuli, the much higher effect of drugs compared to environmental events is hypothesized to alter the thresholds required to activate dopaminergic cells (Koob & Volkow, 2010). After chronic expose to alcohol, a reactive down-regulation of D_2R takes place as a response to the high DA release during ongoing consumption (Volkow et al., 2002). This effect is also associated with increased relapse risk and craving (Heinz et al., 2005a). Imaging studies using (^{18}F)-Fallypride (FAL) PET have shown decreased dopamine release during withdrawal and less $D_{2/3}R$ availability in the striatum in addicted patients compared to healthy controls (Heinz et al., 2004). Processes of

neuroadaptation take place in different regions of the brain, cumulating and influencing behavioural responses (see also figure 3). However, after long-term abstinence, $D_{2/3}R$ availability can recover and increase, as was shown for a small group of patients in Rominger et al. (2012).

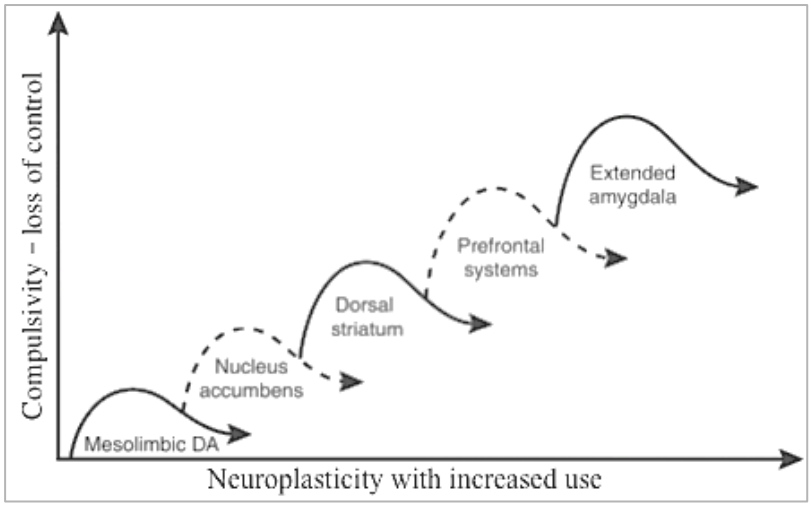

Figure 3. Schematic Drawing describing sequential and cumulative Effects of neuroadaptive Changes in Addiction Development Retrieved and adapted from Koob &Volkow (2010)

It is thought that the activation of the midbrain dopamine system includes multiple roles such as to give incentive salience to stimuli in the environment (Robinson & Berridge, 1993) and to promote performance of goal-directed behaviour (Salamone, Correa, Farrar & Mingote 2007). Most investigations thus focus on dopaminergic midbrain areas (such as the VTA and SNc) as well as the basal ganglia to which they project (in particular the ventral striatum with NAc and the dorsal striatum). These circuits are known to be connected to reward, conditioning and habituation and have been shown to be altered in AUD patients. More recently, preclinical and clinical studies have put an emphasis on the role of the PFC in addictive behaviour (Volkow & Fowler, 2000). Evidence from imaging studies showed that the transition from initial consumption to a chronic relapsing disease involves reprogramming of neural circuits connected to (1) reward and motivation; (2) memory, conditioning and habituation; (3) EF and inhibitory control and others (Koob & Volkow, 2010).

A large number of functions and processes, especially higher-order EF, are ascribed to different regions of the PFC (see also table 4, section II.3.2.1). Thus, PFC disruption, which may also be mediated by dysfunctional DA projections, can help to explain the negative effects on behavioural and learning mechanisms in addictions such as AUD (Chen et al., 2005; Trantham-Davidson et al., 2014; Trantham-Davidson & Chandler, 2015). As Volkow et al. (2004)

postulated, decreased DA function in addicted subjects leads to decreased sensitivity to non-drug-related stimuli (as there is less ascribed salience to natural reinforcers) and disrupts frontal inhibition, which both contribute to impaired control and compulsive consumption. Moreover, the impact of DA modulation on synaptic plasticity and its influence on LTP has been described in three major brain regions innervated by DA: the striatum including the nucleus accumbens, the hippocampus and the prefrontal cortex (Jay, 2003).

II.2.1.2. Measuring Dopamine

The direct investigation of DA, its signalling and metabolites in human brains brings a challenge with it – as measurements used in animals are often invasive (e.g. micro-dialysis or post-mortem brain tissue analysis). With the development of neuroimaging techniques, such as fMRI, MRS, PET and the use of specific (radio-) pharmaceuticals over the past decades, it has become easier to investigate the CNS, its neurotransmitters, metabolites and functioning in a non-invasive way. It is possible to investigate presynaptic (DA transporter, vesicle transporter and DA storage) as well as the postsynaptic DA system (Politis, 2014). For an overview of dopaminergic neuroimaging see figure 4. Amongst the PET radioligands for postsynaptic $D_{2/3}R$ are mainly three molecules that have been used in studies with humans and that continue to appear in recent research. These are ^{11}C-Raclopride (RAC), ^{11}C-FLB 457 and ^{18}F-Fallypride (FAL). Due to its kinetics and moderate in-vivo affinity RAC is only used to display striatal receptor density. However, ^{11}C-FLB 457 and FAL have higher affinity and signal-to-noise ratios

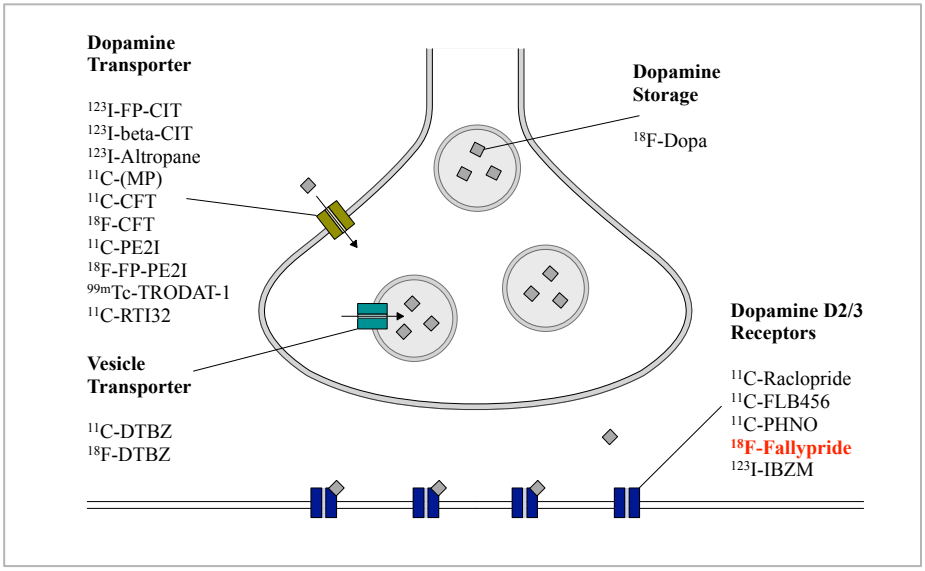

Figure 4. Radiotracers in dopaminergic Neuroimaging

Retrieved and adapted from Politis (2014)

than RAC and can therefore provide reliable measures of extrastriatal $D_{2/3}R$ availability, which is typically much lower than in the striatum. Because of the high affinity of ^{11}C-FLB 457, clearance from the striatum is relatively too slow for ^{11}C decay, leaving a max of 2 hours for imaging time. Thus, FAL is the molecule most suited for investigating striatal and extrastriatal quantification of $D_{2/3}R$ availability in one imaging session (Laruelle, Slifstein, & Huang, 2003; Slifstein et al., 2010). More detailed information about FAL and PET/CT to investigate $D_{2/3}$ receptor availability can be found in section III.3.2 and III.3.3.

II.3. Executive Functions

As stated above, AUD is amongst other aspects characterized by the repeated intake of alcohol despite negative consequences (American Psychiatric Association, 2013). This component is possibly caused and maintained by an impaired flexibility to change and adapt behaviour and by impaired inhibitory control – in short by difficulties in abilities that are attributed to executive functioning. To gain control over adaptive behaviour, attributed values need to be transmitted to higher executive regions of the CNS, such as the PFC – regions disrupted in compulsive drug intake and involved in the development of addiction (Goldstein & Volkow, 2011).

II.3.1. Definitions

There is no universally accepted definition of what exactly falls under the term *executive functions* - only that it collects a series of many different higher-order cognitive abilities. It is a multifaceted neuropsychological construct that enables higher organisms to act and adjust goal-directed behaviour. It is therefore also highly relevant for the avoidance of maladaptive behaviours (Day, Kahler, Ahern, & Clark 2015).

We as humans have the most evolved EF of all species, allowing us to consider and select options and give specific responses to a stimulus. For this, we can take into account situational contexts, our long-term goals and knowledge that we previously acquired (Suchy, 2009). Making use of EF is however connected to a stronger effort than responding with routinized sets of behaviour. Thus, most of the time, even humans do not utilize EF processes but function and behave in well-rehearsed routines. Hence, EF remains *dormant* for most of the time as long as

automated functioning is sufficient. EF processes only take over when the novelty and/or complexity of the situation demands other than automatic or learned responses (Suchy, 2009) for example, when there is no well-established stimulus-response association, when one notices an error in one's functioning or a sub-optimal behaviour (Gilbert & Burgess, 2008). The term EF has become synonymous with these complex networks' abilities to supervise, update, inhibit and adapt behaviour (see also figure 5).

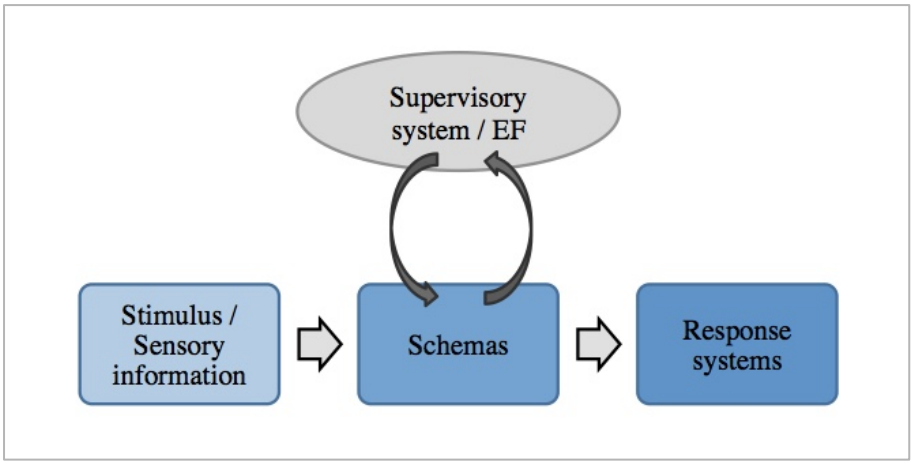

Figure 5. Schematic Diagram of Executive Functioning (EF) as a supervisory/modulating System of Routine Behaviour
Based on Norman & Shallice (1998), adapted from Gilbert & Burgess (2008)

Despite the many discussions about constructs and theories concerning the splitting up of EF specific abilities, there are major subcomponents of EF that can lead to certain predictable behavioural response patterns: (1) set formation, (2) set maintenance, (3) set shifting (Suchy, 2009). Or as proposed in another influential model, which states that EF is comprised of three higher-order factors:

(a) set shifting or also called mental flexibility,

(b) information updating or working memory,

(c) top-down inhibitory control, including behavioural and emotional self-inhibition and interference control such as in selective attention (Diamond, 2013; Miyake et al., 2000).

All of these subcomponents play a role in the development and maintenance of problematic alcohol intake and alcohol related behaviour. Other cognitive components can be added to this model including semantic / phonetic fluency, working memory, planning abilities, judgment, decision-making and insight (Kramer et al., 2014), which leads to a wide and complex network of cognitive abilities. A number of studies have shown the involvement of disrupted EF under the direct influence of alcohol (Guillot, Fanning, Bullock, McCloskey & Berman, 2010;

Montgomery, Ashmore & Jansari, 2011) and in AUD and other SUD (Bernardin, Maheut-Bosser, & Paille, 2014; Day et al., 2015; Fernández-Serrano, Pérez-García, Schmidt Río-Valle, & Verdejo-García, 2010; Soyoung Q. Park et al., 2010; Ratti et al., 2002). Others have shown that subjects with impaired EF may be at risk for SUD development (Nigg et al., 2006) or that EF performance can be a predictor for treatment outcomes (Bates, 2000).

II.3.2. Neuroanatomy and Neurochemistry

The construct of executive functions has its historical roots in the observation of patients with frontal lobe damage in the context of neuropsychological studies. For a long time, it has been known that patients with frontal lobe damage may present a wide range of problems including difficulties with the regulation of their behaviour (e.g. of impulsive actions or emotions), impaired functionality in everyday life, the inability to pursue long term goals or the dysfunctional preservation of inappropriate behaviour (Gilbert & Burgess, 2008). Thus EF has been traditionally associated with the frontal lobes, emerging from observations of clinical *frontal lobe* syndromes in the 1970s. Later on, EF has been particularly associated with the PFC

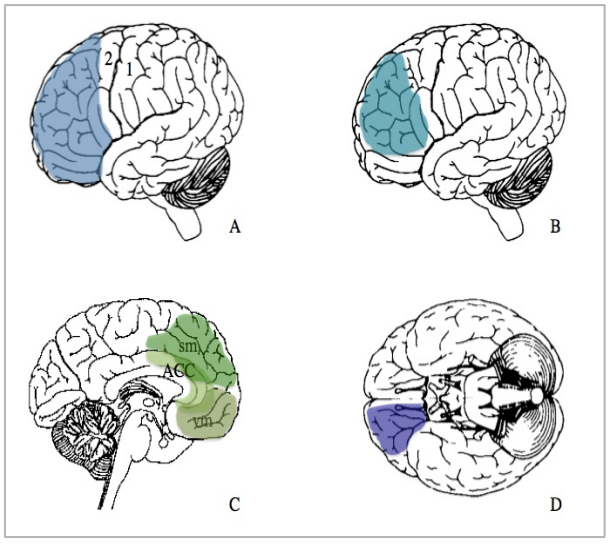

Figure 6. The Human Prefrontal Cortex (PFC)

(A) lateral view; primary (1), secondary and supplementary motor areas (2); (B) dorsolateral PFC (dlPFC); (C) medial view; superomedial (sm), ventromedial (vm) PFC and anterior cingulate Cortex (ACC), (D) ventral view; orbitofronal PFC (OFC).

(Alvarez & Emory, 2006) including all three main convexities (see figure 6): dorsolateral PFC, superomedial PFC (including the anterior cingulate gyrus (ACC)) and the ventral PFC consisting of orbitofrontal and ventromedial PFC (Suchy, 2009). With the possibility of

performing functional neuroimaging, it has become clearer that EF also depends on the integrity of complex networks and functional connections between brain regions that rely on balanced excitatory and inhibitory neurotransmitter systems. It is thus not possible to localize EF solely in the PFC, even if it is highly associated with it, as it is not possible to find a single anatomic correlate for a complex neuropsychological construct. However, a certain association between distinct regions and specific functions can be observed (see table 4). Therefore, EF is very vulnerable to various kinds of CNS injury, to perturbations in the neurotransmitter systems and as a result, sensitive to many psychiatric, neurodegenerative and medical conditions (Goldstein & Volkow, 2011).

