UvA-DARE (Digital Academic Repository)

White matter disruptions in male cocaine polysubstance users: Associations with severity of drug use and duration of abstinence

van Son, D.; Wiers, R.W.; Catena, A.; Perez-Garcia, M.; Verdejo-García, A.

DOI

[10.1016/j.drugalcdep.2016.09.023](https://doi.org/10.1016/j.drugalcdep.2016.09.023)

Publication date

2016

Document Version

Final published version

Published in

Drug and Alcohol Dependence

License

Article 25fa Dutch Copyright Act

[Link to publication](#)

Citation for published version (APA):

van Son, D., Wiers, R. W., Catena, A., Perez-Garcia, M., & Verdejo-García, A. (2016). White matter disruptions in male cocaine polysubstance users: Associations with severity of drug use and duration of abstinence. *Drug and Alcohol Dependence*, 168, 247-254. <https://doi.org/10.1016/j.drugalcdep.2016.09.023>

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (<https://dare.uva.nl>)

Full length article

White matter disruptions in male cocaine polysubstance users: Associations with severity of drug use and duration of abstinence

Dana van Son^{a,*,1}, Reinout W. Wiers^a, Andrés Catena^b, Miguel Perez-Garcia^b, Antonio Verdejo-García^{c,d}

^a Addiction, Development and Psychopathology (ADAPT) lab, Dept. of Psychology, University of Amsterdam, Amsterdam, The Netherlands

^b Mind, Brain and Behavior Research Center (CIMCYC), Universidad de Granada, Granada, Spain

^c Red de Trastornos Adictivos & Institute of Neurosciences F. Olóriz, Universidad de Granada, Granada, Spain

^d School of Psychological Sciences and Monash Institute of Cognitive and Clinical Neurosciences, Monash University, Australia

ARTICLE INFO

Article history:

Received 1 June 2016

Received in revised form

14 September 2016

Accepted 17 September 2016

Available online 6 October 2016

Keywords:

Diffusion tensor imaging

Fractional anisotropy

Polysubstance use

Cocaine

Alcohol

Abstinence

ABSTRACT

Background: Cocaine dependence has been associated with alterations in the brain's white matter integrity, yet relevant questions remain about what alterations are linked to cocaine use and/or polysubstance use, and whether they are amenable to abstinence.

Methods: This study applied a single measurement session of diffusion tensor imaging (DTI) to examine white matter structure in male cocaine polysubstance users ($n = 37$) versus male healthy controls ($n = 38$), along with correlations between DTI measures and patterns of polysubstance use and duration of abstinence. Specifically, we conducted voxel-wise analyses of fractional anisotropy (FA) in the corpus callosum, frontolimbic, striatal and cingulate tracts relevant to drug sequelae.

Results: Cocaine polysubstance users, compared to controls, showed lower FA in the body of the corpus callosum, anterior cingulate, uncinate fasciculus and retrolenticular part of the internal capsule. Duration of cocaine use had a marginal negative association with FA in the corpus callosum, and duration of alcohol use was negatively associated with FA in the internal capsule and the uncinate fasciculus. Duration of cocaine abstinence was positively correlated with FA in the uncinate fasciculus, posterior cingulate and fornix-striatum. In the context of cocaine polysubstance use, chronicity of cocaine use is therefore likely to be associated with lower FA in the corpus callosum, and chronicity of alcohol use with lower FA in the frontal-striatal and frontal-limbic tracts. Longer abstinence was correlated to greater FA in frontal-striatal and frontal-limbic tracts, though the direction of causality remains unclear.

Conclusion: Since the results did not survive multiple comparison-corrected thresholds, more studies are needed to confirm these indications.

© 2016 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Cocaine dependence is a significant public health problem, particularly among adult males (Agabio et al., 2016). Biologically based treatments are lacking (Everitt and Robbins, 2016), and existing psychosocial interventions have their lowest efficacy in cocaine polysubstance users (Dutra et al., 2008). Therefore, broad knowledge of the neurobiological alterations associated with polysubstance use, and the brain networks associated with successful

abstinence, can improve understanding of cocaine dependence and its treatment. Diffusion tensor Imaging (DTI) is a useful technique for non-invasively investigating the structure of white matter by measuring fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD) and mean diffusivity (MD). These measures reflect the degree of alignment of cellular structures within fiber tracts, as well as structural integrity (Betz et al., 2012; Cercignani et al., 2001). Previous studies using DTI have already found FA reductions in cocaine dependent individuals within the genu and the rostral body of the corpus callosum, as well as in frontal, frontal-limbic and parietal regions (Bell et al., 2011; Lim et al., 2008; Ma et al., 2009; Moeller et al., 2005; Romero et al., 2010). The present study aimed to replicate and extend these findings in a sample of cocaine

* Corresponding author at: Leiden University, Department of Clinical Psychology, Wassenaarseweg 52, 2333 AK, Leiden, Netherlands.

E-mail address: d.m.e.van.son@fsw.leidenuniv.nl (D. van Son).

¹ Now at department of Psychology, Leiden University, The Netherlands

dependent individuals with varying levels of abstinence enrolled in therapeutic community treatment.

An important issue in the context of the neurobiological correlates of cocaine dependence is whether white matter alterations are more severe in users who consume more than one drug, so called ‘polysubstance users’, compared to users of a single drug. Studying populations of polysubstance users is usually avoided for methodological reasons. However, polysubstance users are clinically representative due to the high prevalence of this pattern in addiction treatment services (Barrett et al., 2006; Redonnet et al., 2012). High rates of polysubstance use have specifically been documented in cocaine dependent users (Connor et al., 2014). In addition, there is growing scientific interest in the impact that other drugs such as alcohol and tobacco have on the neurobiological abnormalities ascribed to cocaine dependence (Abé et al., 2013; Pennington et al., 2015). In polysubstance users with cocaine as their main drug of choice, structural neuroimaging studies have shown significant reductions of the volume of discrete brain regions such as the prefrontal cortex, striatum and amygdala (Barrós-Loscertales et al., 2011; Moreno-López et al., 2012). It has been also found that a higher number of used substances was related to lower whole brain white matter integrity (Kaag et al., 2016). Prefrontal and Striatal regions have been implicated in reward learning and cognitive control processes that are pivotal to addiction (Kahnt et al., 2009; Koob and Volkow, 2009). However, the function of these regions is ultimately determined by their pattern of white matter input and output (Passingham et al., 2002) and thus white matter studies can lead to a better understanding of the impact of polysubstance use in cocaine dependence.

