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Extrastriatal Dopamine D2/3 Receptor Availability in Alcohol Use Disorder and Individuals at High Risk

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Keywords

Dopamine D2/3 receptor · Extrastriatal · D2/3 receptor availability · Alcohol use disorder · High risk · Positron emission tomography

Abstract

Introduction: Reduced striatal dopamine D2/3 receptor availability in alcohol use disorder (AUD) has been demonstrated in recent clinical studies and meta-analyses. However, only a limited number of studies investigated extrastriatal D2/3 availability in AUD or in at-risk populations. In line with a dimensional understanding of addiction, extrastriatal dopaminergic neuroadaptations have been suggested to be relevant from a pathobiological perspective. **Methods:** We investigated D2/3 receptor availability via ¹⁸F-fallypride positron emission tomography applying a region of interest (ROI) approach. We selected ROIs for the prefrontal cortex (PFC) and the anterior cingulate cortex (ACC). Our sample included 19 healthy controls (low risk [LR]), 19 individuals at high risk (HR) to develop addiction, and 20 recently detoxified AUD patients. **Results:** We found significantly higher D2/3 receptor availability of HR compared to AUD in the left and right rostral ACC (rACC), as well as in the left ventrolateral PFC (vlPFC). We did not ob-

serve a significant difference between AUD and LR. After corrections for multiple comparisons none of the ROIs reached significance throughout the group comparison. The D2/3 receptor availability in the left rACC was inversely correlated with symptom severity assessed with the Alcohol Dependence Scale. **Discussion:** To our knowledge, the present work is the first study investigating extrastriatal D2/3 receptor availabilities in individuals at HR and patients with AUD. The observation that D2/3 receptor availabilities are highest in HR might suggest that their pathobiology differs from subjects with AUD. Future studies are necessary to clarify the individual course of this biomarker over different disease stages and its possible role as a risk or protective factor.

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Introduction

Behavioral as well as neurobiological categorical approaches toward addiction are questioned lately on behalf of a more continuous, dimensional understanding of substance use disorders as represented in the 5th edition of the *Diagnostic and statistical manual of mental disorders (DSM-5)* [1].

This dimensional model of disease is supported by several neurobiological studies suggesting continuous neuroadaptational changes to be associated with the development and maintenance of substance use disorder. More precisely, Koob and Volkow [2] characterize time dependent changes during the progression of addictive disorders, first in the mesolimbic dopaminergic system, subsequently in the nucleus accumbens and the dorsal striatum. Eventually, increasing substance abuse may hereafter cause changes in the prefrontal system and the extended amygdala [2].

Addiction-related changes in the mesolimbic dopamine (DA) system may reflect acute DA release in the nucleus accumbens after, for example, alcohol consumption, leading to reward and consequently reinforcement of addiction-related behaviors [3, 4]. In chronic alcohol abuse, reductions in striatal dopamine D2/3 receptor availability have been shown in several recent positron emission tomography (PET) studies and 2 recent meta-analyses [5, 6]. Specifically, reductions were observed in the nucleus accumbens, the putamen and the caudate nucleus of alcohol use disorder (AUD) compared to healthy controls (HCs) and have been associated with several alcohol-related behaviors such as craving symptoms and increased relapse probabilities in AUD [7–10]. Along with a dimensional approach, our research group has investigated reduced striatal dopamine D2/3 receptor availabilities of AUD patients compared with HCs and observed an intermediate receptor availability of individuals at high risk (HR) [7].

Along with the abovementioned continuous neuroadaptational changes during the development of addiction these striatal impairments which may be related to an impaired reward system, ought to be followed by changes in the prefrontal system, reflecting cognitive and impulse control disruptions on a behavioral level. Dysfunctions of the prefrontal cortex (PFC) and the anterior cingulate cortex (ACC) in addiction are well-documented in several fMRI studies and are associated with increased negative clinical outcomes such as higher relapse probabilities [11–13]. More precisely, the ventrolateral PFC (vlPFC) and lateral orbitofrontal cortex seem to be both involved in automatized behaviors and increased impulsiveness [12]. Moreover, functional connectivity as well as structural morphology of the ACC seem to both have an independent impact on alcohol-related behaviors and may be a potential marker of later relapse in AUD or may predict later alcohol-related problems in adolescents [11, 13].

Prefrontal synaptic plasticity is involved in the pathophysiology of addiction but its implications for the pre-

frontal dopaminergic system have only been examined in preclinical studies up to now [14]. In a recent preclinical study in mice, impairments of the dopaminergic homeostasis in the PFC have been related to cognitive deficits [15]. Another study found an association of prefrontal dopaminergic dysregulation and increased neuroplasticity in cocaine consuming rhesus monkeys [16].