II.3.2.1. Dopaminergic Modulation of Executive Functions

Many modulation processes in the PFC take place via projections of monoaminergic neurons, including dopaminergic neurons. These projections reach out widely to diverse areas, including the hippocampus, striatum, amygdala, thalamus and the neocortex (Robbins & Arnsten, 2009). Especially the field of working memory has been studied in this context, but also other subcomponents of EF have been the centre of investigations (Floresco & Magyar, 2006). Multiple studies have been performed, looking into rodents, primates and humans. Different types of study designs have been used, from observations to interventional studies and genetic analyses. In particular in recent years, PET studies in humans have shown an inverted-U-shape relationship between D_1R binding in the PFC and the performance in EF tests (Takahashi et al., 2008; Vijayraghavan, Wang, Birnbaum, Williams, & Arnsten, 2007) such as the Wisconsin Card sorting test (WCST), a laboratory measure that reflects in particular shifting abilities and mental flexibility. The inverted-U relationship suggests that levels, which lie either above or below the optimum level of dopaminergic activation, lead to impaired performance. Experiments with rodents and monkeys have also shown impaired cognitive functioning in laboratory measures (especially in spatial working memory) after the injection of selective D_1R blockers into the PFC but not always after the injection of D_2R blockers (Floresco, Magyar, Ghods-Sharifi, Vexelman & Tse, 2006; Granon et al., 2000; Sawaguchi & Goldman-Rakic, 1994). The review by Robbins and Arnstern (2009) summarizes and suggests a major role of DA modulation in a set of different tasks of the PFC: spatial and online working memory, reversal learning/extinction and underlying reinforcement learning. As described by Seamans & Robbins (2010) and Floresco & Magyar (2006), D_1R and D_2R can be stimulated optimally at different

Table 4. Processes associated with the Prefrontal Cortex that can be disrupted in Addiction

Retrieved and adapted from Volkow et al., 2011.

Process	Possible disruption in addiction	Probable PFC region
Self-control and behavioural monitoring:response inhibition, behavioural coordination, conflict and error prediction, detection and resolution	Impulsivity, compulsivity, risk taking and impaired self-monitoring (habitual, automatic, stimulus-driven and inflexible behavioural patterns)	DLPFC, dACC, IFG and vlPFC
Emotion regulation: cognitive and affective suppression of emotion	Enhanced stress reactivity and inability to suppress emotional intensity (for example, anxiety and negative affect)	mOFC, vmPFC and subgenual ACC
Motivation: drive, initiative, persistence and effort towards the pursuit of goals	Enhanced motivation to procure drugs but decreased motivation for other goals, and compromised purposefulness and effort	OFC, ACC, vmPFC and DLPFC
Awareness and interoception: feeling one's own bodily and subjective state, insight	Reduced satiety, 'denial' of illness or need for treatment, and externally oriented thinking	rACC and dACC, mPFC, OFC and vlPFC
Attention and flexibility: set formation and maintenance versus set-shifting, and task switching	Attention bias towards drug-related stimuli and away from other stimuli and reinforcers, and inflexibility in goals to procure the drug	DLPFC, ACC, IFG and vlPFC
Working memory: short-term memory enabling the construction of representations and guidance of action	Formation of memory that is biased towards drug-related stimuli and away from alternatives	DLPFC
Learning and memory: stimulus–response associative learning, reversal learning, extinction, reward devaluation, latent inhibition (suppression of information) and long-term memory	Drug conditioning and disrupted ability to update the reward value of non-drug reinforcers	DLPFC, OFC and ACC
Decision making: valuation (coding reinforcers) versus choice, expected outcome, probability estimation, planning and goal formation	Drug-related anticipation, choice of immediate reward over delayed gratification, discounting of future consequences, and inaccurate predictions or action planning	lOFC, mOFC, vmPFC and DLPFC
Salience attribution: affective value appraisal, incentive salience and subjective utility (alternative outcomes)	Drugs and drug cues have a sensitized value, non-drug reinforcers are devalued and gradients are not perceived, and negative prediction error (actual experience worse than expected)	mOFC and vmPFC

PFC = Prefrontal cortex; mPFC = medial PFC; DLPFC = dorsolateral PFC ; vmPFC = ventromedial prefrontal cortex; vlPFC = ventrolateral PFC; ACC = anterior cingulate cortex; rACC = rostral ACC; dACC = dorsal ACC; IFG = inferior frontal gyrus; OFC = orbitofrontal cortex; lOFC = lateral OFC; mOFC = medial OFC

levels of DA presynaptic activity. This may improve different aspects of cognitive abilities. The distinct role of D_2R levels in this context is less clear and needs further investigation. However, $D_{2/3}R$ availability in the striatum has been positively associated with performance on laboratory tests that reflect and rely on EF (Ballard, Dean, Mandelkern & London, 2015; Chen et al., 2005; Christopher et al., 2014). As striatal $D_{2/3}R$ availability is typically lower in AUD patients than in healthy controls (Bühler & Mann, 2011; Heinz, Siessmeier, et al., 2005b) we can suppose that there are also extrastriatal effects of chronic alcohol consumption on $D_{2/3}R$ availability. This is of special interest in areas that are connected to EF and prefrontal integrity. The relation of D_2R availability in the frontal cortex and EF has been shown for example by Vyas et al. (2017) for schizophrenic patients. Better performance in WCST perseverative errors was associated with higher binding potential in the frontal cortex.

II.3.3. Assessing Executive Functions

The variety of laboratory tests that investigate EF performance is as inhomogeneous as the different theories that try to define what EF is. Thus, a large variety of tests have been developed and implicated (Day et al., 2015). Some of them have gained greater acceptance in the researcher community and have been used and validated intensely. The tests administered in this study will be further explained in the methods section (see III.2.2).

II.4. Alcohol and Executive Functions

As proposed by Koob and Volkow (2010), a possible chronologic development of addiction can be seen as a change in firing in mesolimbic DA neurons starting with administration of the drug (e.g. alcohol), leading to LTP first in the VTA and then in the NAc and engaging the dorsal striatum via feedback loops. Long-term changes in the extended amygdala and the PFC may follow (see figure 3). This eventually leads to a continued strong drive for drug-seeking behaviour, reduced inhibitory control and poor decision-making when confronted with alcohol related stimuli even long after withdrawal. Studies in humans and animals have shown that alterations in DA signalling play a part in higher relapse risks, stronger craving and impaired quality of life (Heinz, Siessmeier et al., 2005b; Volkow et al., 2002). On the contrary, individuals with higher levels of D_2R availability and a positive family history of AUD may have a protective factor towards the development of AUD (Volkow et al., 2006).

When talking about EF in AUD a question of causality is inevitable: Does impaired EF lead to compulsive alcohol consumption or does drug consumption lead to EF difficulties? The interconnection between the two is evident but it is not possible to develop an exclusive hierarchic or chronological causality. However, there seem to be certain patterns and regularities in the impairment of EF under the acute and chronic influence of alcohol. In the following, we will focus on aspects of EF that may have a strong impact on AUD and on those that are most affected by alcohol consumption.

II.4.1. Acute Alcohol intake and Executive Functions

The direct influence of acute alcohol intake on EF in healthy subjects has been intensively investigated. These studies help to understand dose- and task-related effects of ethanol on the brain. Additionally, they contribute to identifying the affected functions and brain circuits impaired by acute intoxication. It has been shown that temporary impairments of EF occur in different laboratory measures for set shifting, information update, working memory and response inhibition (see Day et al., 2015). The review by Day et al. (2015) lists 35 studies that investigate the acute effects of alcohol on EF. Examinations throughout the studies reviewed took place under different conditions, between 0.2 and 0.8g ethanol per kg bodyweight or at 0.01 to 0.10% BAC. As summarized by the authors, many sub-functions such as long- and short-term memory, working memory, word generation, mental flexibility, response inhibition and reaction time were affected by acute alcohol intake. The effects occurred mainly in groups with high and medium BAC, leading mainly to perseveration errors that are thought to be more specific to frontal lobe dysfunction.

II.4.2. Chronic Alcohol intake and Executive Functions

A characteristic trait of AUD and chronic, hazardous alcohol consumption is e.g. a certain loss of control over the amount of alcohol that is taken in or the resulting personal problems. In the most pronounced cases of AUD, persistent consumption can lead to substance-induced neurocognitive disorders such as the Wernicke-Korsakoff syndrome (WKS) – with extensive impairment and damage especially to the frontal lobe and important associated functions such as EF (Oscar-Berman, Kirkley, Gansler, & Couture, 2004). The investigation of chronic alcohol intake and its influence on EF performance brings up different challenges. The

influencing factors are by far not as controllable as in studies that investigate the acute influence of alcohol consumption. In those studies, researchers have direct insight and control over e.g. applied doses of alcohol. Investigations of chronic alcohol intake however depend on self-rating and personal insight abilities as well as the willingness of the participants to give honest information about aspects such as consumption patterns and alcohol-related problems. In the following, we will focus on two subgroups of chronic alcohol consumption: (1) heavy drinking, HR subjects with a problematic intake of alcohol as defined in chapter II.1.1.2 and (2) patients with a diagnosis of AUD after acute detoxification.

II.4.2.1. High-Risk Drinking and Executive Functions

In a study published by Heffernan (2002), HR alcohol users reported more memory slips of prospective, short-term and long-term memory than controls, suggesting that heavy drinking has a negative effect on aspects of everyday memory. As shown in the review by Montgomery et al. (2015), many studies that are investigating HR drinking behaviour and EF concentrate on young subjects in their early twenties, who are mainly university students and currebtly performing HED. Those subgroups present a very specific group of HR subjects, not fully representative of the community. Other studies, as the one performed by Houston et al. (2014), however, looked into bigger samples (n=560 subjects) with differing educational backgrounds, different age groups and thus representing a demography more similar to that of the community. The results of that study showed that greater alcohol use was associated with significantly poorer performance in EF tasks such as WCST, TMT (testing mental flexibility and set shifting ability), go/stop tasks (testing response inhibition) and higher scores in a self-reported dysexecutive functioning questionnaire. This effect remained when controlled for age, gender and education (Houston et al., 2014) and is providing evidence that HR drinking behaviour has a negative impact on EF performance.

II.4.2.2 Alcohol Use Disorder and Executive Functions

A large number of studies investigating AUD and the influence of alcohol on the brain have been performed over the past decades. Studies of neuropathology, post-mortem and in vivo neuroimaging using CT and MRI have found global atrophy and structural abnormalities in the CNS of AUD subjects. Furthermore, there is a great body of work showing that alcohol-

dependent subjects exhibit particular deficits, involving alterations in brain circuits connected with the prefrontal cortex, cognitive abilities and EF (Moselhy, Georgiou, & Kahn, 2001; Oscar-Berman & Marinković, 2007; Stavro et al., 2013; Wilcox, Dekonenko, Mayer, Bogenschutz, & Turner, 2014). Additional evidence in support of frontal system dysfunction in AUD is based on work looking into patients with persisting substance-induced amnestic disorder such as WKS (Oscar-Berman & Evert, 1997; Oscar-Berman, Kirkley, Gansler & Couture, 2004; Sullivan, Harris & Pfefferbaum, 2010).

Taking these deficits in working memory, set maintenance, response inhibition and mental flexibility into account, subjects with AUD are challenged in a particular way concerning everyday living, employment situations and personal life. Long-term abstinence from alcohol can improve or resolve previously present deficits in cognitive and EF (Guthrie & Elliott, 1980; Oscar-Berman et al., 2014). However, not all AUD patients show EF impairment. The exploration regarding which processes are spared and which are affected in a given subject might give a basis for a more targeted therapy approach during recovery (Sullivan et al., 2010).

> Normalizing these functions [reduced inhibitory control, emotional disruptions], using empirically based and targeted pharmacological and cognitive-behavioural interventions — in combination with the relevant reinforcers — should become a goal in the treatment of addiction. (Goldstein & Volkow, 2011)

II.5. Hypotheses

Regarding the development and maintenance of problematic alcohol consumption and AUD, it is important to look into different subgroups to simulate the dimensional approach of the disorder. Therefore, the inclusion of a third group that lies between the two extremes on the continuum was plausible.

We aim to determine the relationship between $D_{2/3}R$ availability and EF performance with a special focus on group differences between LR, HR controls and AUD. Non-displaceable binding potential (BP_{ND}) of FAL and EF test results will be compared between the three groups of subjects.

Hypothesis 1.1 (H1.1)

EF performance of HR controls and detoxified AUD patients is impaired when compared to LR control performance.

Hypothesis 1.2 (H1.2)

HR subject will lie on an intermediate performance level between LR and detoxified AUD.

Hypothesis 2.1 (H2.1)

Extrastriatal $D_{2/3}R$ availability is lower in detoxified AUD than in LR and HR controls.

Hypothesis 2.2 (H2.2)

Extrastriatal $D_{2/3}R$ availability measured in HR subject will lie on an intermediate level between LR and detoxified AUD.

Hypothesis 3 (H3)

Extrastriatal $D_{2/3}R$ availability is positively correlated to EF performance.

III. Material and Methods

The present study was performed as a subproject (Project 5 (P5)) of the LeAD Study, a bi-centric study on learning and habituation as predictors of the development and maintenance of alcoholism (www.leadstudie.de; clinical trial number: NCT01679145). The research was conducted in the framework of a collaboration of Charité Universitätsmedizin Berlin and Technische Universität Dresden. P5 was conducted at the Clinic of Psychiatry and Psychotherapy at Charité Berlin and was further carried out at Physikalisch-Technische Bundesanstalt Berlin (PTB). The LeAD study was funded by Deutsche Forschungsgesellschaft (DFG FOR16/17).

The data for P5 were collected in Berlin only. Data storage was centralized and organized in Dresden in a subproject of the LeAD study (see figure 7). P5 was approved by the local ethics committee, performed according to the World Medical Association Declaration of Helsinki and carried out under Prof. Dr. med. Gallinat's and Prof. Dr. med. Felix Bermpohl's supervision. Participants underwent written informed consent and detailed instruction regarding clinical testing and scanning procedure by trained researchers, in particular with regard to imaging procedures and a radiation exposure of approximately 5.8 mSv during PET/CT. All participants were financially compensated after their participation.

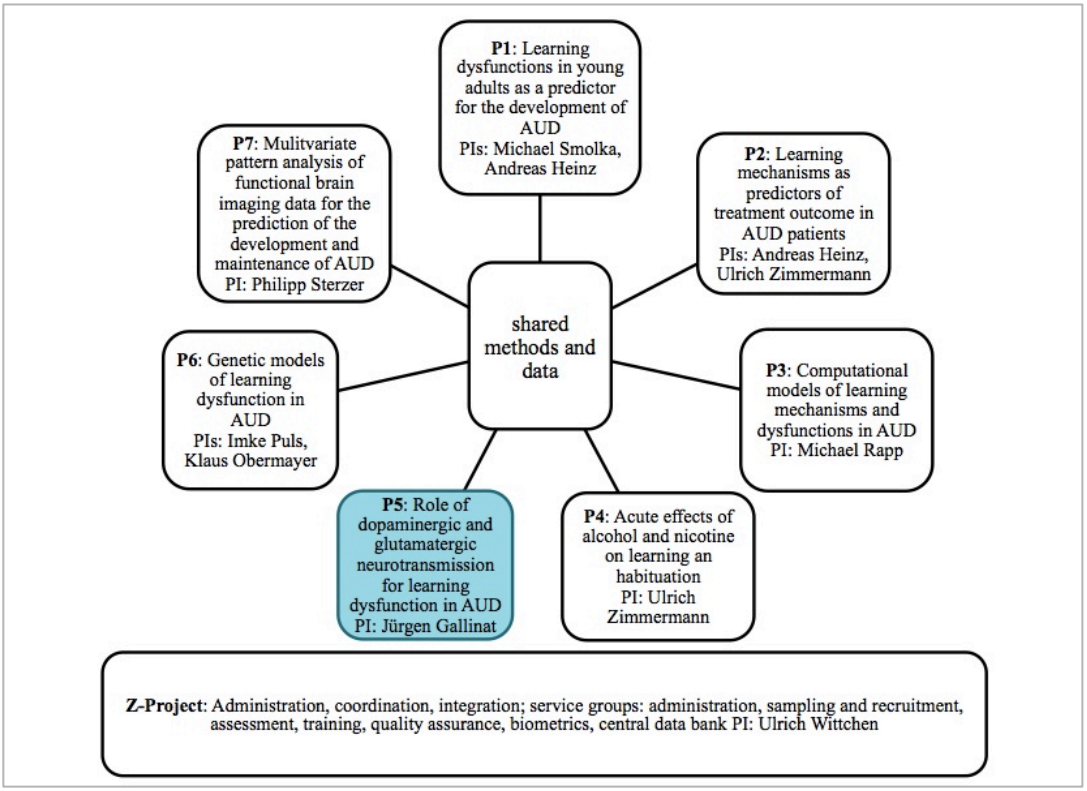

Figure 7. Learning and Alcohol (LeAD) Study with Subprojects, Funding Period I

III.1. Subjects

III.1.1. Recruitment, Inclusion and Exclusion Criteria

The aim was to recruit 20 participants for each group (20 AUD, 20 HR, 20 LR). AUD subjects were recruited during acute detoxification on specialised psychiatric wards in four different hospitals in Berlin (Sankt Hedwig Krankenhaus, Bundeswehr Krankenhaus, Jüdisches Krankenhaus, Charité Universitätsklinikum Campus Mitte (CCM)). Patients had been diagnosed with AUD by independent psychiatrists and information about the LeAD study was obtained during inpatient treatment. AUD patients were referred to P5 from Project 2 (P2) of the LeAD Study. AUD diagnosis was verified through a structured clinical interview performed during the first assessment session in P2 by trained researchers. HR and LR controls were directly recruited from the community via advertisements in supermarkets, local newspapers and online on an internet platform (www.ebay.kleinanzeigen.de).