In addition, recent studies have examined white matter recovery following abstinence. Cocaine dependent individuals who received treatment showed a positive correlation between duration of abstinence (measured with both self-reports and urine tests) and FA values in the corpus callosum, cerebellum, frontal, parietal, temporal, and occipital lobes (Xu et al., 2010). Also, cocaine dependent individuals who were treated with transcranial direct current stimulation (tDCS) during abstinence, had increased DTI parameters in the left ventral-medial prefrontal cortex connection to the nucleus accumbens (Nakamura-Palacios et al., 2016) when compared to cocaine dependent individuals treated with a placebo (sham) tDCS. More directly, Bell and colleagues (2011) looked at 43 cocaine dependent individuals who were abstinent varying from 5 to 102 days and found duration of abstinence to correlate with increased FA in the inferior and superior longitudinal fasciculus, anterior and ventral connections from the thalamus, superior corona radiata, cingulum and the precentral gyrus. Mainly the longitudinal fasciculus and tracts connecting the thalamus and the cingulum are involved in emotional and homeostatic processes (Olson et al., 2007; Pisner et al., 2016).

Given the findings of reduced FA in cocaine dependent individuals compared to healthy controls, and the findings of FA recovery in abstinent cocaine users, this study re-assessed these findings in a relatively larger group of male cocaine polysubstance users undergoing therapeutic community treatment. The main aims were (i) to compare white matter integrity in tracts connecting frontal, callosal, striatal and limbic regions in male cocaine polysubstance users versus male non-drug using controls, and (ii) to determine the extent of the association between white matter integrity, chronicity of substance use and duration of abstinence in the cocaine polysubstance group. We hypothesized that cocaine polysubstance users will show lower white matter integrity compared to non-drug using controls. We also hypothesized negative associations between patterns of use of cocaine and other drugs and white matter integrity, and a positive association between duration of abstinence and white matter integrity.

Table 1

Substance use characteristics in male cocaine polysubstance users (CPU) and male healthy controls (HC).

	CPU	HC
	n = 37	n = 38
Age	29.5 ± 6.6	31.1 ± 5.1
Years of education	9.7 ± 1.62	10.38 ± 1.96
Duration of cocaine use in months	48.6 ± 36.86	–
Current abstinence of cocaine in weeks	36.76 ± 22.60	–
Cocaine use per month (grams)	18.19 ± 29.84	–
Units of alcohol per month	107.58 ± 114.03*	23.83 ± 25.45*
Occurrences of marijuana use per month	88.03 ± 107.01	11.01 ± 12.03
Percent smokers	83.8	44.7
Number of cigarettes per month**	488.48 ± 307.40	352.16 ± 340.56

Note: Mean ± standard deviation of the mean. CPU = cocaine polysubstance users, HC = healthy controls.

* Mean units of alcohol per month (in bold) was significantly different between groups ($p = 0.003$). Due to multiple comparisons, level of significance was adjusted to $p < 0.01$ using Bonferroni correction.

** Use of non-smokers is included as well in the measure of number of cigarettes per month.

2. Material and methods

2.1. Participants

A total of 37 cocaine polysubstance users with a mean age = 29.6, standard deviation (SD) = 6.5, and 38 non-drug users (controls) with a mean age = 31.1, SD = 5.1 were included in this study. Cocaine polysubstance users were recruited in an inpatient addiction treatment clinic (“Proyecto Hombre”), located in Granada, Spain, and were currently undergoing therapeutic community treatment. Due to the low prevalence of women entering drug treatment during the recruitment period, all the participants were male. All the cocaine dependent participants reported cocaine as their main drug of choice and the drug for which they received treatment. Clinical interviews based on Diagnostic and Statistical Manual version IV (DSM-IV) criteria confirmed cocaine dependence diagnosis; in addition, they reported regular use of tobacco, alcohol, marijuana and 3,4-methylelendioxymethamphetamine (MDMA; see Table 1). Cocaine dependent users had to be abstinent for at least 15 days (for any drug but tobacco) to participate in the study, as confirmed by weekly urine tests. In this way, it became possible to rule out acute and residual effects of previously used drugs on brain structure; with the exception of tobacco (83.8% of cocaine-dependent patients and 44.7% of controls were current smokers). Although short-term abstinence was objectively monitored, the total duration of abstinence was substantially longer in most participants, and thus this total length of abstinence was self-reported. This self-report data, which was corroborated by clinicians, was used in correlational analyses concerning the relationship between duration of abstinence and white matter measures (see Results subsection 3.3 below).

The non-drug using controls were recruited through a local employment agency, and were matched to the cocaine participants in terms of being unemployed. Selection criteria for control participants were: (i) the absence of current or past substance use, excluding past or current social drinking (less than ten standard alcohol units per week) and tobacco use; (ii) the absence of documented major psychiatric disorders; (iii) the absence of documented head injury or neurological disorder, and (iv) not using medication with effects on the central nervous system.

Potential participants; both cocaine polysubstance users and healthy controls who had previously been diagnosed with any disorder from DSM-IV Axes I and II (other than substance dependence), or who had neurological or systemic diseases affecting central nervous system (CNS) functioning were excluded. Furthermore, none

of the cocaine polysubstance users were currently following pharmacological substitution treatments.

Any participant with evidence of stroke or space-occupying lesions observed on conventional clinical MRI images, any contraindications to MRI scanning (including claustrophobia and implanted ferromagnetic objects), and history of loss of consciousness for longer than 30 min or loss of consciousness with any neurological consequence, were excluded for both groups.

2.2. Instruments and assessment procedures

All participants signed an informed consent form certifying their voluntary participation. Controls, unlike cocaine polysubstance users, received a €40 compensation for participating in the study. The assessment of the MRI scans lasted approximately 6 min. The study was approved by the Ethics Committee for Research in Humans of the University of Granada.