There are only a limited number of studies on dopaminergic transmission and dopamine D2/3 receptor availability in prefrontal or other extrastriatal brain regions in AUD. Furthermore, these studies showed inconsistent results, with some results indicating increased dopaminergic transmission in AUD and some results indicating reduced dopaminergic transmission, although differences in study design and stimuli used makes direct comparison between studies difficult. For example, reduced prefrontal dopamine neurotransmission has been observed in AUD subjects compared to HC after an acute amphetamine challenge [17]. In contrast, both lowered and increased dopamine D2/3 receptor availabilities were observed in extrastriatal brain areas of AUD compared to HC subjects [18, 19]. Research on risk traits leading to alcohol-related behaviors has revealed higher D2/3 receptor availabilities in the PFC to be associated with more pronounced amygdala responses to unpleasant stimuli [20]. Further, Jaworska et al. [21] observed altered D2/3 availabilities in prefrontal, limbic, and paralimbic brain areas of adolescents with high externalizing traits compared to those with low externalizing traits revealing a potential predisposing vulnerability for future development of AUD.

These heterogenous results reflect the complexity of prefrontal disruptions in AUD as well as individuals at HR. Further, it raises the question whether hypo- or hyperdopaminergic states may be associated with alcohol-related behavior as both states have been presumed to increase the probability of relapse in AUD [22]. Along with a dimensional understanding of the development and maintenance of AUD, it seems necessary to investigate dopaminergic neurotransmission in individuals at HR for a better understanding of the pathophysiological steps and potential risk factors leading to AUD.

The aim of the present study was to investigate dopamine D2/3 availability via ^{18}F -fallypride PET in the prefrontal and ACC of 3 groups: recently detoxified AUD patients, subjects at HR to develop an AUD as well as HCs (low risk [LR]). Along with our previous findings of reduced D2/3 availabilities in the striatum of AUD and HR compared to LR, we hypothesized reduced prefrontal dopamine D2/3 availabilities in AUD patients and HR com-

Table 1. Sample characteristics [31–33]

Variable (available data for LR/HR/AU)	Group			<i>p</i> values for test statistic		
	LR (<i>n</i> = 19)	HR (<i>n</i> = 19)	AUD (<i>n</i> = 20)	LR vs. HR	LR vs. AUD	HR vs. AUD
Gender	3 female, 16 male	2 female, 17 male	3 female, 17 male	1.0 ^d	1.0 ^d	1.0 ^d
Handedness	19 right handed	19 right handed	19 right, 1 left handed	–	1.0 ^d	1.0 ^d
Smokers (including ex-smokers), %	63	94	84	0.008 ^e	0.30 ^e	0.11
	Mean (SD)/median (IQR)*					
Demographic variables						
Education, years (19/17/20)	14.6 (3.1)	17.5 (5.4)	15.1 (3.3)	0.87 ^b	0.69 ^b	0.16 ^b
Age, years (19/19/20)	45.2 (8.7)	42.9 (9.1)	45.4 (8.4)	0.27 ^c	0.91 ^c	0.21 ^c
Duration of abstinence, days (20)	–	–	36.4 (20.1)	–	–	–
Clinical characteristics						
AUD identification test (6/19/0) ^f	4.5 (2.25)*	12.0 (5.0)*	–	<0.001 ^{a, b}	–	–
Severity of AUD (19/14/19) ^f	2.1 (5.2)*	5.2 (5.1)*	17.0 (8.0)*	0.02 ^{a, b}	<0.001 ^{a, b}	<0.001 ^{a, b}
Craving (19/18/16) ^f	2.0 (3.0)*	8.0 (5.0)*	11.0 (12.0)*	<0.001 ^{a, b}	<0.001 ^{a, b}	0.03 ^{a, b}
Craving “thoughts” (19/18/16) ^f	0 (0)*	0.5 (3.0)*	6.0 (6.5)*	0.01 ^{a, b}	<0.001 ^{a, b}	<0.001 ^{a, b}
Craving “impulse” (19/18/16) ^f	2.0 (2.0)*	6.0 (3.0)*	6.6 (7.2)*	<0.001 ^{a, b}	<0.001 ^{a, b}	0.45 ^b
Age at first drink (14/0/16)	14.7 (2.1)	–	14.3 (2.0)	–	0.62 ^b	–
Age of first AUD diagnosis (16)	–	–	32.3 (11.2)	–	–	–
Years since AUD diagnosis (15)	–	–	13.9 (11.0)	–	–	–

* Nonparametric test statistics are displayed as median and interquartile ranges instead of mean and standard deviation. ^a Significant difference. ^b *p* value of Wilcoxon rank-sum test with continuity correction. ^c *p* value of Welch 2 sample *t* test. ^d *p* value of χ^2 test. ^e Pearson's χ^2 test with Yate's continuity correction. ^f Measured via: AUD identification test: AUDIT [23], Severity of AUD: Alcohol Dependence Scale [31], Craving: Obsessive-Compulsive Drinking Score [32], “thoughts”: subscale obsessions, “impulse”: subscale compulsions. This table is retrieved from [33].

pared with LR, with an intermediate dopamine D2/3 availability of HR [7]. Furthermore, we expected alcohol related symptoms to be associated with neurobiological changes. To our knowledge, this is the first PET study investigating extrastriatal D2/3 receptor availability in AUD and individuals at HR in the same sample.