Participants were screened for exclusion criteria via telephone or in person. Exclusion criteria were left-handedness, insufficient knowledge of the German language, major visual or auditory impairments, a history of any substance dependence or current substance use other than alcohol. Only nicotine dependence in HR and LR controls and nicotine and alcohol dependence in AUD patients were accepted. The AUDIT (see III.2.1.) was performed during the telephone screening and allowed direct allocation of controls to the HR subgroup. However, some LR participants were directly referred to us by P2 and did not complete AUDIT during the screening. Additionally, major non-communicable diseases such as non-treated diabetes, high blood pressure, thyroid dysfunctions and infectious diseases like HIV or hepatitis as well as major psychiatric disorders (lifetime history of DSM-IV bipolar or psychotic disorder; current threshold DSM-IV diagnosis of any following disorders: current major depressive disorder, generalized anxiety disorder, posttraumatic stress disorder, borderline personality disorder, or obsessive-compulsive disorder), intake of psychotropic medication, neurological diseases like epilepsy, multiple sclerosis, any form of dementia, WKS, history of traumatic brain injury, meningitis, brain operations or claustrophobia led to exclusion.

AUD patients, furthermore, were to be assessed in early abstinence, i.e. between 72 hours and 21 days after their last drink, and only allowed to show a low severity of withdrawal symptoms according to the Clinical Institute Withdrawal Assessment Scale (CIWA <8) at baseline. As P5 was performing magnetic resonance imaging and spectroscopy (MRI/MRS) and PET/CT on participants, metallic implants such as pacemakers, orthopaedic or complex dental

prostheses and metal clips, pregnancy and nursing infants (for females), recent exposure to radiation (e.g. x-ray exams or CT scans in the last 3 months), were further exclusion criteria. Moreover, we carefully tried to match participants to minimize group differences and respected the inclusion and exclusion criteria.

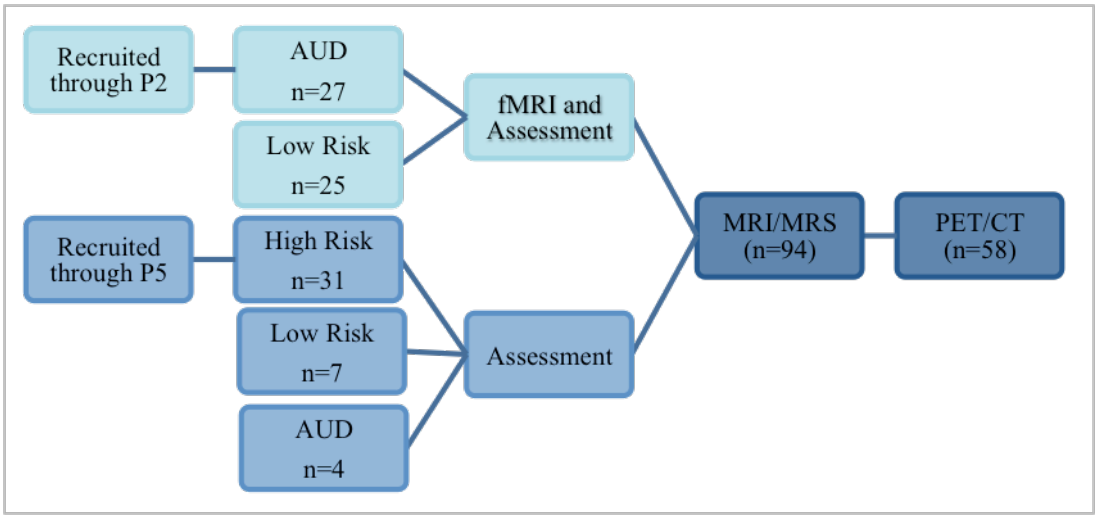

Figure 8. Study Process and Participant Numbers

III.1.2. Group Distinction

The AUDIT was used to distinguish between HR and LR control groups. A subject with an AUDIT score of 8 or higher and no history of a former or current AUD diagnosis or treatment was considered as belonging to the HR group. Subjects with a score of less than 8 or no intake of alcohol were classified as LR subjects. For detailed information on the AUDIT, see section III.2.1.

III.1.3. Description of the Sample

58 subjects completed the whole test battery and were included in the final sample. A total of 96 subjects (31 AUD; 32 HR; 33 LR) were initially recruited. A number of participants did not respond in verbal or written form after the first (2 drop outs) or second assessment (26 drop outs). For study process, see figure 8. Also, a number of participants could not be assessed due to problems with tracer synthesis.

Our final PET sample consisted of 19 LR controls aged between 30.8 and 61.8 years ($Mean_{age}$ in years = 45.22; SD_{age} = 8.65), 19 HR controls between 26.8 and 57.6 years ($Mean_{age}$ in years = 42.89; SD_{age} = 9.08) and 20 patients between 29.4 and 58.3 years ($Mean_{age}$ in years = 45.36; SD_{age} = 8.41). Female subjects were distributed over the different subgroups as follows: 3 LR controls, 2 HR controls and 3 AUD patients. Kruskal-Wallis tests showed no significant group differences concerning age, gender distribution, handedness, BMI, education years and estimated intelligence quotient (IQ) at baseline. Significant differences in OCDS, $H(2) = 26.73$, $p < .05$; ADS, $H(2) = 31.91$, $p < .05$ and AUDIT, $H(1) = 13.40$, $p < .05$, were confirmed. For

Table 5. Descriptive Statistics of final PET Sample

PET SAMPLE		AUD	valid cases	HR	valid cases	LR	valid cases	total	total valid cases
Group size									
	n	20	20	19	19	19	19	58	58
Gender									
	m	17		17		16		50	
	f	3	20	2	19	3	19	8	58
FTND									
	smokers	16		17		10		43	
	ex-smokers	3		0		4		7	
	non-smokers	0	19	2	19	5	19	7	57
EHI									
	right handed	19		19		19		57	58
	left handed	1	20	0	19	0	19	1	
Age at PET									
	M±SD	45.36 ±8.41	20	42.89 ±9.08	19	45.22 ±8.65	19		58
	(min-max)	(29.4 - 58.3)		(26.8 - 57.6)		(30.8 - 61.8)			
BMI at PET									
	M±SD	26.77 ±3.39	20	27.26 ±4.49	19	25.57 ±4.10	19		58
	(min-max)	(19.7 - 32.9)		(20.7 - 38.0)		(20.6 - 34.7)			
Education Years at BL									
	M±SD	15.10 ±3.32	20	17.50 ±5.36	17	14.55 ±3.09	19		56
	(min-max)	(10 - 23)		(12 - 31)		(8 - 21)			
Estimated IQ, MWT-B									
	M±SD	102.65 ±8.40	20	102.32 ±9.67	19	102.89 ±5.54	19		58
	(min-max)	(86 - 120)		(94 -130)		(95 - 112)			
AUDIT Score at BL									
	M±SD	NA		12.32 ±3.02	19	4.67 ±1.21	6		24
	(min-max)			(9 - 18)		(3 - 6)			
ADS Score at BL									
	M±SD	17.05 ±6.33	19	5.97 ±3.22	13	3.21 ±3.76	19		51
	(min-max)	(5 - 30)		(2.08 - 12.50)		(0 - 12)			
OCDS Score at BL									
	M±SD	13.55 ±8.35	16	7.44 ±3.28	18	2.63 ±2.31	19		53
	(min-max)	(3 - 30)		(0 - 12)		(0 - 8)			
Age at first Drink									
	M±SD	14.31 ±2.02	16	NA		14.69 ±2.06	13		16
	(min-max)	(11 - 19)				(10 - 18)			
Age of first AUD Diganosis									
	M±SD	32.27 ±11.23	16	NA		NA			16
	(min-max)	(17 - 57)							
Years since AUD Diagnosis at BL									
	M±SD	13.93 ±11.00	15	NA		NA			15
	(min-max)	(0.96 - 36.00)							
Days of abstinence at PET									
	M±SD	36.4 ±20	20	NA		NA			20
	(min-max)	(9 - 96)							

ADS = Alcohol Dependence Scale; AUD = alcohol use disorder; AUDIT = Alcohol Use Disorder Identification Test; BL = baseline; BMI = body-mass-index; EHI = Edinburgh Handedness Inventory; FTND = Fagerström Nicotine Dependence Scale; IQ = Intelligence Quotient; M = Mean; max = maximum; min = minimum; MWT-B = Mehrfach-Wortschatz-Intelligenztest; OCDS = Obsessive Compulsive Drinking Scale; PET = Positron Emission Tomography; SD = standard deviation.

visual and comprehensive reasons clinical parameters of the sample are shown for all groups (LR, HR, AUD) in table 5.

III.2. Assessment

Subjects meeting inclusion criteria were invited to Charité Berlin to complete in-person interviews, paper and pencil tests as well as digital questionnaires and tests during their first appointment. Directly prior to testing, all participants were screened for alcohol and other substance consumption (including benzodiazepines, amphetamines/MDA, metamphetamines/ MDMA/XTC, opiates, cannabis) via breath and urine test (Mahsan® – Kombi/DOA6 Schnelltest, Mahsan® Diagnostika, Germany) and were only allowed to complete the assessment if tested negative. A positive test led to direct exclusion. All participants had their blood taken for future genotyping and further analysis before starting the session. The assessments took place during the day in special testing rooms at Sankt Hedwig Krankenhaus Berlin (between 2014 and 2015) and CCM (from 2013 until 2016). Participants were allowed to take breaks when needed. Supervision and explanation of tasks was ensured through the presence of at least one and a maximum of two study researchers. Assessments took place at least one day before the imaging assessments. Participants were contacted after a 6 months' interval for a first telephone follow-up (FU). For the 12-month-FU, we invited the subjects once again to Charité Berlin to let them perform a similar battery of tests. The last 12-month-FUs were completed in 2017.

The completion of the battery of questionnaires, neuropsychological and cognitive tests took between two and a half and three hours on average. All three groups were equally assessed in different domains of executive functioning and cognitive performance. Subjects participating in Project 2 additionally completed the WHO composite international diagnostic interview (CIDI), adding another 60 minutes to the session.

III.2.1. Clinical Tests

Alcohol Use Disorder Identification Test (AUDIT)

The AUDIT has been widely used and verified as a reliable tool of early detection of problematic alcohol consumption in a primary healthcare setting (Saunders, Aasland, Babor, de la Fuente, & Grant, 1993). It is a simple questionnaire that comprises 10 items concerning drinking behaviour, alcohol consumption and related problems such as domestic, legal or occupational difficulties related to the consumption of alcohol. For each response, there is a minimum of 0 and a maximum score of 4 points, thus a total minimum of 0 and maximum score of 40. A positive case, as described by Saunders et al., was defined as any of the following: a hazardous daily level of alcohol intake (meaning an average intake of 40 g of pure ethanol for women and 60 g for men), recurrent intoxication (with an intake of 60 g of ethanol daily or 120 g weekly), abnormal drinking behaviour (meeting at least one of the criteria of the alcohol dependence syndrome but not enough to fit its diagnosis), at least one alcohol-related problem in the past year (e.g. traumatic injury caused by intoxication, drunk-driving), an alcohol-related disease or a perceived drinking problem (for example concern mentioned by family, health professionals or friends).

The cut off scores were chosen to fulfil maximal sensitivity and specificity and thus set at a score of 8, resulting in an overall sensitivity of 92% for identifying hazardous and harmful alcohol consumption and a specificity of 94% (Conigrave et al., 2006; Saunders et al., 1993). Reliability and validity were tested in different population samples from primary health care settings in the USA, Kenya, Australia, Bulgaria, Norway and Mexico (Saunders et al., 1993). We used a German translation of the AUDIT (Rist et al., 2003).

Alcohol Dependence Scale (ADS)

The ADS is a widely used, valid and reliable clinical and research tool which provides a quantitative measure of AUD severity (Skinner & Allen, 1982). The total of 25 items cover domains such as impaired control over drinking, awareness of compulsive behaviour, increased tolerance, withdrawal symptoms, salience of drink-seeking behaviour and refer to the past 12 months. The ADS can be used to quantify the severity of AUD with respect to treatment planning for patients but also as a screening tool. A score of 9 or more on the ADS is highly predictive of a DSM / ICD diagnosis of AUD. We used a German translation of the ADS questionnaire (Ackermann, Hollweger & Gordon, 1999). The computer self-administration took about 5 minutes.

Obsessive Compulsive Dependence Scale (OCDS)

The 17-item OCDS is a quick and reliable self-rating instrument that assesses compulsive behaviour with regard to drinking and obsessive thoughts about alcohol that are thought to be crucial dimensions of perceived craving (Anton, Moak & Latham, 1995). This scale has been shown to be sensitive to and specific for the obsessive and compulsive characteristics of drinking-related thought in alcohol-abusing and AUD populations. It is also possible to calculate subscores concerning *obsessions* and *compulsions* as suggested by Anton et al. (1995). Furthermore, it has been shown to be sensitive as a monitoring tool with predictive validity for relapse drinking. We used the German translation according to Mann & Ackermann (2000) in a computed version for self-administration, taking about 5-10 minutes.

Fagerström Nicotine Dependence Scale (FTND)

The FTND is a standard test to evaluate physical nicotine dependence and provide an ordinal measure of severity (Heatherton, Kozlowski, Frecker & Fagerström, 1991). The answers to the 6 items add up to a total score of between 0 and 10 points. We used the German translation according to (Bleich, Havermann-Reinecke, & Kornhuber, 2002) to determine smoking-status.

Edinburgh Handedness Inventory (EHI)

The EHI is a self-administered measurement scale that is frequently used to assess the dominance of a person's right or left hand (laterality) (Oldfield, 1971). The items cover everyday activities. We used our own translation of the EHI to assess handedness with 8 items. All participants with results of *strongly right-handed* and *mixed right-handed* were attributed right-handed, persons *strongly left-handed* and *mixed left-handed* were considered as left-handeder.

Estimated IQ – Mehrfachwahl-Wortschatz-Intelligenztest

Verbal intelligence was assessed by a very common standardized, multiple choice vocabulary test (Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B)) as a representative measure of fluid intelligence. The results are known to correlate fairly well with global IQ levels in healthy adults and to be rather insensitive to confounders such as age, CNS and psychiatric diseases (Lehrl, 2005). Its administration takes about 5 minutes. Subjects are asked to identify the correct word out of a group of words that are phonetically similar. However, only one of the given words in each of the 37 groups is correct. The items are listed in ascending order in terms

of their difficulty. Thus, in the interpretation of the test, the further the participant gets in identifying the right word, the higher the crystallized intelligence. Estimated IQ levels were examined to control for major differences between groups that can reflect on cognitive and EF test performance.

III.2.2. Cognitive Tests – Assessing Executive Functioning

As stated in section II.3.3., many different laboratory tests have been developed to assess EF. For this study, we chose to use a battery of tests that are well known and easy to perform. We administered the battery of neuropsychological tests and questionnaires during the first testing session. Only tests and questionnaires relevant to this investigation will be explained in the following.