2.3. Patterns of drug use

All data regarding time, amount and duration of use of the different drugs was self-reported by participants and collected using the Interview for Research on Addictive Behavior (Verdejo-García et al., 2005). This interview provides an estimation of monthly use of each substance during regular use (amount per month) and total duration of use of each substance (in months). It also provides an estimation of the duration of abstinence (in weeks); given that patients were in residential treatment, indicating abstinence from all drugs including alcohol. The descriptive scores for these variables are presented in Table 1. Units of alcohol per month was significantly different between groups ($F(1,74)=9.43, p=0.003$). No other significant demographic or drug-use differences were found between groups.

2.4. MRI acquisition

Participants were scanned using a 3T whole body MRI scanner (Phillips Achieva, The Netherlands) operating with 8 channels phased-array head coil for reception. Diffusion imaging data were acquired in 32 diffusion gradient directions plus two $b=0$ reference images using the following parameters: repetition time (TR)=8359 ms, echo time (TE)=60 ms, flip angle=90°, b-factor=800 s/mm², voxel size=1.75 × 1.75 × 2 mm, 62 slices on the axial plane with no slice gap, FOV=224 × 224 mm², matrix 128 × 128. Data was transferred to a workstation for image processing and analyses.

2.5. Image processing

DTI images were processed using the FSL software library (FMRIB, <http://www.fmrib.ox.ac.uk/fsl>, Smith et al., 2004). Motion and eddy current corrections were performed by affine registration of the diffusion images to the non-diffusion weighted image. The Brain Extraction Toolbox (BET; part of FSL; Smith et al., 2004) was used to create brain masks. In order to generate FA, AD, RD and MD values for each participant, the diffusion tensor model was fitted to each voxel using the FSL diffusion toolbox (FDT; Behrens et al., 2003).

2.6. Tract-based spatial statistics (TBSS)

Tract-Based Spatial Statistics (TBSS; Smith et al., 2006) was used for the analysis of the preprocessed FA data. First, all FA data was non-linearly registered to the FMRIB58_FA template in standard Montreal Neurological Institute (MNI) space. A mean FA image was

generated and thinned to create a mean FA skeleton using a threshold of >0.2. Each participant's aligned FA data was then projected onto this skeleton and fed into voxel-wise cross subject statistics. This procedure helps to overcome any spurious findings related to anatomical differences because in this way, analyses are restricted to regions of white matter that are shared by all the participants (the skeleton). The same non-linear registration was applied to the diffusivity parameters AD, RD and MD; each parameter was projected onto the FA skeleton for statistical analysis.

Additionally, to a whole brain skeleton selection, specific white matter tracts connecting the regions related to recovery with abstinence were used, as found in previous literature (Bell et al., 2011; Hanlon et al., 2011; Matochik et al., 2003; for a review see Garavan et al., 2013). The ROI masks were based on the JHU ICBM DTI 81 White Matter Atlas. Tracts were selected in both left and right hemispheres for the following regions: internal capsule (anterior limb and retrolenticular part), anterior cingulate gyrus, posterior cingulate gyrus (connected to the hippocampus), the fornix, the fornix-striatum, uncinate fasciculus, tapetum (side part of corpus callosum) and the main parts of the corpus callosum (genu, body, splenium). All selected white matter masks were projected to the FA mean skeleton of each subject in order to include only voxels comprised in the mean FA skeleton, and mean FA, AD, RD and MD values were calculated for each tract of interest.

F-tests were applied to test for significant differences between both groups for the whole brain and selected ROIs in FA. F-tests were chosen because additional covariate analyses were performed. We examined two contrasts: cocaine polysubstance users having greater FA than healthy controls, and healthy controls having a greater FA than cocaine polysubstance users. The results were corrected with a threshold free cluster enhancement approach (TFCE as part of FSL; Smith and Nichols, 2009). Along with FA values, information on each individuals' AD (the 1st eigenvalue), RD (the average of the 2nd and 3rd eigenvalue) and MD was tested by using separate F-tests in the ROIs that showed significantly lower FA in the cocaine polysubstance users. These results were again corrected with TFCE. Because cocaine use was accompanied by tobacco and alcohol use, we included tobacco and alcohol use as a covariate in the analysis.

Linear correlation analysis (SPSS, v 15.0, Chicago, IL, USA) was done between FA, substance use and abstinence measures. The whole brain and ROI FA, AD, RD and MD values were used for further correlational analyses. Five separate correlational analyses were done, therefore the family-wise corrected p -value was set up to 0.01 (0.05/5). For the analysis of substance use we computed the products of all different substances using amount of use * duration of use in months. Duration of abstinence in weeks was analyzed separately. Tests of normality showed that the drug-use data was non-normally distributed, therefore a logarithmic transformation was used to improve normality fitting.

3. Results

3.1. Demographic variables and substance use

The two groups were matched for gender, age and years of education. Demographic information and patterns of substance use are provided in Table 1. There was a significant difference in alcohol consumption between cocaine polysubstance users compared to healthy controls, $F(1,74)=9.428, p<0.01$. No significant difference was found in amount of tobacco use per month between the groups, $F(1,74)=2.552, p=0.12$. Alcohol and tobacco use were inserted as covariates for the ROIs analyses and correlational analyses. Neither alcohol use nor tobacco use had a significant effect on any of the

Fig. 1. Between-group differences in white matter integrity between cocaine polysubstance users and controls. A; y = −28, B; x = −7, C; x = −46, D; z = −2. Note. Selected tracts of interest (green) with significant between-group findings highlighted in (red). A; Coronal view of (top to bottom) left and right Cingulate Gyrus, Body of the Corpus Callosum (CC), the left and right Uncinate Gyrus, left and right Striatal Fornix and the left and right Posterior Cingulate. B; Sagittal view on the left Anterior Cingulate Gyrus (above) and the CC (body of the CC is significant). C; Sagittal view on the left Retrolenticular part of the Internal Capsule (upper spot) and the Uncinate Fasciculus. D; Dorsal view of (top to bottom) the Genu part of CC, left and right Anterior Limb Internal Capsule, the left and right Uncinate Gyrus, left and right Striatal Fornix and the left and right Posterior Cingulate.