Methods

This study was part of a multicenter project investigating learning mechanisms and neurobiological alterations in AUD (www.lead-studie.de; clinical trial number: NCT01679145). The present sample contained data from subprojects 2 and 5 and was approved by the local Ethics Committee (Charité – Universitätsmedizin Berlin; EA1/245/11). All subjects gave written consent before participating in the study.

Participants

The sample contained 58 subjects including 19 LR controls, 19 HR participants, and 20 recently detoxified AUD patients. LR and HR subjects were recruited via local online platforms and advertisements in supermarkets and newspapers. The participants were assigned to the LR/HR group based on the individual score of the Alcohol Use Disorder Identification Test (AUDIT) [23]. The AUDIT is a widely used diagnostic instrument known for its validity

and reliability [24]. Above 8 points, subjects were assigned to the HR group, as this is the critical cutoff score for harmful use [23, 25, 26]. HR participants are at increased risk to develop an AUD by practicing an active drinking pattern and experiencing addiction related symptoms. However, LR and HR subjects did not fulfill diagnostic criteria for alcohol dependence which was confirmed with the standardized testing procedure “Composite International Diagnostic Interview” (CIDI) [27, 28].

AUD patients were recruited in different inpatient psychiatric clinics (Berlin, Germany) where they were currently undergoing withdrawal treatment. Patients had to be abstinent for at least 3 days (72 h) and were only included when experiencing low withdrawal symptoms based on a score below 3 on the Clinical Institute of Withdrawal Assessment of Alcohol (CIWA-Scale) [29]. AUD patients fulfilled DSM-IV criteria for at least 3 years and were diagnosed by a trained clinician [30]. This diagnose was later confirmed during the testing procedure using the CIDI interview.

All participants were instructed not to consume alcohol for at least 24 h before the testing took place and not to consume any medication interacting with the central nervous system 10 days before participating in the study. Alcohol breath tests and urine drug screening were performed at the day of the neuropsychological testing. Exclusion criteria were substance dependence or current substance use (other than nicotine consumption). Moreover, participants with diagnosed bipolar, psychotic, major depressive, generalized anxiety, post-traumatic stress disorder, borderline personality disorder, or obsessive-compulsive disorder based on

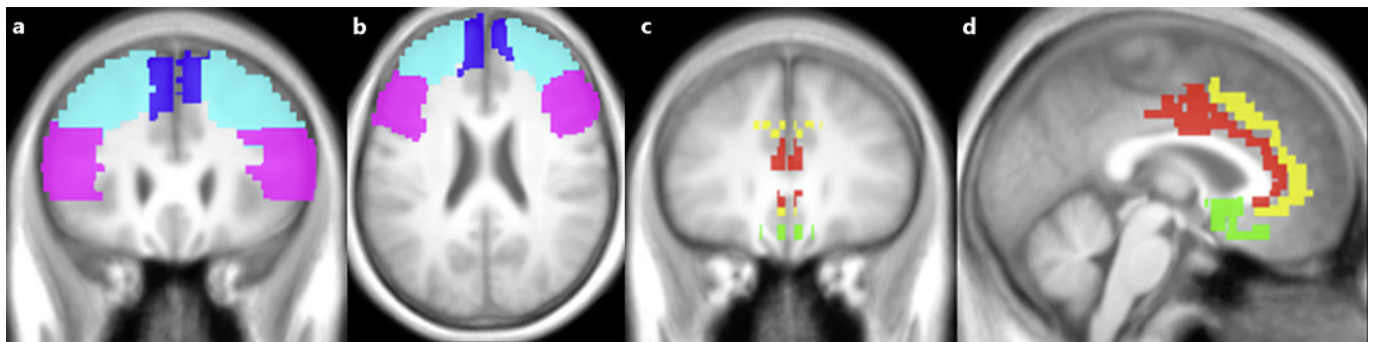


Fig. 1. Selected ROIs and their anatomic localization: coronal view: mPFC in blue, vlPFC in violet, dlPFC in cyan (**a**); axial view: mPFC in blue, vlPFC in violet, dlPFC in cyan (**b**), coronal view: rACC red, dACC yellow, sgACC green (**c**), sagittal view: rACC

red, dACC yellow, sgACC green (**d**). These images were produced using MRIcron (www.mricron.com). The mean MPRAGE picture of the subjects of this sample was used as a template for the displayed ROIs.

DSM-IV criteria were excluded from the study [30]. Further exclusion criteria were neurological diseases, current pregnancy or nursing, and contraindications for magnetic resonance imaging (MRI).