Semantic Fluency – Animal-naming (CIMP)

Fluency represents the ability to maximize the production of information in a limited time span while the subject must avoid repetition. Semantic fluency (or category fluency) is one of three common fluency tasks along with design (e.g. to connect dots with four lines in as many different ways as possible) and phonemic fluency (e.g. to generate as many words as possible starting with a certain letter). During this task, participants were asked to name as many words from a specific category (e.g. animals, clothes, groceries) as possible. The limited timespan for the task was 60 seconds. In the end, the score reflects the number of correctly named animals in the given timespan. Deficits observed in these measures can be a sign of disorganization, lack of initiation (Rabinovici, Stephens, & Possin, 2015) and reflect thus on EF.

Trail-Making Test (TMT) Part A+B

The trail-making test (TMT) has been widely used for the assessment of cognitive impairment (Reitan, 1955). In particular, it has been used to look into alcohol abuse and frontal lobe (dys)function. The TMT is a tool to measure visual attention, motor speed and set shifting/cognitive flexibility. In TMT-A, the participant has to draw a line between a series of numbers, connecting them in the correct sequential order as fast as possible. Part B requires the participant to draw a line connecting numbers and letters in alteration between the ascending and the alphabetical order (1-A-2-B-3-C-...). Thus the participant needs to additionally cope with a

shifting paradigm, divide the attention between numbers and letters and suppress the impulse to follow the more familiar task of connecting numbers in the sequential order only. Various studies have shown that especially the performance in TMT-B can be impaired in AUD patients (Houston et al., 2014; Ratti et al., 2002; Zinn, Stein & Swartzwelder, 2004) even in those that did not show any obvious deficits during clinical observation or in everyday life (Moselhy et al., 2001). Also the performance of HR subjects as described in Houston et al. (2014) can be poorer when compared to LR controls.

Digit Symbol Coding (ZST)

The ZST is a subcomponent retrieved from the Wechsler Adult Intelligence Scale (WAIS) (Aster, von Neubauer & Horn, 2006) that investigates mainly working memory and processing speed. During ZST, participants are asked to translate a sequence of numbers (from 1 to 9) corresponding to digit-symbol pairs as shown at the top of the given work sheet. Subjects are asked to work as fast and as precise as possible. The number of symbols correctly written down in a limited time (120 seconds) is measured. This test reflects mainly processing speed and working memory abilities.

Digit Span Backwards Test (ZNR)

The ZNR mainly investigates working memory and is also retrieved from the WAIS (Aster, von Neubauer & Horn, 2006). For ZNR, subjects hear a sequence of numerical digits at approximate speed of one digit per second and are asked to repeat the sequence in reverse order. After a correct answer, the length of the sequence increases. The longest span of correctly repeated digits is measured. Points for each correct sequence are added up to measure performance.

III.3. Imaging Acquisition

All participants underwent two imaging assessments on two separate days after the clinical assessment. FAL PET/CT took place at Charité Berlin and MRI/MRS at the Physikalisch Technische Bundesanstalt Berlin (PTB). MRS data were not analysed for this particular work. PET measurements were performed in cooperation with the department of Nuclear medicine of Charité Berlin (for radiochemistry, synthesis of the tracer and scanning procedure). For this analysis, the focus will be on acquired PET/CT and structural MRI data. Participants started their PET session either at 9 or 10.30 a.m. during weekdays. The whole scanning process took up a maximum of 5 hours. PET/CT data was acquired in 3 blocks with a break between each block during which participants were allowed to stretch, drink, eat and use the bathroom. Trained medical personnel were present throughout the exam. Subjects participating in P2 also underwent functional magnetic resonance imaging (fMRI) beforehand.

III.3.1. Magnetic Resonance Imaging

Anatomical MRI data was obtained during the second session at PTB prior to PET/CT testing. The whole session lasted approximately 1.5 hours. MRI was carried out on a 3-Tesla scanner (Siemens Verio) using a circularly polarized head coil. T1-weighted images (modified driven equilibrium Fourier transform (MDEFT), echo time (TE) = 3.8 ms, repetition time (TR) = 20.53 ms, 128 contiguous slices of 1.5 mm thickness, 1 mm inplane (x-y) resolution) were acquired. MRI was not one of the primary measurements and will thus not be explained in detail in the present work.

III.3.2. Positron Emission Tomography – Principle and Physical Basis

PET is a type of nuclear medical imaging and provides information that is not available through other procedures. It is used in diagnostics and research to assess metabolic processes in the body and to investigate a multitude of biochemical and physiological parameters. In contrast to classical x-ray examinations or CT it does not generate images by passing x-rays though the body from an external source. PET scanners detect pairs of photons (gamma (γ) rays) that are emitted by a special positron-emitting radioactive tracer from inside the body. In PET images, areas of greater intensity, so called *hot spots*, indicate a higher concentration of the tracer and

thus higher metabolic or chemical activity. Corresponding to that, areas with low intensity represent less activity and less accumulation and are thus called *cold spots* (Mikla & Mikla, 2014). The assessment of different parameters is possible due to different tracers. Nearly all biological molecules (e.g. glucose, amino acids, peptides, enzymes, transporters, receptors, proliferation markers etc.) can theoretically be labelled with positron emitters (Wadsak & Mitterhauser, 2010).

Therefore, the application (intravenous, oral, inhaled) and choice of a radiopharmaceutical plays a central role in the selectivity and functioning of PET. Radiopharmaceuticals are specific molecules that are radiolabeled and thus consisting of two essential parts:

(a) a molecular structure (vehicle or ligand) that determines the distribution and target site inside the organism according to its binding characteristics, pharmacodynamics and pharmacokinetics

(b) a radioactive, positron-emitting isotope which is responsible for the signal that allows tacking from outside the organism via PET (Wadsak & Mitterhauser, 2010).

Isotopes are variants of a chemical element that differ in the number of neutrons in their atomic nucleus. Proton numbers stay the same, as they define the chemical element. Isotopes used for PET typically hold an excess of protons compared to neutrons in their nucleus as these typically decay to a large portion in beta plus (β^+) decay. By definition, the atomic nucleus emits a positron (e^+) and a neutrino during β^+ decay in order to transform a proton into a neutron. In that way, the atom reaches a more stable configuration. The neutrino does not interact with surrounding tissue, whereas the e^+, being the antiparticle of an electron (e^-), has a positive charge and interacts with an e^- of another atom. When e^+ stops moving away from its source and meets an e^-, their entire rest mass energies (511 keV each; 1,22 MeV in total) are fully transformed into two photons travelling at an 180° angle in opposite directions (Mikla & Mikla, 2014). This process is called annihilation coincidence. The simultaneous detection of the two emerging photons, the annihilation coincidence detection (ACD), is the basis of PET (see figure 9). The emission of photons is by definition γ-radiation and can, in contrast to e^+, leave the body. Surrounding detectors inside the PET scanner can register the emitting γ-rays. The site of the annihilation has to be close to or on the line that is projected by the two photons (line of projection, LOP). Its distance to the LOP is defined by the energy of the e^+ and is a limiting factor to the precision of PET resolution. PET cameras can have up to thousands of opposing detectors to localize the annihilation processes as precisely as possible.

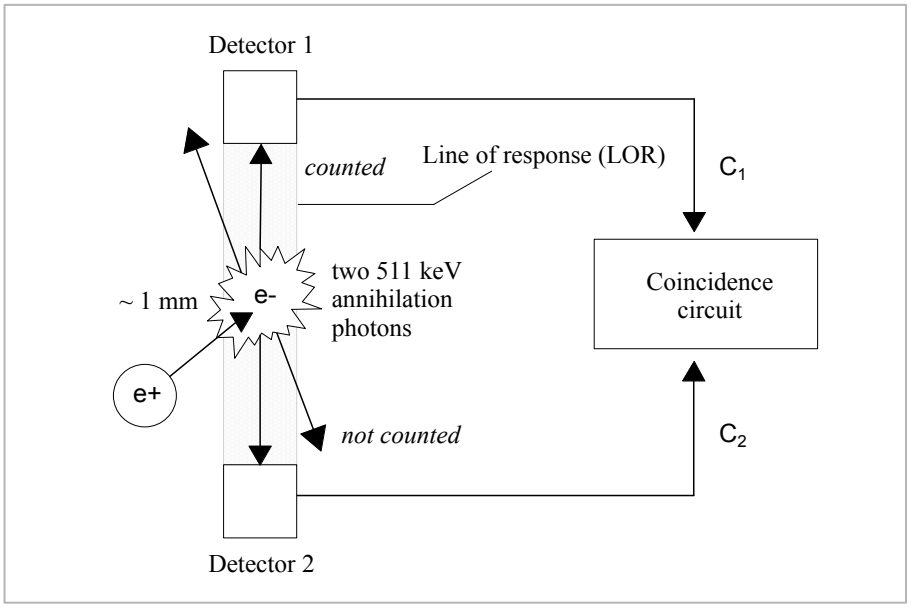

Figure 9. Schematic Illustration of Annihilation Coincidence Detection (ACD). An event is only counted when the two γ-rays are detected simultaneously (within the timing window); C1 and C2 are the single count rates recorded by Detectors 1 and 2. Retrieved and adapted from Mikla & Mikla, 2014

Radiopharmaceuticals cannot be chemically distinguished from their physiological, non-radioactive counterpart and are thus processed and transported indifferently to them in the body (Wadsak & Mitterhauser, 2010). Usually, the amount of applied tracer is very low so that physiological processes are not impaired. This particularity makes it possible to visualize metabolisms and processes in vivo and track the substance throughout the body. Various radiotracers are being used in different fields of nuclear medicine. A widely known tracer is ^{18}F-fluorodeoxyglucose (^{18}F-FDG), which is used mainly in oncology to show processes with increased metabolism like cancers. The isotope ^{18}fluorine (^{18}F) holds one neutron less than the naturally occurring, stable isotope ^{19}F. It has a half-time of 110 minutes and is produced either onsite in cyclotrons or can be distributed regionally and is thus very convenient for clinical exams (Muehllehner & Karp, 2006). By losing a proton, it is transformed into ^{18}oxygen (^{18}O) – a more stable and naturally occurring isotope of oxygen, the preceding element in the periodic table of the elements with one proton less in its core than fluorine. This transformation happens through β^+ decay, the atom emits an e^+ and a neutrino and thus loses a proton from the atomic nucleus. The annihilation process of the emitted e^+ and an e^- in the surrounding tissue transforms their masses into two photons travelling in opposite directions, as mentioned above. These can be detected by the PET scanner and provide information about their origin.

If two photons are detected by opposing detectors within the coincidence timing window τ (typically 6 to 12ns = 6 to 12 x 10^{-9}s), they are assumed to emerge from the same annihilation event and are called a true coincident event. Such a definite time window is needed, as photons have slightly different distances to travel until they reach the detector, depending on the exact localization of the positron-electron annihilation. However, as photons travel at the speed of light, this effect is however very small. Photons within a relatively large energy range (e.g. 250–650 keV) can be detected as valid γ-rays emerging from an annihilation. This may produce mispositioned coincidence events. It should also be noted that not all photons can be detected by the PET camera. Some photons travel in other directions than in that of the detectors or are altered in their direction, others are absorbed by the surrounding tissue. It is also possible that two photons are accidently registered as emitting from the same annihilation process due to very little time differences but do not do so (Compton-scattered annihilation, scattered γ-rays, scattered and unscattered non-annihilation photons). These processes affect the quality and resolution of PET images. The integrated data result in tomographic images that can be reconstructed with algorithms similar to those used for 3D CT image reconstruction. The spatial resolution of the detectors and the kinetic energy of the e^+ limit the total special resolution of PET to 1-2 mm in humans (Mikla & Mikla, 2014).

PET data can be combined with anatomical data from MRI or CT. In PET/CT, the sequentially registered images are taken in one session to be then superposed on a set of 3D images. The alignment of functional (PET) and anatomic data (CT/MRI) allows much higher precision of anatomic localization of a *hot spot* (a metabolically active process). The merging of MRI and PET data from two different sessions is possible as well. In this study, we used MRI for anatomical data and PET for quantitative characterization of the dopamine $D_{2/3}R$ status. It was performed using a time-of-flight PET/CT system Philips Gemini TF 16 (Surti et al., 2007). The high-affinity ligand FAL will be discussed in more detail in the section below.

III.3.3. ^{18}F-Fallypride - showing $D_{2/3}$ Receptor Availability

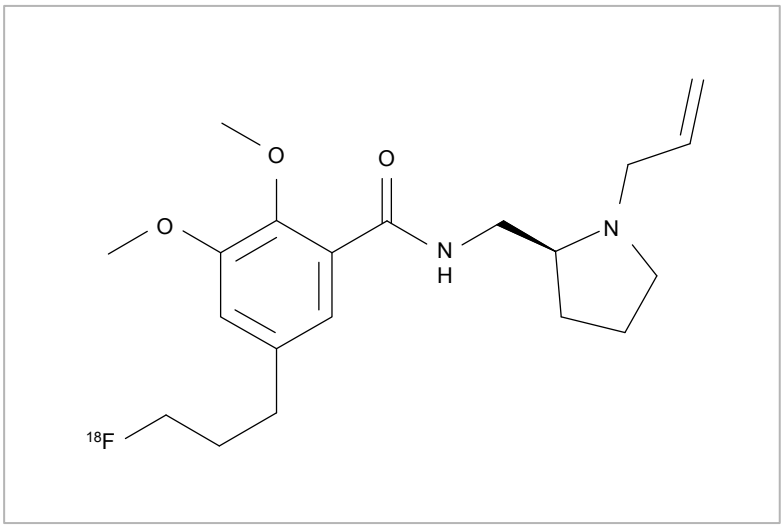

Figure 10. Molecular Structure of ^{18}F-Fallypride ($C_{20}H_{29}FN_2O_3$)

The molecule FAL, as shown in figure 10 above, is a substituted benzamide and a high-affinity $D_{2/3}$R antagonist and radioligand. Its affinity for other receptors such as D_4R is poor (Mukherjee et al., 2002). It is thus used for quantitative characterization of $D_{2/3}$R availability with PET. Due to its high affinity and relatively rapid in vivo reversibility of binding, it can simultaneously provide information about postsynaptic $D_{2/3}$R binding in extrastriatal brain regions with low receptor density and in the striatum with high receptor density in the same scanning session. However, the ligand clears slowly from areas with a high receptor density. Scanning therefore has to be performed up to 4 hours after the application of FAL to be able to trace emission during the wash-out phase in the striatum (also see scanning process, figure 11). To bind to the postsynaptic DA neuroreceptors, the tracer molecules compete with endogenous DA in the brain (Laruelle, 2000).

The FAL that was used for this study was produced at the Department of Nuclear Medicine of Charité Berlin following protocols previously described by Mukherjee, Yang, Das & Brown (1995). For detailed information about production and development processes, kinetics, distribution, sensitivity and advantages, see primary literature (Laruelle et al., 2003; Mukherjee et al., 2002, 1995; Slifstein et al., 2010) as this would exceed the scope of the present work.

III.3.3.1. Non-displaceable Binding Potential and the Simplified Reference Tissue Method

The outcome parameter of our measurements was the non-displaceable binding potential (BP_{ND}) of FAL. The binding potential (BP) is a combined measure of (a) the density of available (free) neuroreceptors and (b) the affinity of a ligand to that specific receptor. As described in Innis et al. (2007), BP_{ND} refers to the ratio at equilibrium of a specifically bound radioligand to that of non-displaceable radioligand in tissue. It is the typical measurement of reference tissue methods, as it compares the concentration of radioligand in receptor-rich to receptor-free regions. The specific binding of a radioligand equals the distribution volume of total ligand uptake in tissue (V_T) minus the distribution volume of the non-displaceable compartment (V_{ND}). The volume of V_{ND} is defined as free ligand in tissue plus non-specific binding in tissue. Thus, BP_{ND} can also be calculated from volumes of distribution measured with arterial plasma concentrations of the radioligand (Innis et al., 2007) and is defined as the following:

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

The unit of BP is ml/cm^3. To estimate $BP_{ND,}$ a reference tissue is needed. This tissue needs to have a negligible density of the receptors that are being investigated. $D_{2/3}R$ density is typically ranked as follows (from high to low): putamen > caudate > thalamus > amygdala > hippocampus = temporal cortex > parietal cortex = occipital cortex = orbitofrontal cortex (Mukherjee et al., 2002). Typical regions that are used as reference tissue are a white matter (WM) region, the superior longitudinal fasciculus (SLF), or traditionally, the cerebellum, as there are close to no DA receptors to be found in these regions. In this case, we used the SLF as a reference, which has been proven feasible for FAL PET (Ishibashi, Robertson, Mandelkern, Morgan, & London, 2013). BP_{ND} estimations were calculated using the simplified reference tissue model (SRTM) according to (Ishibashi et al., 2013). The SRTM is based on a single-tissue compartment model (Ishibashi et al., 2013). SRTM can be used for FAL BP_{ND}. Time-radio-activity curves (TACs) on the basis of regions of interest (ROIs) were generated using predefined standard ROIs available through the Wake Forest University (WFU) PickAtlas (http://fmri.wfubmc.edu/ software/PickAtlas). The binding potential BP_{ND} was the primary PET outcome parameter.