Table 2
Regions where decreased FA was observed in TBSS analysis in cocaine polysubstance users (CPU) compared to healthy controls (HC).

Cluster Location		k	p	MNI			Mean FA (SD)	
				X	Y	Z	CPU	HC
1	Body of Corpus Callosum	4320	0.048	−1	−5	27	0.953(0.024)	0.963(0.016)
2	Cingulate L	814	0.041	−8	−12	31	0.913(0.021)	0.923(0.019)
3	Cingulate R	799	0.042	9	−8	31	0.879(0.027)	0.892(0.025)
4	Uncinate L	493	0.041	−32	−9	−3	0.745(0.020)	0.757(0.028)
5	Retrolenticular part of internal Capsule L	188	0.027	−30	−28	10	0.831(0.023)	0.844(0.025)

SD = standard deviation, L = left, R = right, p = X,Y,Z are derived from MNI space. k = cluster size in voxels, CPU = cocaine polysubstance users, HC = healthy controls. Due to multiple comparisons, level of significance was adjusted to $p < 0.01$ using Bonferroni correction. No p -values reached significance.

tracts that we selected, except for a covariate effect of tobacco use on the right cingulate gyrus; $F(1,74) = 4.61, p = 0.04$.

3.2. Tract based spatial statistics (TBSS) analyses in whole brain and regions of interest (ROIs)

TBSS- analyses demonstrated no significant difference in whole brain FA between cocaine polysubstance users and healthy controls, $F(1,74) = 0.123, p = 0.73$. However, FA reductions were found in cocaine polysubstance users compared to healthy controls, including clusters spanning the corpus callosum (body), left and right anterior cingulate gyrus, the left uncinate fasciculus and the

retrolenticular part of the left internal capsule (Fig. 1). The statistical results of the FA values in these ROIs are presented in Table 2. The biggest FA cluster was more than 4000 voxels and included tracts in the body of the corpus callosum extending to anterior and posterior directions. No region was found in which the controls had significantly lower FA values compared to cocaine polysubstance users. Analyses of the AD, RD and MD in the ROIs that showed significantly lower FA in the cocaine polysubstance user group revealed a significant decrease of RD in the right cingulate only; $F(1,74) = 5.246, p = 0.025$, in the cocaine polysubstance users compared to healthy controls. No significant differences were found between groups in AD or MD.

Table 3

Pearson correlations between measures of drug use behavior (log-transformed) and FA density in different white matter tracts.

	Tract	Pearson Correlation	p	N
Cocaine duration of use in months	Tapetum L	−0.420	0.010	37
Alcohol duration of use in months	Retrolent. Part of Intern.	−0.318	0.007	70
	Capsule R	−0.357	0.002	
	Uncinate R	−0.372	0.002	
	Uncinate L			
Marijuana duration of use in months	Fornix Striata R	0.501	0.005	30
Duration of abstinence cocaine (weeks)	Uncinate R	0.351	0.033	37
	Fornix striatum L	0.362	0.028	
	Posterior cingulate R	0.378	0.021	

3.3. Association between drug use, abstinence and FA measures

In Table 3 the correlations between log transformed measures of drug use behavior and mean FA in the selected white matter tracts are shown. In these analyses, we prioritized clinical relevance, and thus results are considered significant if R-squared values accounted for at least a 10% of the variability of the outcomes. A significant negative correlation was found between the time of cocaine use in months and FA values in the left tapetum ($r = -0.42$, $p = 0.01$). Alcohol time of use in months of all participants had a significant negative correlation with FA values in the right uncinate ($r = -0.36$, $p < 0.01$) the left uncinate ($r = -0.37$, $p < 0.01$) and the right retrolenticular part of the internal capsule ($r = -0.32$, $p < 0.01$). Marijuana time of use in months, however, showed a significant positive correlation with FA in the right fornix striatum ($r = 0.5$, $p < 0.01$). No significant differences in FA were found for tobacco or MDMA use. Also, no correlations were found between drug use and RD, AD or MD measures.

No significant correlations were observed for duration of abstinence at the selected threshold ($p < 0.01$). To reveal potential associations between duration of abstinence and the white matter in the selected tracts, we additionally inspected the results with an uncorrected threshold of $p < 0.05$. This uncorrected threshold was used to mitigate the risk of type II error that can result from applying Bonferroni corrections in small samples sizes (Woo et al., 2014; Nichols and Hayasaka, 2003). Therefore, in these analyses we chose to increase statistical sensitivity for exploratory purposes. With this significance threshold, positive correlations were found between the duration of abstinence and FA in the right posterior cingulate gyrus ($r = 0.38$, $p = 0.02$), the left fornix-striatum ($r = 0.36$, $p = 0.03$) and the right uncinate fasciculus ($r = 0.35$, $p = 0.03$) (see Table 3 and Fig. 2). No correlations were found between time of substance abstinence and RD, AD or MD measures.

4. Discussion

Our results indicate that cocaine polysubstance use in males is associated with deficits in white matter bundles connecting frontal, frontal-limbic, striatal and cingulate regions. Moreover, we found a negative association between cocaine chronicity and white matter in the left tapetum, and between alcohol chronicity and white matter in the retrolenticular part of the internal capsule and in the uncinate fasciculus. These white matter bundles have been implicated in cognitive control and social-emotional functioning (Downey et al., 2015; Kopell and Greenberg, 2008). Cocaine polysubstance users with a longer period of abstinence potentially had greater white matter in the right uncinate fasciculus, the left fornix striatum and the right posterior cingulate, suggesting that abstinence may possibly be linked to progressive recovery in these tracts.

Although we did not find whole-brain differences between cocaine polysubstance users and healthy controls, the discrete tracts showing differences between groups are consistent with

the results of structural neuroimaging studies in cocaine dependent populations, which have shown volume reductions in the regions connected by these tracts. Grey matter studies in cocaine-dependent subjects have shown lower volumes in regions that take part in frontal-limbic and frontal-striatal networks, such as the medial orbito frontal cortex, posterior cingulate, postcentral gyrus and inferior parietal lobule (Moreno-López et al., 2012; Tanabe et al., 2009). They are also consistent with findings of white matter studies, which have shown lower density in the body of the corpus callosum (Ma et al., 2009; Moeller et al., 2005) and in tracts connecting the orbitofrontal cortex with the basal ganglia, such as the inferior fronto-occipital fascicle and anterior cingulate tracts (Romero et al., 2010). We extend these findings by showing significant reductions in the uncinate fasciculus and internal capsule tracts, which are pivotal to frontal-limbic and frontal-striatal connectivity, similar to anterior cingulate tracts. White matter reductions in cocaine polysubstance users might be concentrated in tracts connecting the previously named functions such as cognitive, social and emotional functioning. Whole brain analysis on the other hand is possibly too broad and various for revealing these specific differences.