LR, HR, and AUD participants were matched for gender, education, and handedness. Age in years and nicotine consumption were included as covariates. For detailed sample characteristics, see Table 1. All subjects underwent 3 testing session. During the first session, participants underwent several questionnaires such as the AUDIT, CIDI, the Obsessive-Compulsive Drinking Score (OCDS), and the Alcohol Dependence Scale (ADS).

The OCDS is a self-rating instrument assessing craving via 14 items. There are 7 questions about uncontrollable unwanted intrusive reoccurring thoughts and 7 questions regarding repetitive unstoppable behavior patterns or mental acts about alcohol consumption [32]. The ADS investigates symptom severity of alcohol dependence and contains 29 items correlating with a high number of psychopathological symptoms and physical consequences resulting from harmful alcohol consumption [31, 34]. On the second and third sessions, the subjects underwent MRI and PET measurements.

Neuroimaging

MRI Data Acquisition

MRI scanning was performed at “Physikalisch-Technische Bundesanstalt” in Berlin using a 3-Tesla scanner (Siemens Verio). T1-weighted images (MPRAGE, isotropic resolution 1.0 mm, TR = 2.3 s, TE = 3.03 ms, TI = 900 ms, flip angle 9°) were acquired and used for spatial normalization of the PET images. For visual purposes, we used the mean MPRAGE picture of this sample as a template for the region of interests (ROIs) shown in Figure 1.

PET Data Acquisition and Processing

Dynamic PET-imaging was conducted with a time-of-flight PET-CT system (Philips Gemini TF 16) [35]. The procedure started with intravenous injection of 197 ± 7 MBq (AUD), 199 ± 14 MBq (LR), and 195 ± 7 MBq (HR) of the high-affinity dopamine D2/3 receptor antagonist ^{18}F -fallypride, respectively [36]. Directly after the radiotracer injection PET data were acquired during 4 h in 3 blocks of 30 min duration with breaks in between [37]. For each block, a low-dose CT was performed for attenuation correc-

tion. Then, transaxial PET images were reconstructed via the iterative LOR-RAMLA algorithm of the scanner software with default parameter settings (3 iterations, 33 subsets and “normal” relaxation).

There was a frame-wise realignment to correct head motion during each emission block using the realign tool of the Statistical Parametric Mapping software package (SPM8; Wellcome Department of Imaging Neuroscience, Institute of Neurology, London, UK; <http://www.fil.ion.ucl.ac.uk/spm/>). In the reconstructed PET images, the spatial resolution was about 7 mm full width at half minimum. Coregistration of the second and third PET block to the first block was performed using the coregistration tool of SPM8. Further, the early PET frames from the perfusion phase of each subject were coregistered to the subject’s MPRAGE MRI. The two-step simplified reference tissue model was used with the bilateral superior longitudinal fasciculus (SLF) as reference region. The SLF was defined by the white matter tractography atlas provided by the Laboratory of Brain Anatomical MRI of Johns Hopkins University [38]. This reference region was chosen because it has been suggested that white matter regions increase statistical power of ^{18}F -fallypride PET in comparison with grey matter reference regions [39]. Moreover, the cerebellum did not seem suitable as reference region as cerebellar atrophy is associated with alcohol dependence [40]. For further information and discussion of the use of the SLF as reference region, see our recently published studies [7, 41].

Parametric maps of the nondisplaceable ^{18}F -fallypride binding potential (BP_{ND}) were computed with the simplified reference tissue model and using a global rate constant for tracer clearance from the SLF [42]. BP_{ND} is the ratio of the concentration of specifically bound ^{18}F -fallypride to the concentration of nondisplaceable ^{18}F -fallypride at equilibrium [43].

Furthermore, the individual T1-weighted MR images were spatially normalized into the anatomical space of the Montreal Neurological Institute using the unified segmentation approach [44]. The BP_{ND} map of each subject was normalized into Montreal Neurological Institute space by the same transformational procedure.

Regions of Interest

BP_{ND} of ^{18}F -fallypride was assessed in the dorsolateral, ventrolateral, and medial PFC (dlPFC/vlPFC/mPFC) and the subgenual,

dorsal, and rostral ACC (sgACC/dACC/rACC), separately in the left and right hemisphere. These brain regions were selected based on the frontal brain regions associated with addictive behavior as reviewed by Goldstein and Volkow [12]. The subdivision of the ACC and PFC followed the suggested functional specialization described in [12, 45–47] based on fMRI studies. ROIs and Brodmann areas were selected using the Online Brain Atlas Reconciliation Tool (<http://qnl.bu.edu/obart>) and were generated using the standardized templates from Automated Anatomic Labelling, WFU PickAtlas (<http://fmri.wfubmc.edu/~software/PickAtlas>). Figure 1 shows the selected ROIs and their anatomic localization.