III.3.4. Measurement and Assessment Protocol

All participants signed a special informed consent form concerning PET/CT and were informed in detail about procedures, radiation exposure and related risks beforehand. Radiation exposure from each of the three low-dose CT scans was < 0.5 milli Sievert (mSv) effective dose (ED). The ED associated with the application of FAL was 4.3 mSv, resulting in an estimated total ED of 5.8 mSv associated with the study.

Subjects were prepared with an i.v. catheter in one arm for the tracer application. 200 megabecquerel (MBq) of FAL ($Mean_{InjD}$ = 196.87; SD_{InjD} ± 9.89) was injected intravenously over 30 seconds. PET data was acquired in three bocks with a break between each of them (see figure 11):

(1) 50 min emission scan, 30 min break;

(2) 60 min scan, 60 min break;

(3) 40 min scan.

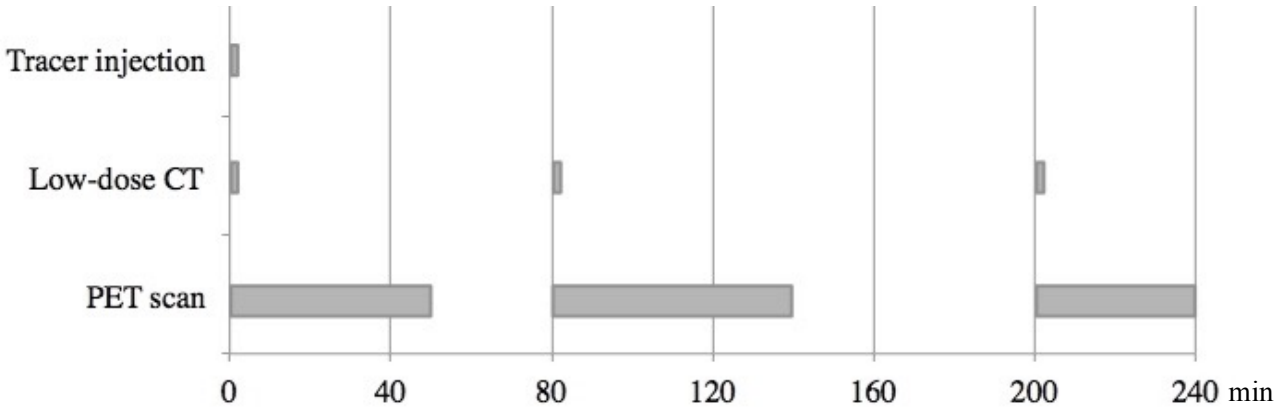

Figure 11. PET/CT Assessment Protocol

A low-dose CT was performed before each block for attenuation correction of the emission data. As participants were allowed to move during breaks, the separate low-dose CT for each block was necessary to avoid spatial mismatch between CT and PET caused by incomplete repositioning. Scanning took place until four hours after injection. Like this, FAL reached the wash-out phase even in high receptor density areas like the striatum.

III.3.5. Processing of PET Data

After the acquisition of PET, the data of the first block was sorted into frames according to the following protocol: 3 x 20 s, 3 x 1 min, 3 x 2 min, 2 x 5 min, 3 x 10 min. PET data of the second and third blocks was sorted into frames of 10 min. The images were reconstructed by the 3D iterative algorithm provided by the system software. Afterwards, the images of the 3 blocks were re-aligned using Statistical Parametric Mapping software package (SPM8, Wellcome Department of Imaging Neuroscience, Institute of Neurology, London; http://www.fil.ion.ucl.ac.uk/spm/). PET data were co-registered to each individual's MRI scan. This method has been previously successfully applied in assessments using PET (Mukherjee et al., 2002) to combine higher-resolution anatomical information with functional information.

The distributions of grey matter (GM), WM and cerebro-spinal fluid (CSF) were calculated for every individual MR brain image and stereotactically normalized into a 3D MNI template space using SPM8. For group comparisons and explorative voxel-wise comparisons, we used SPM with the factor *group* (LR controls, HR controls, detoxified AUD patients). Further, as age showed to have different effects in patients compared to healthy participants (Rominger et al., 2012), we added age as a covariate as regressor of no interest. We used the Rex toolbox to extract all BP_{ND} values (measured by FAL) for several ROIs (see section III.3.5.1) based on our hypotheses. Data were transferred into SPSS to perform further statistical analyses (see section III.4.).

III.3.5.1 Regions of Interest

The extrastriatal ROIs were generated using standard templates from Automated Anatomic Labelling (ALL) (Tzourio-Mazoyera et al., 2002), WFU PickAtlas (http://fmri.wfubmc.edu/-software/PickAtlas). Selection took place according to listed possibly affected regions and Brodmann areas (BA) in Goldstein & Volkow (2011) with the aid of the Online Brain Atlas Reconciliation Tool (http://qnl.bu.edu/obart).

The ROIs for the anterior cingulate cortex (ACC) were subdivided into rostral (rACC / BA 24), dorsal (dACC / BA 32) and subgenual ACC (sgACC / BA 25) in accordance with the suggested functional specialization (Bush, Luu, & Posner, 2000; Lumme, Aalto, Ilonen, Någren, & Hietala, 2007; Ko et al., 2009; Goldstein & Volkow, 2011). ROIs for the vlPFC were generated from corresponding templates from AAL including parts of BA 44 and 45 of the inferior frontal gyrus pars opercularis and triangularis. ROIs for the dlPFC were composed of templates for the superior and middle frontal gyrus including portions of BA 6, 8, 9, 10, 44 and 45. ROIs for the mPFC were generated using the medial superior frontal gyrus template of the AAL atlas, including the GM belonging to the internal surface of the hemisphere anterior and superior to the cingulate gyrus. ROIs for OFC were composed of the orbital parts of the inferior, medial and superior gyrus partly including BA 10, 11 and 47. For an overview of all investigated ROIs and their anatomical localisation see table 6 and figures 12 to 16.

Table 6. Investigated Regions of Interest

Regions of Interest	**Reference**
dlPFC (R/L) vlPFC (R/L) mPFC (R/L) OFC (R/L)	Goldstein & Volkow, 2011; Puig, Rose, Schmidt, & Freund, 2014; Takahashi, 2013; Trantham-Davidson et al., 2014; Vijayraghavan et al., 2007
sgACC (R/L) dACC (R/L) rACC (R/L)	Ko et al., 2009; Lumme et al., 2006; Goldstein & Volkow, 2011

dlPFC = dorsolateral prefrontal cortex; vlPFC = ventrolateral prefrontal cortex; mPFC = medial prefrontal cortex; OFC = orbitofrontal cortex; ACC = anterior cingulate cortex; sgACC = subgenual ACC; dACC = dorsal ACC; rACC = rostral ACC; R = right; L = left

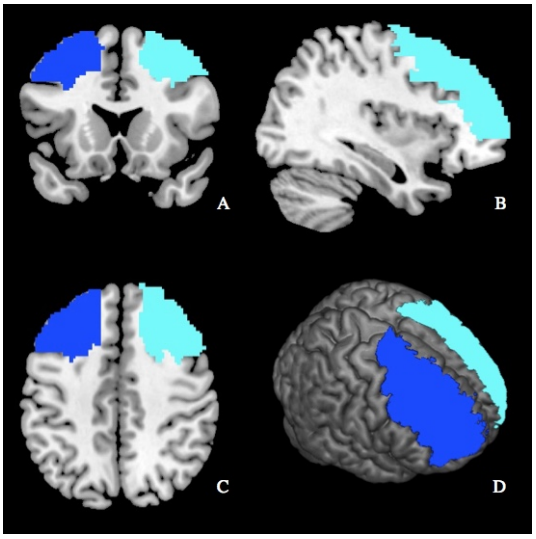

Figure 12. ROI - Dorsolateral Prefrontal Cortex. (A) coronal view (B) sagittal view (C) axial view (D) 3 D view of the region of interest. Cyan = left, blue = right.

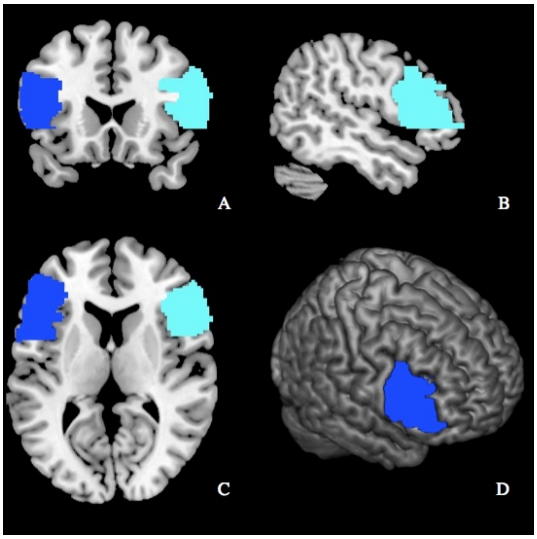

Figure 13. ROI - Ventrolateral Prefrontal Cortex. (A) coronal view (B) sagittal view (C) axial view (D) 3 D view of the region of interest. Cyan = left, blue = right.

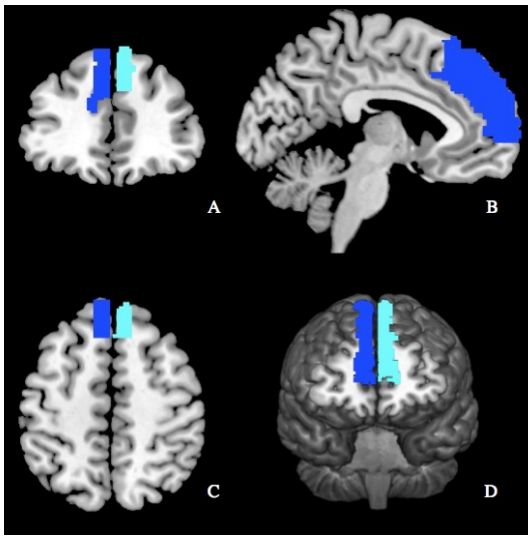

Figure 14. ROI - Medial Prefrontal Cortex. (A) coronal view (B) sagittal view (C) axial view (D) 3 D view of the region of interest. Cyan = left, blue = right.

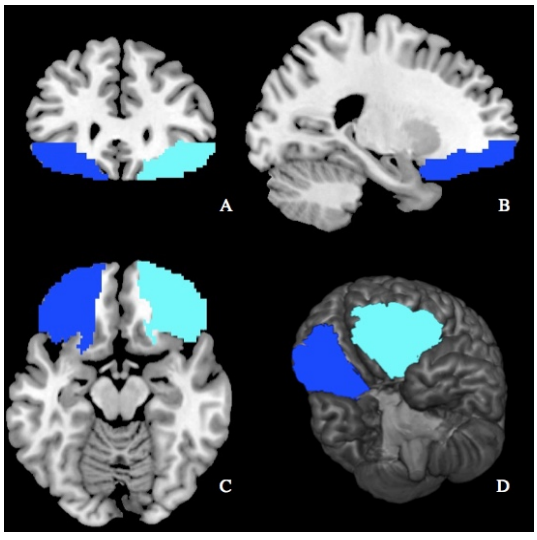

Figure 15. ROI - Orbitofrontal Prefrontal Cortex. (A) coronal view (B) sagittal view (C) axial view (D) 3 D view of the region of interest. Cyan = left, blue = right.

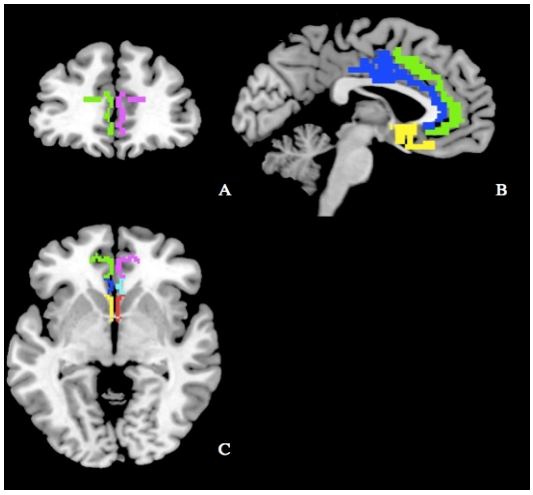

Figure 16. Anterior Cingulate Cortex (A) coronal view (B) sagittal view (C) axial view of the regions of interest. Rostral ACC: blue = right, cyan =left; dorsal ACC: green = right, violet = left; subgenual ACC: yellow = right, red = left.

All images of ROIs were produced using MRIcron (www.mricro.com)

III.4. Statistical Analysis

The statistical analyses were carried out using IBM SPSS Statistics Version 24.0 for Mac OS (IBM Corp., Armonk, NY) along with the description in Fields (2009). In a first step, we tested whether our data was normally distributed on the basis of Kolmogorov-Smirnov (K-S) tests and visually via Q-Q-plot results. We performed normality tests on the complete sample, as we consider AUD development as a continuous process. The K-S tests revealed non-normal distribution for a number of variables. The completion time for TMT-A ($D(57) = 0.13$, $p < .05$), TMT-B ($D(57) = 0.15$, $p < .05$), as well as scores for ADS ($D(51) = 0.14$, $p < .05$) and OCDS ($D(53) = 0.14$, $p < .05$) were significantly non-normal. Furthermore, BP_{ND} in the left vlPFC ($D(58) = 0.13$, $p < .05$) was significantly not normally distributed. As a number of variables were not normally distributed, we decided to use non-parametric tests (a series of Kruskal-Wallis (K-W) tests) as an alternative to the one-way independent ANOVA (univariate analysis of variance). Differences between groups were confirmed by Mann-Whitney tests. Correlation of EF performance parameters, clinical scores and BP_{ND} in different ROIs were tested using Spearman's correlation test. A two-sided p-value < .05 was set as the threshold for significance. Due to the explorative character of the study, we did not correct for multiple comparisons except in post hoc tests (Mann Whitney tests, Bonferroni correction) of significant results. The p-values of statistical tests are thus to be understood as exploratory ones with no confirmatory generalization of the results.

IV. Results

IV.1. Executive Function Test Performance

Hypothesis 1.1 (H1.1): EF performance of HR controls and detoxified AUD patients is impaired when compared to LR control performance.

Hypothesis 1.2 (H1.2): HR subject will lie on an intermediate performance level between LR and detoxified AUD.

When looking into estimated IQ, TMT-A, semantic fluency and digit-symbol-coding performance using Kruskal-Wallis tests, no significant group differences were observed (see table 7). However, maximum digit span backwards remembered by the participants was significantly different between the groups ($H(2) = 7.86$, $p < .05$). Also TMT-B performance showed a trend to differ between groups ($H(2) = 5.26$, $p = .073$).

Mann-Whitney tests were used to follow up these findings (see tables 8 and 9). A Bonferroni correction was applied for this step. As we compare LR with HR, LR with AUD as well as HR and AUD, all effects are reported at a .0167 level of significance ($p < .05/3$). It appeared that LR controls performed less strong on the digit span backwards test than HR controls ($U = 87.50$, $z = -2.75$, $p = .006$) and showed a trend to perform less strong than patients ($U = 122.50$, $z = -1.93$, $p = .054$). LR differed trendwise in terms of performance from AUD patients ($U = 87.50$, $z = -2.75$, $p = .022$); LR showed a decreased performance in TMT-B. Test performance compared between LR and HR in TMT-B and HR and AUD did not reach statistical significance.