By adding monthly tobacco use (number of cigarettes) and amount of alcohol units per month as covariates, we demonstrated that the use of these substances did not influence white matter alterations, except alterations in the right cingulate, which were also explained by tobacco use, and not cocaine use alone. Previous studies have found decreases, but increases as well in FA related to tobacco use (Yu et al., 2015) specifically in the cingulate tracts (Baeza-Loya et al., 2016). This is in line with our spurious finding that RD decreased significantly in the right cingulate. RD has been related to demyelination and dysmyelination (Alexander et al., 2007) therefore in a tract with decreased FA we expected stronger demyelination so increased RD instead of decreased. Since several substances seem to have an effect on white matter changes in the cingulate tracts, our contradicting results of FA and RD values are hard to interpret. Additionally no other correlations between tobacco use and RD, AD or MD values were found, making it difficult to draw any conclusions. Important to mention is that although we did not find a significant covariation effect in any of the other ROIs, the percent smokers and amount of alcohol use was higher in cocaine polysubstance users than among healthy controls. Hence, it could be that tobacco and alcohol use still partly explained the relation between polysubstance use and white matter abnormalities.

Correlational analyses of cocaine and alcohol use when maintaining a less stringent significance criterion show an impact on white matter densities. Mainly cocaine use was associated with lower FA in the tapetum, while alcohol time of use in months showed a marginally significant negative correlation with FA values in the right and left uncinate and the right retrolenticular part of the internal capsule, which are limbic/thalamic tracts. These findings are consistent with previous results showing degenerated cortical-limbic circuits among alcohol users (e.g., Bjork and Gilman, 2014;

Fig. 2. Correlations between duration of abstinence in weeks and mean FA in the associated tracts. Data was log-transformed to be normally distributed.

Suzanne and Kril, 2014). No correlations were found for tobacco and MDMA time of use in months. Because these results did not survive the significance threshold corrected for multiple comparisons, they should be taken with caution and should be replicated in future studies. Consistently however, substance use is generally thought to induce brain damage through the combined effect of multiple biological factors including substance-related effects on brain physiology and brain genes expression (Kendler et al., 2003). For example, cocaine might significantly increase the risk on a restriction of blood supply in the brain, so called 'cerebral ischemia'; both grey and white matter integrity become vulnerable this way to a variety of neurovascular effects with long term use of cocaine (Buttner, 2012). Furthermore, abnormalities in white matter could be due to the disturbance of myelin productions on a genetic level; cocaine induced white matter alterations have been related to epigenetic effects in myelin-related genes (Nielsen et al., 2012; Smith et al., 2014). Albertson et al. (2004) found a decrease of the expression of several myelin related genes in the nucleus accumbens of cocaine users. In alcohol dependent subjects, similar genetic disturbances were found to negatively influence the molecular network of myelin, including a widespread downregulation myelin-related genes and deficits in oligodendrocyte myelin glycoprotein (Liu et al., 2004; Manzardo et al., 2015). Therefore, it is biologically plausible that cocaine and alcohol detrimental effects explain the observed associations. Furthermore, the uncinate fasciculus is a white matter bundle that effectively connects the limbic system with the frontal regions of the brain and is an important connection in the emotion and stress related network (Ghashghaei and Barbas, 2002). This possibly explains regained emotional control when alcohol dependent individuals recover from their dependence (Bochand and Nandrino, 2010).

The positive correlation between marijuana use and white matter in the Fornix Striatum was unexpected. However, it should be noted that Jacobus et al. (2015) also found increased cortical thickness in marijuana users, and higher marijuana use was associated with increased thickness in the entorhinal cortex. Both the fornix and the entorhinal cortex are major white matter 'pathways' into and out of the hippocampus (Galani et al., 2002).

No significant correlations were observed for duration of abstinence using the most stringent threshold, however the uncorrected threshold ($p < 0.05$) revealed correlations of duration of abstinence with FA reductions in white matter tracts relevant to frontal-limbic and frontal-striatal networks: the uncinate fasciculus, the fornix striatum and the posterior cingulate. Again these results should be carefully interpreted due to the risk of Type II error. Still, the findings resonate with functional neuroimaging findings showing that the function of these networks is relevant to maintain abstinence (Hanlon et al., 2013). This is in line with findings of increased FA with cocaine abstinence in frontal-limbic, mainly cingulate tracts (Bell et al., 2011; Xu et al., 2010). Recovery of FA in these regions are in line with findings of improved functions after sustained cocaine abstinence including short-term memory, visuospatial skills and flexibility after cocaine dependence (Di Sclafani et al., 2002; Pace-Schott et al., 2008) and sustained attention and working memory after MDMA abstinence (Iudicello et al., 2010; for a review see Schulte et al., 2014). Paulus et al. (2005), as well, found different activation of the posterior cingulate, insular, middle frontal, and temporal cortices in abstinent amphetamine-dependents compared to relapsing patients. Moreover, activation in the left posterior cingulate cortex correlates with worse treatment outcome in recovering cocaine dependent patients (Kosten et al., 2005). Because both the posterior cingulate and the uncinate fasciculus are important for communication between cortical regions and the limbic system (Vogt et al., 2006; Zhang et al., 2011), they are involved in a variety of emotional processes, such as emotional memory and social cognition (Downey et al., 2015; Jalbrzikowski et al., 2014) which are relevant to drug abstinence. Yet, because we used a lenient threshold and abstinence was measured on one single time point, the clinical relevance of these findings remains unclear and the causal direction of these relationships should be further examined in longitudinal studies, e.g., better white matter integrity could have enabled an achievement of longer abstinence.