Statistical Analyses

Statistical analyses were performed with IBM SPSS Statistics Version 25. A non-normal distribution was revealed for the BP_{ND} in most of the ROIs by the Kolmogorov-Smirnov test and visually via Q-Q-plots. Therefore, we applied nonparametric tests for all analyses. The Kruskal-Wallis (K-W) test was performed to compare the BP_{ND} of ¹⁸F-fallypride between the 3 groups (LR/HR/AU) in the dlPFC, vlPFC, mPFC, sgACC, dACC, and rACC in each hemisphere separately throughout the whole sample.

Smoking status and age have been included as covariates in our analyses as both factors seem to independently affect the dopamine D2/3 receptor availability [19, 48–50]. Age was correlated with the BP_{ND} of ¹⁸F-fallypride in the bilateral rACC ($r_s = -0.342, p = 0.009$), bilateral dACC ($r_s = -0.292, p = 0.026$), and bilateral sACC ($r_s = -0.259, p = 0.049$).

For pairwise comparisons of the BP_{ND} of ¹⁸F-fallypride in the respective ROIs in the 3 reference groups, Mann-Whitney tests were conducted as post hoc tests. For this direct group comparison, a Bonferroni correction was applied and so all the effects of the post hoc tests are reported at a two-sided 0.0167 level of significance.

To investigate the distribution pattern of BP_{ND} in the ROIs which had reached significance in the K-W test, a Mack-Wolfe test was performed to test for an inverted U-shape alternative ($\theta_1 \leq \theta_2 \geq \theta_3$) across groups. Further, a Jonckheere-Terpstra trend test was performed to test for a linear relationship ($\theta_1 \leq \theta_2 \leq \theta_3$). Whereas a linear relationship would rather support a dimensional continuous approach where the neurobiological changes increase with higher symptom severity, an inverted U-shape distribution would support a different distribution pattern in favor of a specific role of HR individuals (LR \leq HR \geq AU). For further discussion regarding quadratic and linear effects, see our previous study [7]. Furthermore, we performed correlations of the dopamine D2/3 availability with the ADS score (symptom severity) as well as with the OCDS (craving symptoms) using Spearman's rho as a nonparametric correlation coefficient and reporting a two-tailed significance level.

Results

The K-W test revealed significant differences of BP_{ND} in the left ($H(2) = 6.59, p = 0.037$) and right ($H(2) = 8.66, p = 0.013$) rACC throughout the whole sample. Moreover, significant differences of BP_{ND} were observed in the left vlPFC ($H(2) = 6.30, p = 0.043$). Further, a trend to-

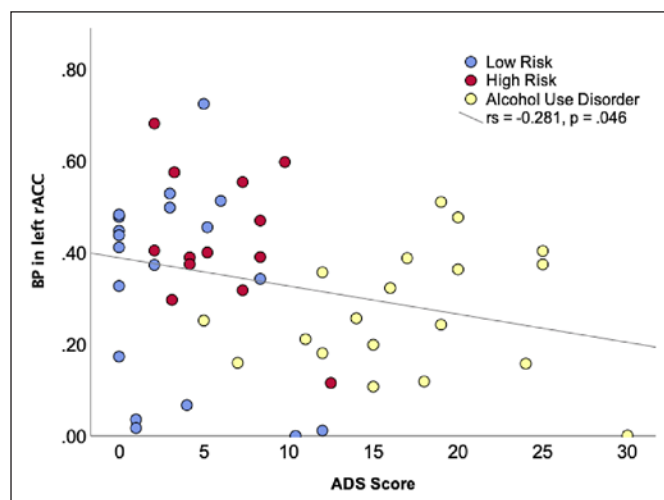


Fig. 2. Scatterplot of BP_{ND} of ¹⁸F-fallypride and the sum of the Total Score of the Alcohol Dependence Scale (ADS) measuring the severity of alcohol-related symptoms in the left rACC.

ward significance was present in the left dlACC ($H(2) = 5.71, p = 0.058$) as well as in the left dlPFC ($H(2) = 5.77, p = 0.056$) throughout the whole sample. Note that after we corrected for multiple comparisons using the False Discovery Rate none of the ROIs reached significance throughout the group comparison.

Post hoc tests revealed significantly higher levels of BP_{ND} in HR subjects compared to AU patients in the left ($U = 90.00, r = -2.81, p = 0.004$) and right rACC ($U = 85.00, r = -2.95, p = 0.003$) as well as in the left vlPFC ($U = 91.00, r = -2.78, p = 0.005$). Differences in the post hoc tests were reported at a significance level of 0.0167 due to a Bonferroni correction. There were no significant differences observed between the LR and HR as well as between the LR and the AU patients.