Table 7. Comparing Executive Function Test Performance amongst Groups

TEST STATISTICS a,b

	Estimated IQ	TMT-A time	TMT-B time	Semantic Fluency Score	Digit Symbol Coding Score	Digit Span Backwards Score
Test Statistics, *H*(2)	1.514	.394	5.257	2.437	.137	7.858
Asymp. Sig.	.469	.821	.072	.296	.934	.02

a Kruskal Wallis Test
b Grouping Variable: Group

Table 8. Post Hoc Comparison between Low-Risk and High-Risk Controls

TEST STATISTICS a

	TMT-B time	Digit Span Backwards Score
Mann-Whitney U	130.500	87.500
Z	-1.460	-2.753
Asymp. Sig. (2-tailed)	0.144	.006

a Grouping Variable: Group

Table 9. Post Hoc Comparison between Low-Risk Controls and Patients

TEST STATISTICS a

	TMT-B time	Digit Span Backwards Score
Mann-Whitney U	108.500	122.500
Z	-2.291	-1.925
Asymp. Sig. (2-tailed)	.022	.054

a Grouping Variable: Group

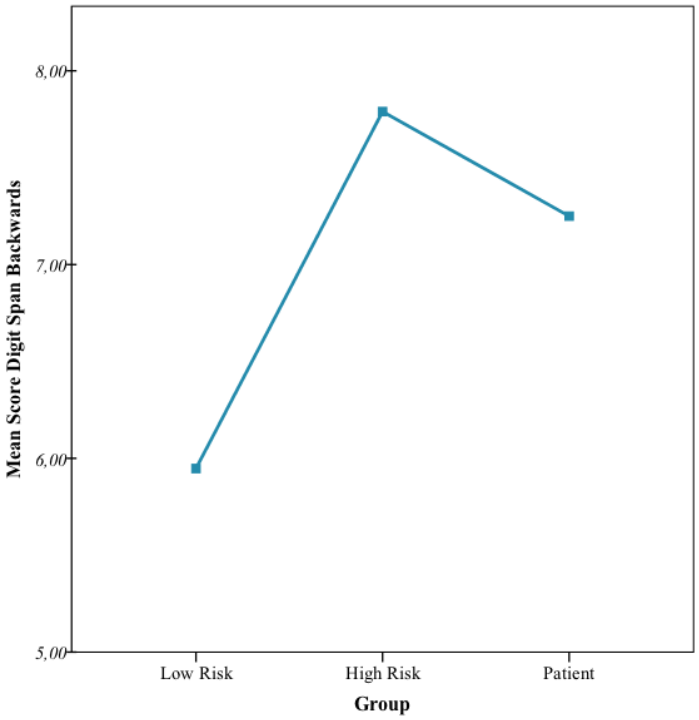

Figure 17. Digit Span Backwards Performance amongst Groups
Significant Difference between Low Risk an High Risk Group ($p < .0167$); Difference between Low-Risk and Patients trending towards significance ($p = .054$)

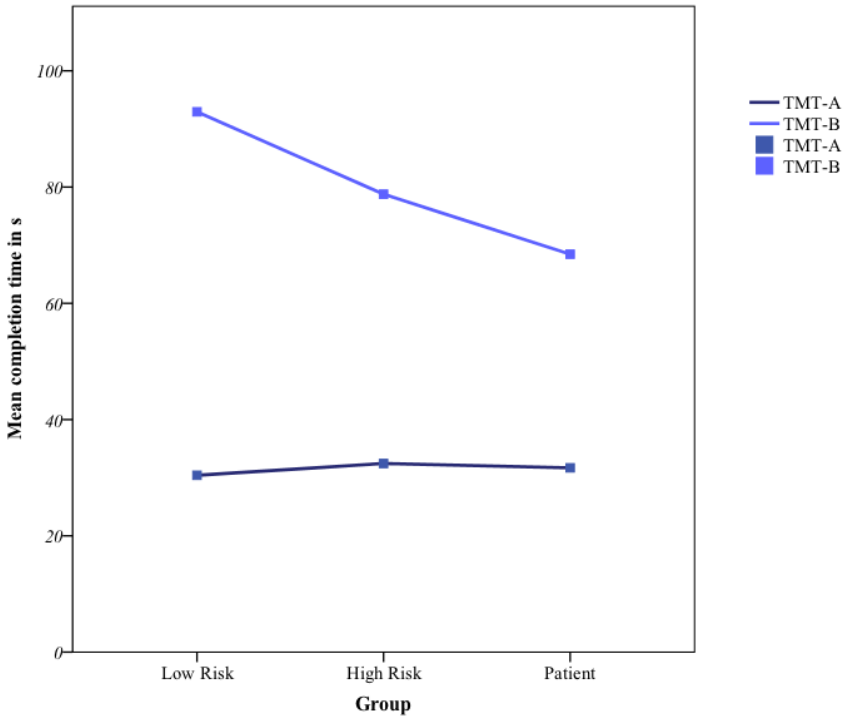

Figure 18. Trail making Test Part A (TMT-A) and B (TMT-B) Performance amongst Groups Differences in TMT-B Performance trending towards significance for Low-Risk versus Patient Group ($p = .022$; significance reported at $p < .0167$)

IV.2. Extrastriatal Dopamine Receptor Availability

Hypothesis 2.1 (H2.1): Extrastriatal $D_{2/3}R$ availability is lower in detoxified AUD than in LR and HR controls.

Hypothesis 2.2 (H2.2): Extrastriatal $D_{2/3}R$ availability measured in HR subject will lie on an intermediate level between LR and detoxified AUD.

As shown in table 10 and figure 19, extrastriatal BP_{ND} was significantly different in the rostral ACC of the right hemisphere ($H(2) = 7.44$, $p < .05$). The analysis also showed marginally significant results for group differences in the left rostral ACC ($H(2) = 5.91$, $p = .05$), the left dlPFC ($H(2) = 5.54$, $p = .06$) and left vlPFC ($H(2) = 5.63$, $p = .06$). Trends were observed on both hemispheres for the dorsal ACC (left: $H(2) = 4.91$, $p = .09$; right: $H(2) = 5.22$, $p = .07$) and for the right dlPFC ($H(2) = 4.60$, $p = .10$). BP_{ND} levels in the other ROIs did not differ significantly between LR, HR controls and AUD. For the post hoc tests, we decided to adapt the p-value to correct for multiple comparisons ($p < .05/3 = p < .0167$). The findings were followed up by Mann-Whitney tests (see table 11, 12, 13) showing significantly higher levels of

extrastriatal BP_{ND} in HR controls compared to AUD patients in the bilateral dorsal ACC (left: U = 103.00, z = -2.44 right: U = 101.00, z = -2.50; both $p < .0167$) and rostral ACC (left: U = 94.00, z = -2.70 right: U = 83.00, z = -3.00; both $p < .0167$) as well as in the left dlPFC (U = 99.00, z = -2.56, $p < .0167$) and left vlPFC (U = 96.00, z = -2.64, $p < .0167$). Differences between extrastriatal BP_{ND} in LR controls compared to AUD and LR to HR controls did not reach statistical significance, but showed trends to lie between the two latter (see figure 19, table 12 and 13).

Table 10. Comparing Groups and Regions of Interest

TEST STATISTICS a,b

	dlPFC_L	dlPFC_R	vlPFC_L	vlPFC_R	mPFC_L	mPFC_R	OFC_L
Test Statistics, *H*(2)	5.541	4.608	5.631	3.740	3.422	2.724	2.711
Asymp. Sig.	.063	.100	.06	.154	.181	.256	.258

	OFC_R	rACC_L	rACC_R	dACC_L	dACC_R	sgACC_L	sgACC_R
Test Statistics, *H*(2)	2.509	5.912	7.435	4.910	5.223	1.534	1.159
Asymp. Sig.	.285	.052	.024	.086	.073	.465	.56

a Kruskal Wallis Test
b Grouping Variable: group

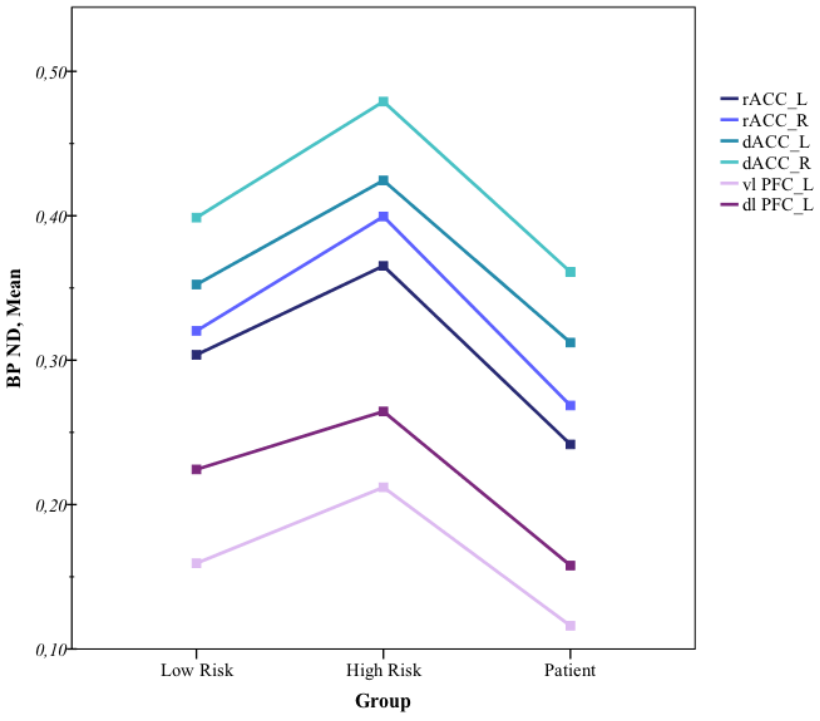

Figure 19. Non-displaceable Binding Potentials (BP_{ND}) of ^{18}F-Fallypride in selected Subregions of the Prefrontal Cortex (PFC) Significant Differences between High Risk and Patient group in bilateral dACC and rACC, left dlPFC, left vlPFC (all reported at $p <$ .0167).

Table 11. Post Hoc Comparison between High-Risk and Patients

TEST STATISTICS a

	dACC_L	dACC_R	rACC_L	rACC_R	dlPFC_L	dlPFC_R	vlPFC_L
Mann-Whitney U	103.000	101.000	94.000	83.000	99.000	110.000	96.000
Z	-2.444	-2.501	-2.697	-3.006	-2.557	-2.248	-2.641
Asymp. Sig. (2-tailed)	.015	.012	.007	.003	.011	.025	.008

a Grouping Variable: group (significance level at $p < .0167$)

Table 12. Post Hoc Comparison between Low-Risk and Patients

TEST STATISTICS a

	dACC_L	dACC_R	r_ACC_L	r_ACC_R	dlPFC_L	dlPFC_R	vlPFC_L
Mann-Whitney U	157.000	162.000	146.000	152.000	151.000	165.000	167.000
Z	-.927	-.787	-1.236	-1.068	-1.096	-.702	-.646
Asymp. Sig. (2-tailed)	.354	.431	.216	.286	.273	.482	.518

a Grouping Variable: group (significance level at p < .0167)

Table 13. Post Hoc Comparison between Low-Risk and High-Risk

TEST STATISTICS a

	dACC_L	dACC_R	r_ACC_L	r_ACC_R	dlPFC_L	dlPFC_R	vlPFC_L
Mann-Whitney U	154.000	146.000	164.000	145.000	154.000	141.000	143.000
Z	-.774	-1.007	-.482	-1.036	-.774	-1.153	-1.095
Asymp. Sig. (2-tailed)	.439	.314	.630	.300	.439	.249	.274

a Grouping Variable: group (significance level at p < .0167)

ACC = Anterior Cingulate Cortex; rACC= rostral ACC; dACC= dorsal ACC; PFC = Prefrontal Cortex; vlPFC = ventrolateral PFC; dlPFC = dorsolateral PFC; mPFC = medial PFC; OFC = Orbitofrontal Cortex; L = left; R = right

IV.3. Correlation of Executive Function Performance and Dopamine Receptor Availability

Hypothesis 3 (H3): Extrastriatal $D_{2/3}R$ availability is positively correlated to EF performance.

Table 14. Correlations between Binding Potential and Test Scores I

CORRELATIONS

		TMT-B time	Digit Span Backwards Score	OCDS thoughts	OCDS ImpulseTo Act	OCDS total Score	ADS Score
rACC left							
	r_s	.289 *	-.018	-.232	-.15	-.212	-.260
	Sig. (2-tailed)	.028	.893	.095	.269	.127	.065
rACC right							
	r_s	.202	.061	-.234	-.173	-.205	-.264
	Sig. (2-tailed)	.128	.651	.091	.202	.141	.061
dACC left							
	r_s	.297 *	.033	-.211	-.158	-.206	-.244
	Sig. (2-tailed)	.024	.809	.130	.245	.138	.085
dACC right							
	r_s	.251	.032	-.190	-.126	-.155	-.212
	Sig. (2-tailed)	.058	.809	.173	.356	.267	.134
dlPFC left							
	r_s	.251	-.022	-.253	-.109	-.188	-.224
	Sig. (2-tailed)	.057	.872	.068	.422	.177	.114
vlPFC left							
	r_s	.176	.021	-.236	-.078	-.161	-.208
	Sig. (2-tailed)	.186	.875	.089	.57	.25	.142
	N	58	58	53	56	53	51

r_s = Spearman's rho * Correlation is significant at the 0.05 level (2-tailed).

ACC = Anterior Cingulate Cortex; rACC= rostral ACC; dACC= dorsal ACC; sgACC = subgenual ACC; PFC = Prefrontal Cortex; vlPFC = ventrolateral PFC; dlPFC = dorsolateral PFC; mPFC = medial PFC; OFC = orbitofrontal Cortex; L = left; R = right; TMT-B = Trail making Test Part B; OCDS = Obsessive Compulsive Drinking Scale; ADS = Alcohol Dependence Scale

Due to the dimensional understanding of AUD, we pooled the groups and performed the analysis on the complete sample. There was a significant positive relationship between the BP_{ND} in the left rACC and TMT-B completion time, r_s = .29, p (two-tailed) < .05. The correlation between left dACC and TMT-B completion time also reached significance, r_s = .30, p (two-tailed) < .05 (see figure 20 and table 14). BP_{ND} is thus negatively correlated with EF performance in our observations.