With regard to the association between abstinence on FA values, one of the main limitations is that this study only included male participants and the results may therefore not generalize to female cocaine polysubstance users. Additionally, we measured absti-

nence at a single time-point, and that we did not find significant findings when we strictly corrected results for multiple comparisons. Also, abstinence was checked by weekly urine tests, which might not have been frequently enough to have an accurate and correct indication of complete abstinence. It would be a stronger design to longitudinally examine white matter changes over a follow up period, more frequent urine tests (e.g., twice a week), and more targeted analyses (e.g., deterministic tractography) are probably necessary to capture accurate associations between abstinence and white matter recovery. Furthermore, both the cocaine polysubstance users and the healthy control participants were only included if they did not meet any of the DSM IV criteria. Polysubstance users in general have a higher likelihood of lifetime diagnosis of psychological disorders as described in the DSM IV. Therefore even though our included participant group has the strength of being a purer sample without co-morbidities, they might not be clinically representative for the overall polysubstance population. In conclusion, our results point to an overall disruption of white matter integrity in cocaine polysubstance users versus controls in tracts connecting frontal-striatal and frontal-limbic areas. Future studies to both male and female polysubstance users are needed to clarify the specific involvement of these white matter tracts in drug abstinence.

Conflicts of interest

The authors declare that no conflicts of interest with respect to the research, authorship, and/or publication of this manuscript are present.

Author contribution

AVG and MPG designed and collected data for the study. DVS, RW, AVG and AC contributed to the experimental design. DVS and AC undertook the statistical analysis and DVS wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

