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White matter alterations in cocaine users are negatively related to the number of additionally (ab)used substances

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ABSTRACT

Diffusion tensor imaging studies have provided evidence for white matter (WM) alterations in cocaine users. While polysubstance use is a widespread phenomenon among cocaine users, its role in WM alterations in cocaine users is currently unknown. This study examined the relation between the number of substances that are used(cocaine, alcohol and marijuana) and WM alterations in 67 male non-drug users and 67 male regular cocaine users, who were classified into five groups based on the number of used substances. Diffusion-weighted images were acquired on a 3.0 T magnetic resonance imaging scanner. Using tract-based spatial statistics we demonstrated that there was a negative relation between the number of used substances and fractional anisotropy, a global measure of WM integrity. Also, we demonstrated a positive relation between the number of used substance and radial diffusivity within the prefrontal lobe, suggesting an increase in demyelination with the number of used substances. We did not find a dose-effect between the level of substance use and WM alterations. The results of the current study may reflect the presence of a pre-existing vulnerability to polysubstance use resulting from prefrontal WM abnormalities and related impaired cognitive control although WM alterations because of polysubstance use cannot be fully excluded. This study is an important first step in understanding the problems related to polysubstance use among cocaine users.

Keywords cocaine, DTI, polysubstance use, prefrontal cortex.

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INTRODUCTION

Cocaine (ab)use is associated with neurotoxic molecular and metabolic changes in the brain. The mechanism by which cocaine may cause neural damage is suggested to be complex and seems to involve interactions with several neurotransmitter systems and modulation of transcription factors (Pereira *et al.* 2015). Recent diffusion tensor imaging (DTI) studies have demonstrated cortical white matter (WM) alterations in cocaine users (Lim *et al.* 2002, 2008; Moeller *et al.* 2005, 2007; Ma *et al.* 2009; Lane *et al.* 2010; Romero *et al.* 2010). However, while the majority of cocaine users are polysubstance users, using more than one type of drug simultaneously or at different moments close in time, mostly alcohol and cannabis (European Monitoring Centre for Drugs and Drug Addiction 2009; Connor *et al.* 2014), very little attention has been paid to the role of polysubstance use in brain alterations in cocaine users. There are several reasons for people to use multiple substances rather than a single substance, ranging from enhancing the effect of one substance on the central nervous system to ameliorating the adverse effect of one substance with another substance (Connor *et al.* 2014). However, the use of different types of substances within a short period of time, especially cocaine and alcohol may also lead to more severe neurotoxic effects (Pereira *et al.* 2015). In addition, polysubstance users are at elevated risk of poor treatment outcome (Connor *et al.* 2014). Unfortunately, most DTI studies in cocaine users failed to exclude or explore the effect of polysubstance use within this population. Therefore, it is presently

unknown to what extent WM alterations previously demonstrated in cocaine users are related to cocaine use *per se* or to polysubstance use within this population.

There are some studies on the effect of polysubstance use in patients with an alcohol use disorder. In contrast to their expectations, several of these studies showed that heavy alcohol use, in combination with marijuana use (Hamelink *et al.* 2005; Medina *et al.* 2007; Jacobus *et al.* 2009) or amphetamine use (Lawyer *et al.* 2010; Mon *et al.* 2014) was related to fewer abnormalities in gray and WM structures compared with heavy alcohol use alone. Some of these studies postulate that the absence of gray and WM alterations in alcohol users with comorbid marijuana use could be the result of the neuroprotective properties of the cannabinoids in marijuana (Hamelink *et al.* 2005), whereas others claim that gray and WM alterations in polysubstance users might be masked as a result of tissue inflammation and/or reactive astrogliosis induced by amphetamines (Chang *et al.* 2007). In a recent study we demonstrated a similar attenuating effect of marijuana use on cortical gray matter abnormalities in cocaine users (Kaag *et al.* 2014). However, larger studies on polysubstance use in cocaine users are currently lacking. Hence, it remains unclear whether the number of other substances used by cocaine users is associated with a linear increase in neurotoxicity, or that other relations exist, including non-linear amplifications or reductions of the harmful effects on brain structure (O'Neill 2015).