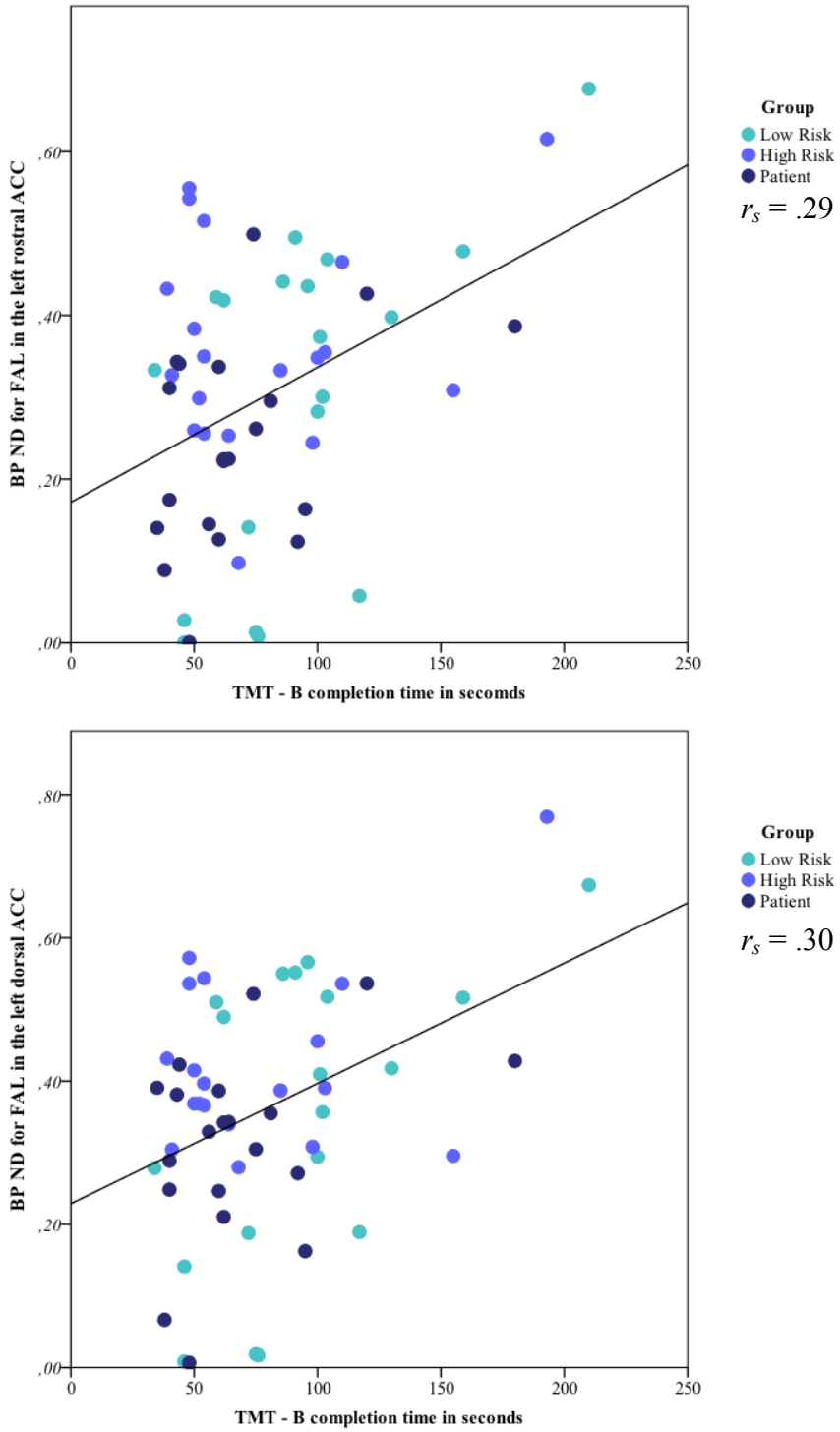

Figure 20. Scatterplots: Correlation of non-displaceable Binding Potential (BP_{ND}) for ^{18}F-Fallypride (FAL) in the left Anterior Cingulate Cortex (ACC) and Trail-Making-Test (TMT-B) completion time. r_s = Spearman's rho

Table 15. Correlations between Binding Potential and Test Scores II

CORRELATIONS

		TMT-B time	Digit Span Backwards Score	OCDS thoughts	OCDS ImpulseTo Act	OCDS total Score	ADS Score
rACC left							
	r_s	.289 *	-.018	-.232 *	-.15	-.212	-.260 *
	Sig. (1-tailed)	.014	.447	.047	.135	.064	.033
rACC right							
	r_s	.202	.061	-.234 *	-.173	-.205	-.264 *
	Sig. (1-tailed)	.064	.325	.046	.101	.071	.030
dACC left							
	r_s	.297 *	.033	-.211	-.158	-.206	-.244 *
	Sig. (1-tailed)	.012	.404	.065	.123	.069	.043
dACC right							
	r_s	.251 *	.032	-.190	-.126	-.155	-.212
	Sig. (1-tailed)	.029	.404	.086	.178	.134	.067
dlPFC left							
	r_s	.251 *	-.022	-.253 *	-.109	-.188	-.224
	Sig. (1-tailed)	.029	.436	.034	.211	.089	.057
vlPFC left							
	r_s	.176	.021	-.236 *	-.078	-.161	-.208
	Sig. (1-tailed)	.093	.437	.044	.285	.125	.071
	N	58	58	53	56	53	51

r_s = Spearman's rho * Correlation is significant at the 0.05 level (1-tailed).

ACC = Anterior Cingulate Cortex; rACC= rostral ACC; dACC= dorsal ACC; sgACC = subgenual ACC; PFC = Prefrontal Cortex; vlPFC = ventrolateral PFC; dlPFC = dorsolateral PFC; mPFC = medial PFC; OFC = orbitofrontal Cortex; L = left; R = right; TMT-B = Trail making Test Part B; OCDS = Obsessive Compulsive Drinking Scale; ADS = Alcohol Dependence Scale

When performing one tailed-tests, we observed additional significant results for the left dlPFC (r_s = .25) and right dACC (r_s = .25), (all p (one-tailed) < .05), see table 15 and figure 21. No correlation between $D_{2/3}$ receptor binding and EF performance was observed in other cortical regions that are thought to be important for executive functioning.

Looking into clinical questionnaires, OCDS scores on the obsessive thoughts scale were significantly negatively correlated with BP_{ND} in the left rACC (r_s = -.23), right rACC (r_s = -.23), left dlPFC (r_s = -.25) and left vlPFC (r_s = -.24) (all p (one-tailed) < .05). Scores obtained in the ADS at baseline showed a significant negative relationship with BP_{ND} in the left rACC (r_s = -.26), right rACC (r_s = -.26) as well as the left dACC (r_s = -.24) (all p (one-tailed) < .05).

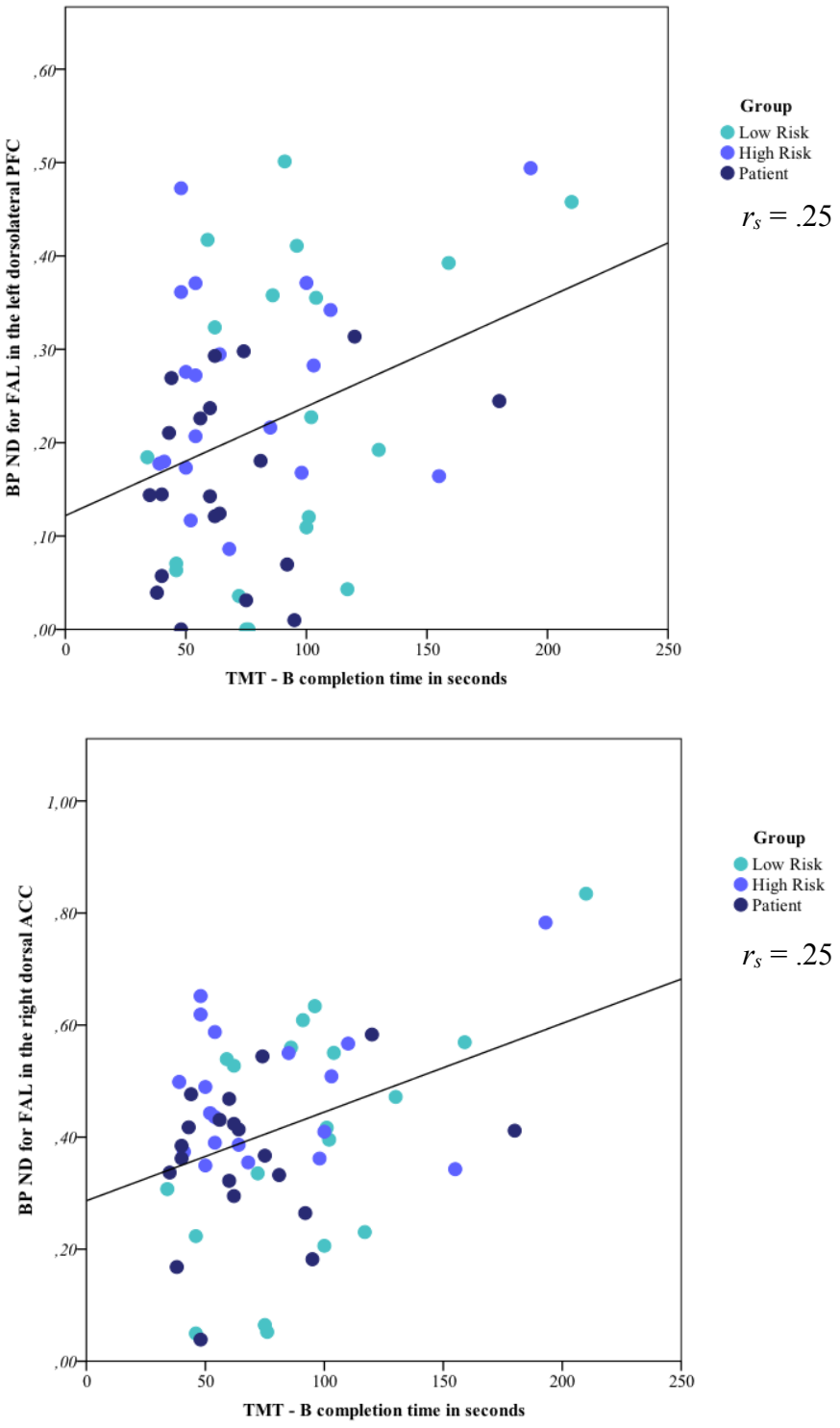

Figure 21. Scatterplots: Correlation of non-displaceable Binding Potential (BP_{ND}) for ^{18}F-Fallypride (FAL) and Trail-Making-Test (TMT–B) completion time ACC = Anterior Cingulate Cortex; PFC = Prefrontal Cortex; r_s = Spearman's rho

For visual purposes, we also show the whole brain activation pattern amongst all participants measured with FAL PET with its effects of interest (EOI) in figure 22 below (threshold $p < .01$).

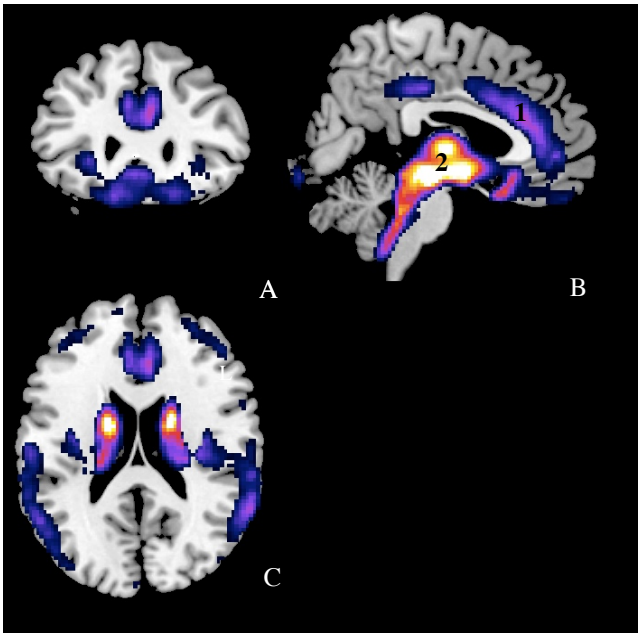

Figure 22. Fallypride PET map - Whole Brain Activation throughout all Groups measured with ^{18}F-Fallypride PET. (A) coronal view (B) sagittal view (C) axial view; (1) Activation in the Anterior Cingulate Cortex; (2) striatal and mid-brain activation. Results are thresholded with $p < .01$.

V. Discussion

V.1. Summary

The present study investigated the relationship of extrastriatal $D_{2/3}R$ availability and EF performance in alcoholic patients and control groups. Of special interest was the focus on the dimensional approach of AUD for which we included an HR control group of subjects with problematic alcohol consuming patterns as defined above (chapter II.1.1.2.). The inclusion of regularly consuming participants adds another level to the investigation, as this has not been a main focus in alcohol research so far. EF performance amongst the groups differed in two tasks, TMT-B and digit-span backwards, with the latter showing a significantly better performance of AUD compared to LR. Surprisingly, TMT-B performance showed a trend to be less strong amongst controls when compared to HR and AUD. Neuroimaging investigations showed a difference in extrastriatal $D_{2/3}R$ availability in several ROIs (bilateral dACC, bilateral rACC, left dlPFC and left vlPFC). The main difference was observed between HR and AUD, with patients showing significantly less BP_{ND} than HR in a number of these areas (bilateral rACC, bilateral dACC, left dlPFC and left vlPFC). Extrastriatal $D_{2/3}R$ availability in the left rACC, bilateral dACC and left dlPFC was positively correlated with TMT-B completion time and thus negatively correlated with EF performance measured by this test. Clinical scores such as OCDS obsessive thoughts and ADS showed a negative correlation with extrastriatal $D_{2/3}R$ availability.

V.2. Executive Function Test Performance

In contrast to our hypotheses (*H1.1* and *H1.2*) and to previous reports (Goldstein & Volkow, 2011; Houston et al., 2014; Ratti et al., 2002; Zinn et al., 2004) AUD patients and HR controls performed better on EF tests than LR controls in the present study. This is a new aspect and leading us to reject *H1.1* and *H1.2* for this sample. To some extent, these observations may be related to the following possible explanations.

First, when investigating EF and stating impaired EF, findings could always be due to other influencing factors such as stress, lack of sleep, loneliness, lack of physical exercise or motivation, etc. All of these factors can influence the examined person's ability to display the EF of which he/she is actually capable (Diamond, 2013). Observed impairments and lesions may

thus decrease and increase throughout consumption, withdrawal, lasting sobriety and relapse in the sense of dynamic lesions. Another challenge in the assessment of EF is the high sensitivity to any kind of disruption and the relatively low specificity of the tests. Second, EF is not a strict construct or trait and can be influenced and improved by training. Hence, subjects who have performed similar or even the same tests previously in their lives have an advantage compared to those who have never performed any of these tasks. In our case, it is possible that especially participants from the AUD group have already taken similar tests. They have spent time in clinics and therapeutic settings, where the evaluation of abilities with the help of standardized tests as those used in the present study are frequently administered. Third, it is possible that due to the complex study design with its demanding assessment sessions, we provoked a selection bias. Possibly only highly functional AUD patients with rather intact EF patterns were able to complete all of the assessments and were thus included in the final sample. Patients with impaired EF might have not been able to manage planning ahead and committing to multiple appointments over a number of days leading to exclusion from the final sample. Fourth, impairment of EF is not referable solely to alcohol consumption – their development can be disrupted in adolescence or be influenced by other factors such as age, gender, IQ and education years or other diseases and disorders (for an overview of differential diagnoses of impaired EF, see Rabinovici et al. (2015)). The influence of these factors was limited by defining explicit exclusion and inclusion criteria, aiming for the least possible differences when comparing groups in core descriptive factors (see table 5, chapter III.1.3.). However, it is possible that other factors than alcohol consumption presented by our participants explain the differences observed in EF performance. Last, on a suggestive level of interpretation, it is also probable that the weaker performance of LR controls may be due to a lack of motivation to show their intact functionality. Healthy controls might not show as much interest in the study content and findings, as they are not as personally involved as affected individuals (namely the AUD group). This could have a non-negligible impact on each participant's motivation and the individual test performance which is highly linked to motivation. AUD patients who have gone through detoxification in specialised wards and who are aware of their disorder, are possibly eager to find out more about AUD and might even see this as an opportunity to prove their functionality (despite their diagnosis) in this kind of setting.

This study underlines the diversity of EF performance amongst participants and shows that high functionality amongst AUD patients is not excluded. In general, it might be possible that patients profit from detoxification treatments in terms of an amelioration of EF performance, which, however, we cannot verify with this study. If one interprets these results on a very

speculative level, it might be conceivable that our sample of AUD consists of individuals that possibly had a higher IQ before suffering from an AUD and lost some of these cognitive capacities due to chronic alcohol consumption. Possibly they have residual EF capacities at their disposal that were neither affected by the consumption nor reflected in the IQ and that allow them to do better on the EF tests than the control groups.

However, the conclusion of our results according to Bates (2000) suggests that this particular patient sample has a higher probability to successfully complete treatment, to stay abstinent for a longer time period and to have better social and professional outcomes. This could be assumed for our sample, as a number of the participants were already measured quite late after their last drink ($Mean_{daystoPET}$ = 36.4; $SD_{daystoPET}$ = 20; min = 9, max = 96 days), indicating a certain level of resilience and commitment, as they were able to remain reliably abstinent for the assessments and testing sessions. For a verification of this hypothesis, an analysis of our follow-up data might give more insight.