References

- Abé, C., Mon, A., Hoefer, M.E., Durazzo, T.C., Pennington, D.L., Schmidt, T.P., Meyerhoff, D.J., 2013. Metabolic abnormalities in lobar and subcortical brain regions of abstinent polysubstance users: magnetic resonance spectroscopic imaging. *Alcohol* 48, 543–551.
- Agabio, R., Campesi, I., Pisanu, C., Gessa, G.L., Franconi, F., 2016. Sex differences in substance use disorders: focus on side effects. *Addict. Biol.* 12, 1030–1042.
- Albertson, D.N., Pruetz, B., Schmidt, C.J., Kuhn, D.M., Kapatos, G., Bannon, M.J., 2004. Gene expression profile of the nucleus accumbens of human cocaine abusers: evidence for dysregulation of myelin. *J. Neurochem.* 88, 1211–1219.
- Alexander, A.L., Lee, J.E., Lazar, M., Field, A.S., 2007. Diffusion tensor imaging of the brain. *Neurotherapeutics* 4, 316–329.
- Baeza-Loya, S., Velasquez, K.M., Molfese, D.L., Viswanath, H., Curtis, K.N., Thompson-Lake, D.G.Y., Baldwin, P.R., Ellmore, T.M., De La Garza, R., Salas, R., 2016. Anterior cingulum white matter is altered in tobacco smokers. *Am. J. Addict.* 25, 210–214.
- Barrós-Loscertales, A., Garavan, H., Bustamante, J.C., Ventura-Campos, N., Llopis, J.J., Belloch, V., Parcet, M.A., Ávila, C., 2011. Reduced striatal volume in cocaine-dependent patients. *Neuroimage* 56, 1021–1026.
- Barrett, S.P., Darredeau, C., Pihl, R.O., 2006. Patterns of simultaneous polysubstance use in drug using university students. *Hum. Psychopharmacol.* 21, 255–263.
- Behrens, T.E.J., Woolrich, M.W., Jenkinson, M., Johansen-Berg, H., Nunes, R.G., Clare, S., Matthews, P.M., Brady, J.M., Smith, S.M., 2003. Characterization and propagation of uncertainty in diffusion-weighted MR imaging. *Magn. Reson. Med.* 50, 1077–1088.
- Bell, R.P., Foxe, J.J., Nierenberg, J., Hoptman, M.J., Garavan, H., 2011. Assessing white matter integrity as a function of abstinence duration in former cocaine-dependent individuals. *Drug Alcohol Depend.* 114, 159–168.
- Betz, J., Zhuo, J., Roy, A., Shanmuganathan, K., Gullapalli, R.P., 2012. Prognostic value of diffusion tensor imaging parameters in severe traumatic brain injury. *J. Neurotrauma* 29, 1292–1305.
- Bjork, J.M., Gilman, J.M., 2014. The effects of acute alcohol administration on the human brain: insights from neuroimaging. *Neuropharmacology* 84, 101–110.
- Bochand, L., Nandrino, J.L., 2010. [Levels of emotional awareness in alcohol-dependent patients and abstinent alcoholics]. *L'Encephale* 36, 334–339.
- Buttner, A., 2012. Neuropathological alterations in cocaine abuse. *Curr. Med. Chem.* 19, 5597–5600.
- Cercignani, M., Inglese, M., Pagani, E., Comi, G., Filippi, M., 2001. Mean diffusivity and fractional anisotropy histograms of patients with multiple sclerosis. *Am. J. Neuroradiol.* 22, 952–958.
- Connor, J.P., Gullo, M.J., White, A., Kelly, A.B., 2014. Polysubstance use: diagnostic challenges, patterns of use and health. *Curr. Opin. Psychiatry* 27, 269–275.
- Di Sclafani, V., Tolou-Shams, M., Price, L.J., Fein, G., 2002. Neuropsychological performance of individuals dependent on crack-cocaine, or crack-cocaine and alcohol, at 6 weeks and 6 months of abstinence. *Drug Alcohol Depend.* 66, 161–171.
- Downey, L.E., Mahoney, C.J., Buckley, A.H., Golden, H.L., Henley, S.M., Schmitz, N., Warren, J.D., 2015. White matter tract signatures of impaired social cognition in frontotemporal lobar degeneration. *Neuroimage Clin.* 8, 640–651.
- Dutra, L., Stathopoulou, G., Basden, S.L., Leyro, T.M., Powers, M.B., Otto, M.W., 2008. A meta-analytic review of psychosocial interventions for substance use disorders. *Am. J. Psychiatry* 165, 179–187.
- Everitt, B.J., Robbins, T.W., 2016. Drug addiction: updating actions to habits to compulsions ten years on. *Ann. Rev. Psychol.* 67, 23–50.
- Galani, R., Obis, S., Coutureau, E., Jarrard, L., Cassel, J.C., 2002. A comparison of the effects of fimbria-fornix, hippocampal, or entorhinal cortex lesions on spatial reference and working memory in rats: short versus long postsurgical recovery period. *Neurobiol. Learn. Memory* 77, 1–16.
- Garavan, H., Brennan, K.L., Hester, R., Whelan, R., 2013. The neurobiology of successful abstinence. *Curr. Opin. Neurobiol.* 23, 668–674.
- Ghashghaei, H.T., Barbas, H., 2002. Pathways for emotion: interactions of prefrontal and anterior temporal pathways in the amygdala of the rhesus monkey. *Neuroscience* 115, 1261–1279.
- Hanlon, C.A., Dufault, D.L., Wesley, M.J., Porrino, L.J., 2011. Elevated gray and white matter densities in cocaine abstinence compared to current users. *Psychopharmacology (Berl)* 218, 681–692.
- Hanlon, C.A., Beveridge, T.J., Porrino, L.J., 2013. Recovering from cocaine: insights from clinical and preclinical investigations. *Neurosci. Biobehav. Rev.* 37, 2037–2046.
- Iudicello, J.E., Woods, S.P., Vigil, O., Cobb Scott, J., Cherner, M., Heaton, R.K., HIV Neurobehavioral Research Center (HNRC) Group, 2010. Longer term improvement in neurocognitive functioning and affective distress among methamphetamine users who achieve stable abstinence. *J. Clin. Exp. Neuropsychol.* 32, 704–718.
- Jacobus, J., Squeglia, L.M., Meruelo, A.D., Castro, N., Brumback, T., Giedd, J.N., Tapert, S.F., 2015. Cortical thickness in adolescent marijuana and alcohol users: a three-year prospective study from adolescence to young adulthood. *Dev. Cogn. Neurosci.* 16, 101–109.
- Jalbrzikowski, M., Villalon-Reina, J.E., Karlsgodt, K.H., Senturk, D., Chow, C., Thompson, P.M., Bearden, C.E., 2014. Altered white matter microstructure is associated with social cognition and psychotic symptoms in 22q11.2 microdeletion syndrome. *Front. Behav. Neurosci.* 8, 393.
- Kaag, A.M., Wingen, G.A., Caan, M.W., Homberg, J.R., Brink, W., Reneman, L., 2016. White matter alterations in cocaine users are negatively related to the number of additionally (ab) used substances. *Addict. Biol.*, Epub ahead of print.
- Kahnt, T., Park, S.Q., Cohen, M.X., Beck, A., Heinz, A., Wrase, J., 2009. Dorsal striatal-midbrain connectivity in humans predicts how reinforcements are used to guide decisions. *J. Cogn. Neurosci.* 21, 1332–1345.
- Kendler, K.S., Prescott, C.A., Myers, J., Neale, M.C., 2003. The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. *Arch. Gen. Psychiatry* 60, 929–937.
- Koob, G.F., Volkow, N.D., 2009. Neurocircuitry of addiction. *Neuropsychopharmacology* 32, 217–238.
- Kopell, B.H., Greenberg, B.D., 2008. Anatomy and physiology of the basal ganglia: implications for DBS in psychiatry. *Neurosci. Biobehav. Rev.* 32, 408–422.
- Kosten, T.R., Scanley, B.E., Tucker, K.A., Oliveto, A., Prince, C., Sinha, R., Potenza, M., Skudlarski, P., Wexler, B.E., 2005. Cue-induced brain activity changes and relapse in cocaine-dependent patients. *Neuropsychopharmacology* 31, 644–650.
- Lim, K.O., Wozniak, J.R., Mueller, B.A., Franc, D.T., Specker, S.M., Rodriguez, C.P., Silverman, A.B., Rotrosen, J.P., 2008. Brain macrostructural and microstructural abnormalities in cocaine dependence. *Drug Alcohol Depend.* 92, 164–172.
- Liu, J., Lewohl, J.M., Dodd, P.R., Randall, P.K., Harris, R.A., Mayfield, R.D., 2004. Gene expression profiling of individual cases reveals consistent transcriptional changes in alcoholic human brain. *J. Neurochem.* 90, 1050–1058.
- Ma, L., Hasan, K.M., Steinberg, J.L., Narayana, P.A., Lane, S.D., Zuniga, E.A., Kramer, L.A., Moeller, F.G., 2009. Diffusion tensor imaging in cocaine dependence: regional effects of cocaine on corpus callosum and effect of cocaine administration route. *Drug Alcohol Depend.* 104, 262–267.
- Manzardo, A.M., McGuire, A., Butler, M.G., 2015. Clinically relevant genetic biomarkers from the brain in alcoholism with representation on high resolution chromosome ideograms. *Gene* 560, 184–194.
- Matochik, J.A., London, E.D., Eldreth, D.A., Cadet, J.L., Bolla, K.I., 2003. Frontal cortical tissue composition in abstinent cocaine abusers: a magnetic resonance imaging study. *Neuroimage* 19, 1095–1102.
- Moeller, F.G., Hasan, K.M., Steinberg, J.L., Kramer, L.A., Dougherty, D.M., Santos, R.M., Valdes, I., Swann, A.C., Barratt, E.S., Narayana, P.A., 2005. Reduced anterior corpus callosum white matter integrity is related to increased