Moreover, the Jonckheere-Terpstra trend test did not reveal a significant linear relationship across groups, whereas the Mack-Wolfe test revealed a significant inverted U-shape (LR \leq HR \geq AU) distribution in the right rACC ($Z = 2.49, p = 0.012$) as well as in the left vlPFC ($Z = 2.20, p = 0.024$). There was a trend toward a significant inverted U-shape distribution in the left rACC ($Z = 1.83, p = 0.077$). Individual data of the BP_{ND} in the respective ROIs of LR, HR, and AU participants are displayed in Figure 2.

The sum of the ADS score was significantly negatively correlated with the BP_{ND} in the left rACC ($r_s = -0.281, p = 0.046$) throughout the whole sample. This correlation is visually displayed as a scatterplot in Figure 3. Addition-

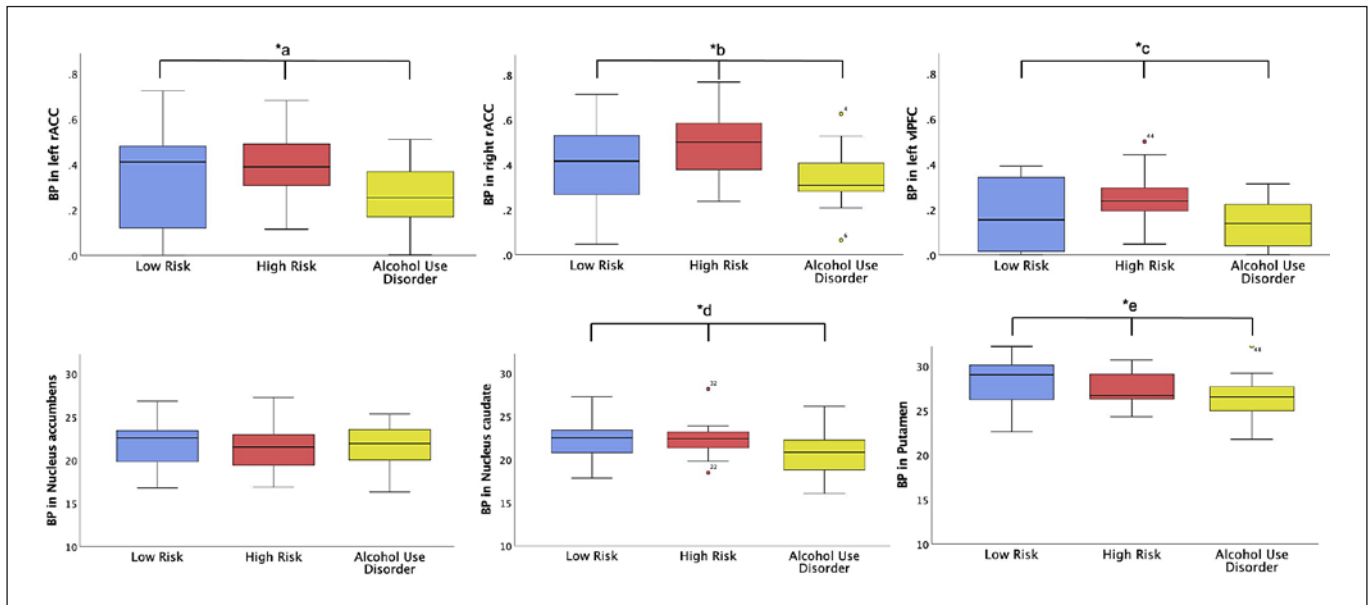


Fig. 3. Trend toward significant inverted U-shape distribution (LR > HR < AUD) $Z = 1.83, p = 0.077$ (*a); significant inverted U-shape distribution (LR > HR < AUD) $Z = 2.49, p = 0.012$ (*b); significant inverted U-shape distribution (LR > HR < AUD) $Z = 2.20, p = 0.024$ (*c); direct group comparisons are reported in the results

section. Second row: showing the results from our previous study for comparison [4]. *d Marginally significant negative quadratic relationship ($p = 0.06$; quadratic contrast; LR > HR < AUD). *e Significant negative linear relationship (linear contrast; $p < 0.05$; LR < HR < AUD).

ally, there was a trend toward a significant correlation between the ADS score and the BP_{ND} in the right rACC ($r_s = -0.256, p = 0.070$). No significant correlation was observed between the ADS score and the BP_{ND} in the vlPFC and dlPFC as well as in the dACC, respectively. No significant correlations were observed within subgroups. Moreover, the sum of the OCDS was not significantly correlated with the BP_{ND} neither in the ACC nor in the PFC in the selected ROIs.

Discussion

In this study, dopamine D2/3 receptor availability in the PFC and ACC of recently detoxified patients with AUD, individuals at HR for developing an AUD and HCs with a low level of alcohol consumption was investigated. We observed a significant higher D2/3 receptor availability in HR compared to AUD subjects in the left and right rACC as well as in the left vlPFC. Although there was no significant difference between LR and AUD subjects as well as between LR and HR subjects, a significant inverted U-shape distribution (LR < HR > AUD) across groups was present in the left, right, and bilateral rACC as well as in the left vlPFC. Further, the

severity of alcohol-related symptoms was correlated significantly with the BP in the left rACC in the whole sample. There was no correlation of BP in the selected ROIs with craving symptoms.