In the current study we use DTI to investigate the relation between polysubstance use and WM microstructure in two general population samples including 67 healthy non-drug (ND) users and 67 cocaine users, with most of the latter group also using marijuana, large amounts of alcohol or a combination of both. All subjects were classified into groups based on their self-reported substance use, resulting in six different groups including non-substance users, light drinkers, cocaine users, cocaine users who also use marijuana, cocaine users who use large amounts of alcohol and cocaine users who use both marijuana and large amounts of alcohol.

Three different relationships between the number of substances that were consumed and WM alterations can be hypothesized: First, we can hypothesize that the negative effects of alcohol and/or marijuana use in addition to cocaine use will simply add up, resulting in a linear relation between the number of substances used and the loss in WM integrity. Second, if the negative effects of using cocaine are amplified or attenuated by the use of alcohol and/or marijuana, we expect an exponential relationship between the number of substances used and the loss of WM integrity. These models were used to investigate the relationship between poly-substance use and total fractional anisotropy (FA) across the whole brain. In addition we performed, voxel-wise analyses using tract-based spatial statistics (TBSS) to test for regional specific effects of polysubstance on WM integrity. Although FA is a frequently used measure for WM integrity, being sensitive to several neural features including axon size, density and myelination (Beaulieu 2002; Mori & Zhang 2006; Wozniak & Lim 2006), it is not very specific. Therefore, we also investigated the relationship between polysubstance use and mean diffusivity (MD), which can be decomposed in axial diffusivity (AD; a measure sensitive for axonal integrity and deletion), and radial diffusivity (RD; a measure sensitive for myelin integrity) (Song *et al.* 2002, 2005; Sun *et al.* 2006). Finally, simple regression analyses were conducted to explore the dose–response relationship between the level of substance use (cocaine, alcohol and marijuana) and WM integrity.

METHODS

Participants

A total of 67 regular cocaine users and 67 ND users were included in this study. All participants were males (aged 18–49) recruited through local advertisement in the greater Amsterdam area, the Netherlands. All cocaine users were actively using cocaine and currently non-treatment seeking. Cocaine users were included when using cocaine at least once per week for a minimum period of 6 months. All participants were psychiatrically evaluated using the MINI International Neuropsychiatric Interview (Sheehan *et al.* 1998). Exclusion criteria for all participants were as follows: major medical or neurological disease, lifetime history of psychotic or bipolar disorder or the presence of a contraindication to magnetic resonance imaging scanning and psychotropic medication use. Non-drug using participants were also excluded if they used or had been using illicit drugs, more than 21 units of alcohol per week or nicotine. Alcohol, cocaine and cannabis use in the 6 months before study inclusion was quantified using the timeline-follow back procedure (Sobell & Sobell 1992). We relied on self-reported measures of substance use, for all the reported substances, including nicotine.

The study was approved by the Ethical Review Board of the Academic Medical Centre of the University of Amsterdam, the Netherlands. All subjects gave written informed consent.

Characterization of polysubstance use severity

In accordance with previous literature, cocaine users were classified as heavy alcohol users if they used at least 21 units of alcohol per week (i.e. Griffiths 1996) and as marijuana users if they used marijuana at least once a week (i.e. Medina *et al.* 2007; Buckner & Schmidt

2008). Using these criteria we ended up with four different subgroups of cocaine users: cocaine users who only used cocaine (C, $n = 23$), cocaine users who were heavy alcohol users (CA, $n = 17$), cocaine users who also used marijuana (CM, $n = 13$) and cocaine users who were both heavy alcohol and marijuana users (CAM, $n = 14$). The majority of ND using participants were light alcohol drinkers, i.e. less than 21 units of alcohol per week (ND, $n = 57$), but a small portion did not drink alcohol at all. This last group of non-alcohol or drug users was considered as a separate group in the analysis (NAD, $n = 10$). Using this classification we ended up with six groups with an increase in the number of substances that were used, but matched on the use of other substance(s). Because the majority of cocaine users also smoked nicotine, whereas nicotine use was an exclusion criterion for the ND users, classifying nicotine smokers in a separate subgroup would result in too small groups. Therefore, subgroups of smoking and non-smoking cocaine users are missing in the current study. Because the CM and CA group both use two types of substances, these groups were considered as one group in all analyses. It should be noted that we did not include a group of 'pure' heavy alcohol users. Substance use characteristics can be found in Table 1.