- impulsivity and reduced discriminability in cocaine-dependent subjects: diffusion tensor imaging. *Neuropsychopharmacology* 30, 610–617.
- Moreno-López, L., Catena, A., Fernández-Serrano, M.J., Delgado-Rico, E., Stamatakis, E.A., Pérez-García, M., Verdejo-García, A., 2012. Trait impulsivity and prefrontal gray matter reductions in cocaine dependent individuals. *Drug Alcohol Depend.* 125, 208–214.
- Nakamura-Palacios, E.M., Lopes, I.B.C., Souza, R.A., Klauss, J., Batista, E.K., Conti, C.L., Moscon, J.A., de Souza, R.S.M., 2016. Ventral medial prefrontal cortex (vmPFC) as a target of the dorsolateral prefrontal modulation by transcranial direct current stimulation (tDCS) in drug addiction. *J. Neural Transm. (Vienna)* (Epub ahead of print).
- Nichols, T., Hayasaka, S., 2003. Controlling the familywise error rate in functional neuroimaging: a comparative review. *Stat. Methods Med. Res.* 12, 419–446.
- Nielsen, D.A., Huang, W., Hamon, S.C., Maili, L., Witkin, B.M., Fox, R.G., Cunningham, C.A., Moeller, F.G., 2012. Forced abstinence from cocaine self-administration is associated with DNA methylation, changes in myelin genes in the corpus callosum: a preliminary study. *Front. Psychiatry* 3, 10–3389.
- Olson, I.R., Plotzker, A., Ezzyat, Y., 2007. The enigmatic temporal pole: a review of findings on social and emotional processing. *Brain* 130, 1718–1731.
- Pace-Schott, E.F., Morgan, P.T., Malison, R.T., Hart, C.L., Edgar, C., Walker, M., Stickgold, R., 2008. Cocaine users differ from normals on cognitive tasks which show poorer performance during drug abstinence. *Am. J. Drug Alcohol Abuse* 34, 109–121.
- Passingham, R.E., Stephan, K.E., Köster, R., 2002. The anatomical basis of functional localization in the cortex. *Nat. Rev. Neurosci.* 3, 606–616.
- Paulus, M.P., Tapert, S.F., Schuckit, M.A., 2005. Neural activation patterns of methamphetamine-dependent subjects during decision making predict relapse. *Arch. Gen. Psychiatry* 62, 761–768.
- Pennington, D.L., Durazzo, T.C., Schmidt, T.P., Abé, C., Mon, A., Meyerhoff, D.J., 2015. Alcohol use disorder with and without stimulant use: brain morphometry and its associations with cigarette smoking, cognition, and inhibitory control. *PLoS One* 10, e0122505.
- Pisner, D.A., Smith, R., Alkozei, A., Klimova, A., Killgore, W.D., 2016. Highways of the emotional intellect: white matter microstructural correlates of an ability-based measure of emotional intelligence. *Soc. Neurosci.* (Epub ahead of print).
- Redonnet, B., Chollet, A., Fombonne, E., Bowes, L., Melchior, M., 2012. Tobacco, alcohol, cannabis and other illegal drug use among young adults: the socioeconomic context. *Drug Alcohol Depend.* 121, 231–239.
- Romero, M.J., Asensio, S., Palau, C., Sanchez, A., Romero, F.J., 2010. Cocaine addiction: diffusion tensor imaging study of the inferior frontal and anterior cingulate white matter. *Psychiatr. Res. Neuroimag.* 181, 57–63.
- SPSS, S., 1996. for Windows Release 10.01. SPSS Inc Chicago, IL, USA.
- Schulte, M.H., Cousijn, J., den Uyl, T.E., Goudriaan, A.E., van den Brink, W., Veltman, D.J., Wiers, R.W., 2014. Recovery of neurocognitive functions following sustained abstinence after substance dependence and implications for treatment. *Clin. Psychol. Rev.* 34, 531–550.
- Smith, S.M., Nichols, T.E., 2009. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage* 44, 83–98.
- Smith, S.M., Jenkinson, M., Woolrich, M.W., Beckmann, C.F., Behrens, T.E., Johansen-Berg, H., Bannister, P.R., De Luca, M., Flitney, D.E., Niazy, R.K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J.M., Matthews, P.M., 2004. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* 23, 208–219.
- Smith, S.M., Jenkinson, M., Johansen-Berg, H., Rueckert, D., Nichols, T.E., Mackay, C.E., Watkins, K.E., Ciccarelli, O., Cader, M.Z., Matthews, P.M., Behrens, T.E., 2006. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *Neuroimage* 31, 1487–1505.
- Smith, H.R., Beveridge, T.J., Nader, M.A., Porrino, L.J., 2014. Regionally-specific alterations in myelin proteins in nonhuman primate white matter following prolonged cocaine self-administration. *Drug Alcohol Depend.* 137, 143–147.
- Suzanne, M., Kril, J.J., 2014. Human alcohol-related neuropathology. *Acta Neuropathol.* 127, 71–90.
- Tanabe, J., Tregellas, J.R., Dalwani, M., Thompson, L., Owens, E., Crowley, T., Banich, M., 2009. Medial orbitofrontal cortex gray matter is reduced in abstinent substance-dependent individuals. *Biol. Psychiatry* 65, 160–164.
- Verdejo-García, A.J., López-Torrecillas, F., de Arcos, F.A., Pérez-García, M., 2005. Differential effects of MDMA, cocaine, and cannabis use severity on distinctive components of the executive functions in polysubstance users: a multiple regression analysis. *Addict. Behav.* 30, 89–101.
- Vogt, B.A., Vogt, L., Laureys, S., 2006. Cytology and functionally correlated circuits of human posterior cingulate areas. *Neuroimage* 29, 452–466.
- Woo, C.W., Krishnan, A., Wager, T.D., 2014. Cluster-extent based thresholding in fMRI analyses: pitfalls and recommendations. *Neuroimage* 91, 412–419.
- Xu, J., DeVito, E.E., Worhunsky, P.D., Carroll, K.M., Rounsaville, B.J., Potenza, M.N., 2010. White matter integrity is associated with treatment outcome measures in cocaine dependence. *Neuropsychopharmacology* 35, 1541–1549.
- Yu, D., Yuan, K., Zhang, B., Liu, J., Dong, M., Jin, C., Luo, L., Zhai, J., Zhao, L., Zhao, Y., Gu, Y., Xue, T., Liu, X., Lu, X., Qin, W., Tian, J., 2015. White matter integrity in young smokers: a tract-based spatial statistics study. *Addict. Biol.* 21, 679–687.
- Zhang, A., Leow, A., Ajilore, O., Lamar, M., Yang, S., Joseph, J., Medina, J., Zhan, L., Kumar, A., 2011. Quantitative tract-specific measures of uncinate and cingulum in major depression using diffusion tensor imaging. *Neuropsychopharmacology* 37, 959–967.