Group Comparison

In line with our hypotheses, we observed significant differences of the dopamine D2/3 availability between the 3 groups with different alcohol consumption patterns. Alterations of the postsynaptic dopamine receptors were present in the left and right rACC as well as in the left vlPFC. These results are in line with the results of several recent fMRI studies suggesting dysfunctions of the ACC and PFC to be crucial for clinical outcomes in addiction [11–13]. Moreover, it has been suggested that deficits in the prefrontal synaptic plasticity contribute to the development and maintenance of addiction [14]. Thus, pre-clinical studies have observed associations between prefrontal dopaminergic dysregulation and cognitive symptoms, as well as cocaine consumption, respectively [15, 16]. However, there are only very few studies that investigated extrastriatal postsynaptic dopamine receptors in addiction [17, 20, 21]. Moreover, many PET studies focus on acute effects of alcohol on D2/3 receptor binding in prefrontal areas [17, 51].

These results must be interpreted with caution as when correcting for multiple comparisons statistical significance was not reached. This may be due to small sample size and technical limitations of PET-imaging in assessing extrastriatal dopaminergic receptor availability. As there are only a few studies that investigated extrastriatal dopamine D2/3 receptor availability in AUD, the precise selection of ROI is very difficult and an exploratory approach with many ROIs is inevitable.

In contrary to our hypotheses, we did not observe a linearly reduced dopamine D2/3 receptor availability with higher alcohol consumption between the 3 reference groups (LR < HR < AUD), but rather an inverted U-shape distribution across groups (LR < HR > AUD) in the left and right rACC as well as left vlPFC. Further, even though in other ROIs, there was no significant difference between groups, the same pattern is visible (LR < HR > AUD). Interestingly, those results are similar compared to previous findings in the same sample in the nucleus caudate where a similar distribution of BP was observed in HR and AUD subjects [7]. However, alongside with the trendwise quadratic relationship in the nucleus caudate, a linear relationship was present in the putamen [7]. For an overview of the extrastriatal BP_{ND} through the reference groups and the previously published results of the striatal BP_{ND}, see Figure 3 [7]. A linear distribution favors a continuous neuroadaptational change associated with higher alcohol consumption, whereas an inverted U-shape distribution emphasizes the special role of HR individuals and raises the question whether this biomarker of higher BP may work as a risk or protective factor for the development of AUD.

Interestingly, we found different effects of BP in the left and right vlPFC whereas in the rACC the results revealed similar effects in both hemispheres. As the observed differences are very small above and underneath the level of significance, the reason for this weak laterality effect may be explained by the relatively small sample size. There are only very few PET studies that reported laterality effects in D2/3 receptors up to now, although [18] observed an altered laterality in the temporal lobe and decreased extrastriatal D2/3 receptors in alcoholics.

Individuals at HR

Whereas in the direct group comparison, there was no significant difference between the BP of LR and AUD subjects as well as LR and HR subjects, dopamine D2/3 availability was altered in the rACC and vlPFC of HR compared to AUD subjects. This finding is in line with the work of Jaworska et al. [21] which observed higher

levels of extrastriatal postsynaptic D2/3 receptors in adolescents at HR for developing an addiction. Further, Pfeifer et al. [51] observed higher extrastriatal BP to be correlated with the subjective effect of liking alcohol after an acute intravenously applied alcohol infusion revealing a potential clinical link. Thus, higher levels of extrastriatal BP seem to be potentially associated with a higher risk for addiction.

On the other hand, it has also been discussed in the literature that higher availability of dopamine D2/3 receptors may serve as a protective factor preserving them from developing a manifest AUD. This is concomitant with the work of Volkow et al. [52] who observed higher levels of striatal dopamine D2 receptors in nonalcoholic family members of families with many alcohol-dependent members suggesting higher levels of D2 receptors as potential protective factors. Also, in their study, high levels of D2 receptors were found to be associated with glucose metabolism in the ACC and PFC suggesting an association of these cortical brain areas with dopaminergic dysfunctions.

Moreover, it is unclear whether alterations of D2/3 receptors are an adaptation process due to the dopamine excess in harmful alcohol consumption or if the availability of D2/3 receptors may be a genetic predisposing factor for AUD. Many genetic and epigenetic influences on the dopamine D2 receptor expression are known to play a role in addiction [53–56]. Thus, higher D2/3 receptors in individuals at HR in this study may be the result of an adaptation or may be a genetically predisposed factor.

Taken together, it is unclear whether higher levels of extrastriatal BP may be a risk factor or a protective factor for the development of AUD as the current literature is inconsistent. Clearly more research is necessary to investigate postsynaptic D2/3 receptors in individuals at HR and particularly investigate long-term clinical outcomes such as relapse probabilities.