Magnetic resonance imaging acquisition

Images were acquired on a 3 T whole body magnetic resonance imaging scanner (Phillips Achieva), with a 32 channel SENSE head coil. Three-dimensional T1-weighted images were acquired with the following parameters: repetition time (TR) = 8.24 ms, echo time (TE) = 3.79 ms, flip angle = 8°, slice thickness = 1 mm, scan resolution = 240 mm × 240 mm, field-of-view (FOV) (anterior–posterior/feet–head/right–left) = 240/240/220 mm and voxel size = 1 mm. A single shot diffusion-weighted imaging (DWI) recording was acquired with the following parameters: TR = 7542 ms, TE = 86 ms, number of slices = 60, FOV = 224 × 224 × 120, acquisition matrix = 112 × 112, slice thickness = 2 mm, along 32 gradient directions, $b = 1000$ second/mm^2 with a non-diffusion weighted baseline.

Image processing

The pre-processing of the DTI data was executed on the Dutch Grid, using a web interface to the e-Bioinfra gateway (Olabarriaga *et al.* 2010; Shahand *et al.* 2011). Head motion and deformations induced by eddy currents were corrected for by an affine registration of the DTI data to the non-diffusion weighted image. The gradient directions were corrected by the rotation component of the transformation. The DWIs were resampled isotropically. Rician noise in the DWIs was reduced by an adaptive noise filtering method (Caan *et al.* 2010). Diffusion tensors were estimated in a non-linear least squares sense. Mean WM FA, AD, RD and MD were calculated.

Statistical analysis of diffusion measures

Univariate regression analysis was used to test the relation between mean FA and the number of substances used, with age included as a covariate of non-interest. To test whether the negative effect of the number of used substances simply adds up, attenuates or amplifies the negative effect of cocaine, one linear and two non-linear models were tested: a linear decay model, a (slow) resilient exponential decay models (EDM) and a (fast) sensitive exponential decay model. In the (slow) resilient EDM model, the WM damage because of the first one or two substances of abuse is small, but this quickly (exponentially) increases with the number of substances used. In the (fast) sensitive EDM model, the WM damage

Table 1 Substance use characteristics

	NAD	*ND*	*C*	*CA*	*CM*	*CAM*
	$n = 10$	$n = 57$	$n = 23$	$n = 17$	$n = 13$	$n = 14$
Age	35.3 (8.9)	31.5 (8.3)	31.3 (7.2)	31.7 (6.8)	29.6 (6.6)	35.0 (9.4)
IQ	101 (9.11)	105 (8.40)	101 (8.10)	99 (11.24)	101 (7.23)	101 (7.44)
Units of alcohol per week[a]	—	3.8 (5.05)	9.7 (10.0)	47.4 (35.8)	8.8 (11.0)	36.9 (28.4)
Grams of cocaine per week[b]	—	—	1.4 (1.2)	1.6 (1.4)	1.6 (1.5)	2.2 (1.7)
Occasions of marijuana use per week[c]	—	—	0.0 (0.04)	0.0 (0.1)	6.9 (6.4)	9.7 (16.3)
Percent smokers	0	0	73.9%	70.6%	84.6%	100%
Number of cigarettes per day	—	—	13.5 (12)	20.0 (17.25)	15.0 (14)	16.3 (18.13)

All values represent mean ± standard deviation. NAD: no alcohol or drug use, ND: alcohol but no drug use, C: cocaine use, CA: cocaine and heavy alcohol use, CM: cocaine and marijuana use, CAM: cocaine, heavy alcohol and marijuana use. [a]ND differed in alcohol use from all other groups, but as intended, C and CM were matched on alcohol use. [b]Group C, CA, CM and CAM were matched on cocaine and nicotine use. [c]Group CM and CAM were matched on marijuana use.