V.3. Extrastriatal Dopamine Receptor Availability

The effects of alcohol consumption on extrastriatal DA transmission have not been in the focus of AUD research until recently. This is also reflected by the limited number of references when searched for on PubMed (e.g. 12 results for studies with humans when searching for *((extrastriatal) AND dopamine) AND alcohol* in a search performed on November, 6th, 2017). This study is adding a rather large body of work, as FAL PET was performed on a relatively large sample (N = 58) to evaluate influences of alcohol on extrastriatal DA.

An especially interesting finding of the present study is the pronounced difference in extrastriatal $D_{2/3}R$ availability between HR and AUD participants despite the absence of significant differences when comparing LR to AUD or to HR. This is partly in line with previous research that failed to find differences in baseline receptor availability between healthy controls and abstinent patients (Narendran et al., 2014). As proposed in *H2.1*, we observed lower extrastriatal $D_{2/3}R$ availability in AUD compared to a control group. This possibly reflects a reactive down-regulation of DA receptors due to high dopaminergic stimulation during long lasting and excessive consumption patterns. Multiple studies were able to show similar effects mainly in striatal regions in recently abstinent alcoholics (Heinz et al., 2004; Thiruchselvam, Malik, & Foll, 2017; Volkow et al., 2002). Yet, in this study this observation was made when comparing AUD with HR controls and not with LR controls.

As we suggested in *H2.2*, beginning neuroadaptations in terms of a loss in DA receptor availability during chronic alcohol abuse (represented by our HR group) was not confirmed by our findings and led to the rejection of the hypothesis. Our results rather indicate the opposite: we saw an elevated level of free receptors in extrastriatal regions in participants with problematic alcohol intake when comparing with AUD and LR. A possible explanation for this condition may be linked to a crucial and fundamental difference between HR participants and the other groups: HR controls were maintaining an active drinking status in the days and weeks previous to our examinations, whereas AUD were obliged to be abstinent and LR did not drink alcohol or just very small amounts. As mentioned above, the results showed an additional trend towards higher levels of free DA receptors in HR than in healthy controls. This relationship was, however, not statistically significant and the mechanisms that lead to these observations are not easily determined.

Nevertheless, there have been studies that observed similar results for chronic alcohol abuse. Leggio et al. (2014) showed the crucial role of D_3R in mice for the reinforcing mechanisms of alcohol intake. These mechanisms are probably pronounced stronger in HR controls than in LR subjects and account at least partly for their alcohol related behaviour. Furthermore, recent findings seem to suggest upregulation of D_3R in extrastriatal areas (e.g. hypothalamus, substantia nigra, ventral pallidum) across different SUDs (Thiruchselvam et al., 2017) which would be consistent with our findings in HR subjects. This would, however, not be in line with our findings in AUD. Thus, our investigations leave the following question unanswered: at what point does the decrease in receptor availability occur or start if we consider AUD as a dimensional construct?

From another point of view, one could suggest that higher levels of extrastriatal $D_{2/3}R$ availability might be a preliminary trait and that high levels could be a protective factor to prevent HR controls from actually developing a manifest AUD condition. It might be one of the compensational mechanisms that HR have to prevent their risky consumption pattern from further development towards AUD. The protective characteristic of higher $D_{2/3}R$ availability has been proposed amongst others by Volkow et al. (2006) for non-affected members of alcoholic families. On the contrary, LR controls showed levels of $D_{2/3}R$ availability to be between those of HR and AUD which does not support this theory (it is worth noting that the differences between HR and LR and AUD and LR were not statistically significant). Hence, other protective mechanisms and compensations such as genetic, epigenetic and social and behavioural factors are very likely to play a role in individual resilience towards AUD (see chapter II.1.3. on

aetiology and risk factors). Higher levels of $D_{2/3}R$ availability amongst HR also implicate the same questions as the more frequently discussed and observed lower levels of $D_{2/3}R$ availability in AUD: is this state a pre-existing or an acquired condition? Currently, we cannot fully answer this question.

Focussing on the affected regions in the brain, our study revealed changes in important extrastriatal regions: the ACC, the dlPFC and vlPFC. Alterations in these regions seem coherent when looking into AUD research. Further, many of the deficits in behaviour and learning associated with addictions can be connected to these regions.

The ACC has traditionally been seen as part of the limbic system of the human brain and it receives projections of the mesocorticolimbic pathway. It is thought to be highly involved in higher cognitive and emotional processing (Bush et al., 2000). Distinct roles are associated with the ACC, such as the involvement in maintaining divided attention, the development of novel responses, and the adaption of on-going behaviour through conflict monitoring and evaluation of errors (Carter & van Veen, 2007; MacDonald, Cohen, Stenger, & Carter, 2000). The latter seems to be engaged by negative feedback or conflicting responses. The conflict-control-loop theory (Carter & van Veen, 2007) promotes ACC activation through errors and negative feedback, causing dlPFC activation and reinforced attention, influencing the behavioural outcomes and actions. This emphasises the connectivity of ACC and dlPFC. Furthermore, the distinction of the ACC into a cognitive part (the dACC) and an emotional / affective ventral part (the rACC) has been widely accepted (Bush et al., 2000). Various functions have been ascribed to the subdivisions: the dACC seems to be involved, amongst others, in the modulation of attention and EF by sensory and response selection, error detection, motivation and working memory. Along with its functions, the ACC's high interconnectivity with the lateral PFC, the parietal and motor and supplementary motor cortex should be pointed out (Bush et al., 2000). The emotional / affective subdivision shows strong connections to other parts of the brain such as the OFC, insular cortex, NAC, amygdala and hippocampus. This part is believed to be involved in the assessment of salience of emotional and motivational information as well as in emotion regulation (Bush et al., 2000). As described by Goldstein & Volkow (2011), the dorsal PFC (including dACC and dlPFC) is thought to be predominantly involved in top-down control and the vlPFC in automatic response tendencies (for example, drug-related attention bias) and impulsivity. An altered DA modulation in these regions due to the consumption of addictive drugs (such as alcohol) seems plausible.

It is thus not surprising to find alterations between HR and AUD present in both the rostral and the dorsal parts of the bilateral ACC as well as in the left dlPFC and vlPFC.

Finally, also technical factors and uncertainties need to be included in this discussion. As FAL binds D_2R and D_3R, it is difficult to distinguish between these two receptor subtypes and state if an increased/decreased BP_{ND} is due to increased/decreased D_2R or D_3R availability or both. A number of studies have tried to map receptor distributions, to determine and distinguish the role of D_2R and D_3R. Results showed rather low concentrations of D_3R in the cortical areas (Hall et al., 1996). Furthermore, it is not entirely clear if an increase in BP_{ND} reflects a higher absolute number of DA receptors or rather relatively more available/free receptors due to lower DA concentrations. Or inversely, if lower levels of BP_{ND} reflect a lower total number of DA receptors or solely less free receptors due to higher DA concentrations (Hirth et al., 2017). Moreover, modulation of transmission at receptor level such as phosphorylation of the receptor itself or its associated G-proteins, altered pathways or internalisation of the receptor are possible but cannot be distinguished in the analysis. Altogether, BP_{ND} reflects the individual relationship between free DA receptors and DA concentrations relative to a reference region with a negligible concentration of DA receptors. This needs to be considered when interpreting results. To clarify the relation between DA concentrations and free receptor concentrations, the observation of tracer displacement, alterations in BP_{ND} during task performance (e.g. response inhibition tasks as shown in Albrecht, Kareken, Christian, Dzemidzic, & Yoder, 2014) or direct measures of DA release (e.g. with a combination of different measures as indicated in figure 4) might be helpful for future investigations.

Possible explanations for the lack of observed differences between LR controls and the two other groups may thus be also linked to methodological and technical procedures (see limitations in section V.5.). Furthermore, it is conceivable that our a priori chosen and generated ROIs were not the right ones to detect the suspected alterations. However, on the basis of previous research we carefully chose and defined ROIs that are thought to be affected in alcohol consumption as well as in EF disruption.

Still, it can be said that alcohol consumption has an impact on extrastriatal $D_{2/3}R$ availability and influences DA transmission, especially in subregions of the ACC, dlPFC and vlPFC.

V.4. Correlation of Executive Function Performance and Dopamine Receptor Availability

It has been widely reported that DA has an important role on the modulation of PFC activities (Robbins & Arnsten, 2009) to which EF are usually attributed. Our results suggest that certain aspects of EF such as working memory, set shifting and mental flexibility, indexed by the TMT-B, may be related to extrastriatal $D_{2/3}R$ availability in individuals who abuse alcohol. Yet, EF performance was negatively correlated with receptor availability. These data were contrary to our hypothesis (*H3*) and led to its rejection. However, other authors have also made similar observations. In a recent study Vyas et al. (2017) described an association of better EF performance (WCST) with low extrastriatal BP_{ND} in schizophrenic patients which correspond to our investigation into AUD. The authors, however, showed that in healthy volunteers EF performance was positively correlated with BP_{ND} (thus with higher DA receptor availability and supposedly less DA function). They suggest that this relationship might represent an adaptation of disturbed DA circuitry connections. A similar interpretation may be possible for AUD in our study. Another study by Lumme et al. (2007) showed a positive correlation between the BP of [^{11}C]-FLB 457 in the right ACC and non-perseverative errors in the WCST. These data are in line with our findings that indicate a positive correlation of FAL BP_{ND} in the left ACC, right dACC and left dlPFC with TMT-B completion time throughout all groups. Nevertheless, Lumme et al. investigated 32 healthy, non-smoking controls with no history of psychiatric disorders.

As illustrated by our observations and other studies, lower extrastriatal $D_{2/3}R$ availability could predict better performance in EF tasks. The exact role of $D_{2/3}R$ availability in EF is, however, not clearly delimited as animal studies showed no relevant impairment of cognitive functions during a D_2R bloc situation (Floresco et al., 2006; Granon et al., 2000).

Taken together, the complex and apparently heterogeneous involvement of DA in the modulation of PFC functioning and extrastriatal regions in AUD is very likely but not finally clarified.

It is worth noting that a positive correlation of BP_{ND} and EF performance has also been previously described for striatal regions (Ballard et al., 2015; Chen et al., 2005; Christopher et al., 2014). An investigation of this association would have exceeded the scope of this work but would present an interesting focus for future investigations, as the named studies did not investigate AUD patients. Negative correlations of extrastriatal $D_{2/3}R$ availability with OCDS thoughts and ADS scores are in line with observations made in nicotine-dependent subjects (Fehr et al., 2008), especially in regard to the ACC.

V.5. Limitations of this Work

Before giving perspectives on the basis of this work, a few limitations to this study have to be discussed. First, when investigating EF, we are confronted with an overall, general challenge. The high variability of EF constructs and of operational design of EF tests, make it hard to compare and interpret findings from different studies (Day et al., 2015). Second, the great variance concerning the moment of PET assessment (between 9 and 96 days after drinking the last alcoholic beverage) in AUD might have an influence on receptor availability: it is thought that availability normalises with long-term abstinence (Rominger et al., 2012). Long time spans between first assessments and PET were inevitable to a certain extent as problems with tracer synthesis and limited time slots to use the PET scan were not predictable. Hence, it would have been of interest to analyse the time of abstinence (between last drink and PET measurement) in controls as well as in AUD to increase the comparability and interpretability of the findings. The inclusion of the duration of abstinence as a covariate seems inevitable for future studies, especially if we assume that AUD patients can recover from hypodopaminergic states. Still, this great range was partly due to one outlier with 96 days and three more with more than 50 days between the cognitive assessment and PET. Moreover, other studies did present a similar range of days between their first day of abstinence and the PET assessment (Heinz et al., 2009). Third, observed relationships between neuroimaging results and test performance are based on correlations that exclude conclusions concerning their causality. Moreover, our conclusions are limited, as the correlation coefficient r_s (Spearman's rho) was rather small (from .289 to .297 for two-tailed tests and between .251 and .297 for one-tailed tests) within our significant results. Therefore, a cautious interpretation of the results is inevitable. Fourth, the present group of AUD patients seems to represent a rather high-functioning group in terms of EF. Due to the rather complex design of this study, the selection of probably highly functional AUD was inevitable in some ways. However, lower functioning AUD patients would be predicted to show stronger differences in EF-associated measures. Fifth, another limitation is the separation of neuropsychological assessment and the evaluation of BP_{ND} in the scanning session. Accordingly, correlations between $D_{2/3}R$ availability and EF performance can only reflect trait features. In addition, the spatial resolution of the detectors and the kinetic energy of the e^+ limit the total resolution of PET to 1-2 mm in humans (Mikla & Mikla, 2014). This has and influence on registered emission and especially on the allocation of detected emission to a specific site in the human brain. Especially in smaller ROIs, like the subdivisions of the ACC, this mechanism could have an impact on results. Nonetheless, BP_{ND} values determined with WM as the reference

region (the SLF in our study) are normally higher than BP_{ND} values determined with the cerebellum as a reference (Ishibashi et al., 2013). Also, there is a controversy that FAL might not be the optimal substance to investigate cortical regions, as high variability in BP_{ND} and low baseline values are often observed (Slifstein et al., 2010). For our investigation the use of FAL was highly suitable and justified, as we were interested in investigating striatal (Spitta, 2018) and extrastriatal $D_{2/3}R$ availability.

And lastly, the inclusion of a number of women in each group (LR = 3, HR = 2, AUD = 3) can be criticized. We did not differentiate between sexes when evaluating drinking habits and assigning participants to HR and LR groups. The percentage of women in each group is, however, relatively low and not significantly different between the groups. This is why we decided to apply the same criteria to men and women in the context of this study. The inclusion of sex differences, nicotine dependence, other individual characteristics and pre-morbid vulnerabilities (e.g. genetic predispositions) might be crucial for interpreting the observed results more accurately.

V.6. Conclusion and Perspectives

AUD is a complex, relapsing disorder that is often associated with deficits in higher cognitive control mechanisms and alterations of the dopaminergic neurotransmitter system. This study is adding more evidence to a growing body of work with very heterogeneous results concerning dopaminergic responses to chronic alcohol consumption and its impact on EF performance. The findings may contribute to a deeper understanding of the multiple pathomechanisms involved in AUD. The differences observed in $D_{2/3}R$ availability between the groups show that besides the striatal differences which are more often investigated, further alcohol consumption-induced changes in frontal areas are likely. Such changes might be of great interest for the general understanding of AUD, other addictions and related diseases, but also for the development of new treatments and interventions. However, clearly more research is needed, also in terms of prediction of the course of the disease, as a majority of patients (up to 80%) with AUD suffer a relapse.

For future research, it might be interesting to investigate in the direction of, and with respect to, some of the following aspects. It would be of great interest to use standardized ROIs for investigating extrastriatal regions. Up to now, this is possible to a limited extend as the ALL

and WFU PickAtlas provide a number of ROIs, but there is no official consensus on regions such as the so called *dlPFC* or the *vlPFC*. A standardized nomenclature of ROIs across studies would facilitate the comparability of different studies and would make it easier to reproduce, verify and interpret findings. As already stated by other authors, especially longitudinal and interventional studies are needed to clarify if the differences in the dopaminergic system observed in SUD either precede or result from chronic use and abuse and where alterations principally take place (DA release vs. receptor concentrations). Furthermore, especially the relationship between prefrontal glutamate and extrastriatal $D_{2/3}R$ availability could present a future focus as the DA system is believed to be potentially regulated by glutamatergic transmission (Gleich et al., 2015). It would also be of great interest to investigate striatal alterations in $D_{2/3}R$ availability throughout the groups, that we observed in our sample (Spitta, 2018) in combination with EF performance. Finally, the future perspectives of EF as a target of therapy in the treatment of AUD need to be investigated and evaluated. Presumably, DA modulation on extrastriatal regions such as the PFC could have an impact on the success of treatment outcomes and relapse.

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VII. Appendix

VII.1. Affidavit

„Ich, Kristin Zacharias, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: *„Extrastriatal $D_{2/3}$ Receptor Availability and Executive Functioning in Alcohol Use Disorder"* 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 29.09.2019

Unterschrift

VII.2. Curriculum Vitae

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

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