Correlations with Clinical Parameters

Dopamine D2/3 receptor availability in the left rACC was correlated with severity of alcohol-related symptoms measured via ADS. This is in line with the finding of impaired dopaminergic neurotransmission and alcohol-related behaviors of several recent PET studies in striatal or extrastriatal brain regions [7–9, 19, 57]. Interestingly, this is the first PET study investigating dopamine D2/3 receptor availability and alcohol related symptoms in the ACC in AUD. However, previous PET studies in the ACC have focused on behavioral alterations such as interruptions, for example, in executive functioning [46, 47].

It must be considered that the correlation coefficient (Spearman's R) when correlating the sum of the ADS with the DR2/3 availability in the left rACC was -0.281 , with a consequently small to medium effect size. This emphasizes that conclusions about this clinical association are limited and of course do not allow any claims about causalities.

Interestingly, we did not observe significant correlations of craving symptoms measured via the OCDS and BP neither in the ACC nor in the PFC. This finding was contrary to our hypotheses as there is evidence for a potential role of the ACC in regulating craving symptoms. Particularly, previous studies have shown that a reduction of ACC connectivity reduces craving in addiction [58–60]. Further, another study was able to observe a correlation of the extent of craving symptoms with changes of the dopamine D2/3 availability in the ACC following cocaine-cue induced dopamine release [61].

A potential explanation why we did not observe correlations with craving symptoms may be that only patients experiencing low withdrawal symptoms based on a score below 3 on the CIWA-Scale were included in this study. Thus, the selected patients might not have been experiencing high craving symptoms and a potential selection bias cannot be excluded. Further, AUD patients were currently undergoing inpatient alcohol withdrawal treatment when participating in the study and thus were not exposed to alcohol-related stimuli during the study, which may have had an influence on the results as well.

To better evaluate clinical parameters associated with D2/3 availability in the ACC and PFC of AUD subjects, more research is necessary. One potential approach may be the combination of the assessment of functional parameters associated with prefrontal disruptions such as executive functioning and impulsivity together with alcohol-related symptoms in AUD and HR to better identify risky behavior patterns potentially contributing the addiction development.

Strength and Limitations

^{18}F -fallypride is a well-established method for assessing dopamine D2/3 availability, but free endogenous dopamine may influence the BP during the data acquisition [62, 63]. However, study participants were not exposed to any direct positive stimuli which might have caused a DA release during the data acquisition, we suggest that this confounder is insignificant. Moreover, ^{18}F -fallypride has been described to be the appropriate and preferable radiotracer for the measurement of BP in extrastriatal brain areas in comparison with other tracers such as ^{11}C -raclopride [37, 64]. Thus, the selection of ^{18}F -fallypride is clear-

ly a strength of the present study. Although we carefully selected our ROIs based on previous studies, there are still various ROI approaches being applied in prefrontal as well as anterior cingulate cortical areas in comparable PET studies which may influence the comparability of results.

Moreover, a potential confounder might have been the variability of the abstinence duration in AUD patients due to technical difficulties (min 9, max 96, mean 36.4, and SD 20.1). Nevertheless, other comparable PET studies had similar abstinence rates in their patient groups [65].

Another limitation may be the fact that we cannot rule out that the individuals in the LR group may have under or overreported their drinking behavior. As LR and HR subjects were practicing an active drinking pattern and self-rating questionnaires were used in this study, it was very difficult to objectify the alcohol consumption.

Conclusion and Perspectives

This is to our knowledge the first study investigating extrastriatal dopamine D2/3 availability in AUD and individuals at HR. With 58 study participants, this is a relatively large sample for a PET study and a dimensional study approach with 3 represented levels of alcohol consumption patterns through our reference groups. With this work, we were able to show higher dopamine D2/3 receptors in individuals at HR for alcohol addiction compared to AUD patients and an inverted U-shape distribution of dopamine receptors across groups (LR < HR > AUD) in the left and right rACC as well as in the left vlPFC. Interestingly, we did not find a gradual receptor reduction with increased substance use, which emphasizes the special role of individuals at HR and raises the question whether the observed higher BP levels may function as risk or protective factors in addiction. Therefore, the intraindividual change of extrastriatal D2/3 receptor availability during the development of AUD should be investigated to better understand its pathobiological role and its possible predictive function for the clinical outcome. Future work may additionally evaluate behavioral correlates such as impulsivity or executive functioning to gain new insights about the role of extrastriatal dopaminergic transmission in AUD.

Statement of Ethics

All subjects have given written informed consent before participating in the study, and the study was approved by the local Ethics Committee (Charité – Universitätsmedizin Berlin; EA1/245/11).

Conflict of Interest Statement

G.S., T.G., K.Z., O.B., R.B., and J.G. have no conflicts of interest to declare.

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