because of the first one or two substances of abuse is already substantial, but this additional effect becomes smaller (exponentially decrease) with the number of substances of abuse used. These models are described by the following formula's (Fig. 1a): $y = -ax + b$ for the linear decay model, $y = \mathrm{a} - \mathrm{b} * 2^{\mathrm{x}}$ for the slow resilient EMD and $y = -\mathrm{a} + \mathrm{b} * 2^{-\mathrm{x}}$ for the fast sensitive EMD, and give the contrast weights for each group, that were used in the DTI analyses to test the relationship between the number of substances that were used and WM integrity. The x in these models represent the number of substances used, ranging from 0 for the NAD group, 1 for the ND group, 2 for the C group, 3 for the CA and CM group and 4 for the CAM group. In these formulas a and b are constants that are chosen in such way that the total contrast coefficient equals zero, resulting in the following formulas: $y = -x + b$ for the linear mode, $y = 1{,}55–0{,}25 * 2^{\mathrm{x}}$ for the slow resilient EDM and $y = -1{,}55 + 4 * 2^{-\mathrm{x}}$ for the fast sensitive EDM. For the linear model the resulting contrast weights (y values) were 2, 1, 0, −1 and −2; for the slow resilient EDM these y values were 2.45, 0.45, −0.55, −1.05 and −1.3; and for the fast sensitive EDM these y values were 1.3, 1.05, 0.55, −0.45 and −2.45 (Fig. 1b). The model with the strongest effect size on mean diffusion measures was used to test the relation between regional FA, MD, RD and AD using TBSS. *F*-tests were applied to test for significant differences between groups. Only in case of a significant group effect, these *F*-tests were followed with a pair-wise comparison between groups. Because cocaine use was strongly confounded with nicotine use we could, however, not include nicotine use as a covariate in the analysis, but the effects of the level of nicotine, alcohol, cocaine and marijuana use on FA, MD, RD or AD was tested in four regression analysis with age included as a covariate of non-interest.

Voxel-wise statistical analysis of DTI data

Regional specific effects were tested using TBSS (Smith *et al.* 2006) implemented in the FMRIB Software Library 4.1.6 (University of Oxford, Oxford, UK). All FA data were then aligned into a common space using the nonlinear registration tool FNIRT, which uses a b-spline representation of the registration warp field (Rueckert *et al.* 1999). Subsequently, the mean FA image was created and skeletonized to create a mean FA skeleton, which represents the centers of all tracts. Each subject's aligned FA data were then projected onto this skeleton, and the resulting data fed into voxel-wise cross-subject statistics.

The model was assessed using Randomize, a TBSS statistical tool that computes non-parametric permutations using the generalized linear model. Threshold-free cluster enhancement was used to identify 'clusters' of significant results in the dataset(Nichols & Ph 2010). The number of permutations was set to 500 using age as a confound regressor. Significance values were reported at $p < 0.05$ with correction for false discovery rate (Zalesky 2011). The principal WM tracts were derived from the John Hopkins University WM probabilistic tractography and

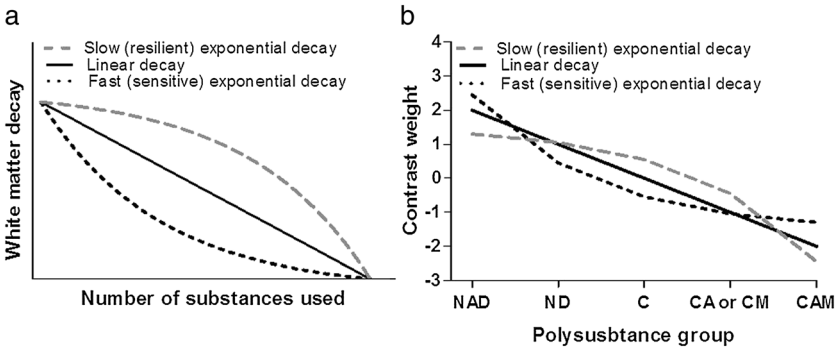

Figure 1 (a) The models to test the relation between the number of substances used and FA value. This plot is a graphical illustration of the three different models used to test the relation between the number of used substances and FA value. The linear decay model: $y = -ax + \mathrm{b}$, the slow resilient exponential decay model: $y = a - b^{\mathrm{x}}$ and the fast sensitive exponential decay model: $y = -a + b^{-\mathrm{x}}$. (b) The x in these formulas represent the number of substances used whereas the a and b values in these formulas are constants that are chosen in such way that the total contrast coefficients (y) equal zero. The resulting y-values were 2, 1, 0, −1 and −2 for the linear decay model, 2.45, 0.45, −0.55, −1.05 and −1.3 for the fast sensitive exponential decay model and 1.3, 1.05, 0.55, −0.45 and −2.45 for the slow resilient exponential decay model

ICBM–DTI −81 WM label atlases (Wakana *et al.* 2007; Hua *et al.* 2008).

RESULTS

Substance use characteristics

All groups were of similar age ($\chi^2 = 4.1$, $p = 0.53$). While there was a significant difference in alcohol consumption between the ND using controls and both cocaine using groups with moderate alcohol use (C and CM) ($\chi^2 = 22.1$, $p < 0.001$), there was no significant difference in alcohol consumption between these two cocaine using groups ($\chi^2 < 0.001$, $p = 0.99$). There was no significant difference in cocaine or nicotine consumption between all four cocaine using groups ($\chi^2 = 3.9$, $p = 0.27$, $\chi^2 = 4.2$, $p = 0.239$, respectively), nor did the CM and CAM group differ in marijuana use ($\chi^2 = 1.5$, $p = 0.26$). Thus, the classification of participants led to six different groups that as intended differed with the previous group in one substance but were relatively well matched on other substance(s).

Relation between the number of substances used and white matter diffusion measures

All three models that tested the relationship between FA and the number of used substances were significant. However, the linear model had the largest effect size ($F_{1,128} = 6.86$, $p = 0.01$, partial $\eta^2 = 0.051$), the slow resilient exponential model had the smallest effect size ($F_{1,128} = 5.92$, $p = 0.02$, partial $\eta^2 = 0.040$) and the effect size of the fast sensitive model was in between the other two effect sizes ($F_{1,128} = 6.40$, $p = 0.02$, partial $\eta^2 = 0.048$). The linear model also showed a significant relation between the number of substances used and radial diffusivity ($F_{1,128} = 4.07$,$p = 0.046$, $\eta^2 = 0.031$). The model was non-significant for MD or AD.

Although the results suggest that the relation between the number of substances that were used and FA value is best described by a linear model, the effect sizes of the models are small and the difference between the model fits of the three models is negligible. However, because there is no strong indication for a non-linear relationship between the number of used substances and FA value, we used the linear model as the most parsimonious one to present our results.

Relation between the number of substances used and regional diffusion measures

Tract-based spatial statistics was used to test the linear relation between the number of substances used and regional deviations in FA value across the WM tracts. The number of substances used showed a significant negative linear relation with FA in the majority of WM tracts, including the corpus callosum, the internal and external capsule, the corona radiatia, the thalamic radiation, the corticospinal tract, the cingulum (hippocampus and cingulate gyrus), the forcepts major, the inferior fronto-occipital fasciculus, the inferior longitudinal fasciculus, the superior longitudinal fasciculus and the uncinated fasciculus, all bilateral (Fig. 2).

The same linear model was used to test the regional effect of polysubstance use on MD, RD and AD. The TBSS analysis revealed a significant positive linear relation between the number of substances used and RD within two clusters that included the anterior thalamic radiation, the anterior and superior radiate, the cingulum, the inferior fronto-occipital fasciculus, the superior longitudinal fasciculus, uncinated fasciculus and the genu, body and splenium of the corpus callosum. Similar to our findings on total MD and AD, the TBSS analysis revealed no significant linear relation between the number of substances used and regional deviations in MD or AD. There were no significant group differences on FA, RD, MD or AD.

Relation between the levels of substance use and mean diffusion measures

Four regression analysis were performed to explore the relation between the level of nicotine, alcohol, cocaine and cannabis use per week and FA, MD, RD or AD value, with age included as a covariate of non-interest. None of these analyses showed a significant relation between the level of weekly nicotine, alcohol, cocaine or cannabis use, corrected for the use of the other substances and FA, MD, RD or AD, suggesting that there is no independent (linear) relation between the amount of one substance used and WM integrity measures. This further emphasizes that it is the number of substances used and not the level of one substance used that is related to WM abnormalities.

DISCUSSION

In this study we aimed to investigate the relation between polysubstance use and WM integrity in general population samples of ND users and regular cocaine users. Based on their self-reported use of alcohol, cocaine and marijuana, participants were categorized in one of six groups ranging from a group that did not use alcohol or drugs to a group that (ab)used cocaine, marijuana and alcohol. It was found that FA, a global measure of WM integrity, decreased with the number of substance used. In addition, TBSS analysis demonstrated that this negative relation between FA and the number of substances used was a widespread phenomenon throughout the brain. A

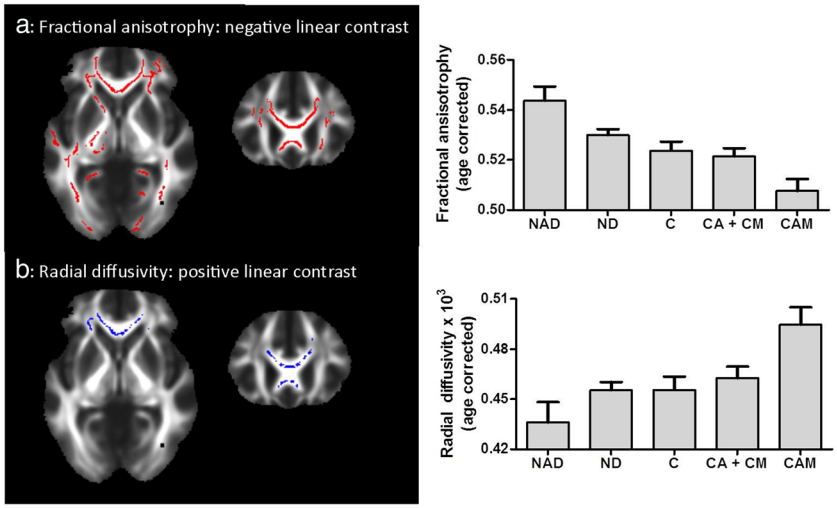

Figure 2 (a) The left figure displays the wide range of brain regions that show a negative linear relationship between the number of substances used and FA value. The plot on the right displays the mean FA value within the clusters that showed a significant linear relation between the number of substances used and FA value. (b) The left figure displays the prefrontal brain regions that show a positive linear relationship between the number of substance used and RD value. The plot on the right displays the mean RD value within the clusters that showed a significant linear relation between the number of substances used and RD value

similar TBSS analysis showed that there was a positive relation between the number of substances used and RD, a measure of demyelination, within the frontal WM, whereas there were no significant relations between the number of substances used and MD or AD. Finally, we found that the level of use of the various substances was not related to WM integrity.

Lower FA value is generally thought to reflect compromised fiber tracts (Lim & Helpern 2002). Thus, the current study suggests that WM integrity decreases with the number of substances used. Whereas FA is a global measure of WM integrity, which is highly sensitive to microstructural changes (Hasan *et al.* 2004), RD and AD are assumed to be more specific markers for demyelination (RD) and axonal morphology (AD) (Gulani *et al.* 2001; Song *et al.* 2002, 2005).

The positive relationship between the number of substances used and RD, but not AD, suggest that alterations in FA are assumed to be related to demyelination rather than to axonal damage (Song *et al.* 2002), although interpretations of DTI indices in terms of underlying neural pathologies should be carried out with caution (Wheeler-Kingshott & Cercignani 2009). While polysubstance use was associated with smaller FA value across a wide range of brain regions, polysubstance use was associated with increases in RD only in the frontal regions of the brain. Moreover, because RD has a much smaller signal-to-noise ratio compared with FA (Hasan *et al.* 2004), this may suggest that abnormalities in WM integrity related to polysubstance use are largest within the prefrontal regions, whereas changes in RD might be too small to be detected in the other parts of the brain.

The current study is not the first to demonstrate WM alterations in cocaine users, but it is, to our knowledge, the first to demonstrate that there is a negative relation between the number of other substances that are used by cocaine users and WM integrity. Our results are in line with previous studies in cocaine users (Lim *et al.* 2002, 2008; Moeller *et al.* 2005, 2007; Ma *et al.* 2009; Romero *et al.* 2010). While these studies claim that these WM alterations are an important characteristic of cocaine use disorder, the results of the current study suggest that WM alterations previously demonstrated within these population are at least partly explained by polysubstance use and not cocaine use per se.

In rats it has been demonstrated that cocaine exposure induces increases in neuro-inflammation and a decrease in myelin and FA within the corpus callosum (Narayana *et al.* 2009, 2014). It may therefore be speculated that the negative relation between the number of used substances and WM alterations is the result of a similar process. However, the absence of any significant relation between the level of substance use and diffusion measures suggests that polysubstance use *per se* is not the driving force behind the WM abnormalities in this population of cocaine users. An alternative explanation therefore could be that WM alterations represent a pre-existing vulnerability to use multiple drugs. Support for this statement comes from several DTI studies that demonstrated WM alterations in non-affected siblings of addicted individuals (Ersche *et al.* 2012) and subjects with a family history of alcohol addiction (Herting *et al.* 2010). Thus, WM alterations in polysubstance users may be a pre-existing vulnerability to deficient cognitive control, resulting in (uncontrolled) polysubstance use.

The prefrontal cortex (specifically the orbital frontal cortex) plays a critical part in addiction related behavior such as drug-craving, impulsive-compulsive behavior and impaired decision making (Crews & Boettiger 2009). Previous studies demonstrated that lower FA and higher RD in cocaine users are both related to impairments in decision-making (Lane *et al.* 2010) and

shorter self-reported abstinence after treatment (Xu *et al.* 2010). Although the current study design does not allow us to conclude that these WM alterations in polysubstance users are a cause or a consequence of polysubstance use, the finding of reduced WM integrity in cocaine users, who also use other substances compared with cocaine users who do not use other substances, could (partly) explain why treatment outcome is poorer in polysubstance users compared with single substance users (Dutra *et al.* 2008; Connor *et al.* 2014), because reduced WM integrity has been shown to be associated with early relapse in cocaine use (Xu *et al.* 2010).

Here we demonstrated that there is a negative relation between the number of used substances and WM alterations. However, previous studies in alcohol users suggested that co-morbid substance use is associated with less abnormalities in gray and WM structure (Hamelink *et al.* 2005; Medina *et al.* 2007; Jacobus *et al.* 2009; Lawyer *et al.* 2010; Mon *et al.* 2014). There are two important differences between our study and these previous studies in alcohol users that could explain these differences: first, we tested for a linear effect, whereas the other studies tested for a group effect. Another important difference is that the previous studies compared only three groups: ND users, users of one substance (alcohol) and users of two substances (alcohol + marijuana or amphetamine), whereas we added two additional groups to this comparison: non-alcohol or drug users and users of three types of substances (alcohol, cocaine and marijuana). Hence, this different methodological approach could explain why we did not find an attenuating effect of using marijuana in addition to cocaine. Alternatively, it could be that the added effect of polysubstance use on alcohol use is different from the added effect of polysubstance use on cocaine use. Therefore, we would like to stress the importance of studying the effect of polysubstance use in different populations (e.g. cocaine users, alcohol users and marijuana users) to further clarify the effect of using multiple substances on WM integrity.

In the current study we collected DTI data of 67 ND users and 67 regular cocaine users. This large population enabled us to investigate the effect of polysubstance use in a general population sample. While this is an important strength of the current study, using a naturalistic design like this also has several limitations. First, individuals were classified into groups based on the type and number of substances used. These groups were assigned a value that was used to calculate the contrast weights in the analysis, ranging from 0 (no alcohol or drugs use to 3 (heavy alcohol, cocaine and marijuana use). According to this classification, we assumed that the difference between not drinking alcohol at all and drinking less than 21 units of alcohol per week is similar to the difference between drinking less than 21 units per week and drinking less than 21 units per week in combination with using cocaine. Thus, the nature of the relation between the number of substances used and WM reductions could have been affected by these assigned values. Related to this, it should be noted that although the linear model showed the strongest effect size, the effect sizes of the three models were small and very similar. The conclusion on the linear nature relation between polysubstance use and WM integrity should therefore be taken with caution. Second, while we hoped that the different groups would differ from each other only in one type of substance used, ND users used significantly less alcohol compared with cocaine users. Third, it would have been more sophisticated if we had included subgroups of non-smokers, as none of the ND users smoked nicotine whereas the majority of cocaine users smoked nicotine. However, tobacco use was highly confounded with cocaine use. Therefore, it was not useful to include cigarette smokers with no other substance use as a separate group in the analysis. Although we did not find a significant relation between the number of cigarettes smoked and WM measures, the percent smokers among cocaine users who used both alcohol and marijuana was larger than among cocaine users who did not use heavy alcohol or marijuana. Previous studies have demonstrated both increases and decreases in FA related to smoking (Savjani *et al.* 2014; Yu *et al.* 2015). Hence, it could be that the use of cigarettes partly explains the relation between polysubstance use and WM integrity. Fourth, the two extreme groups (non-alcohol or drug users and cocaine users also using heavy alcohol and marijuana) were relatively small. However, the standard error of these measures was not larger within these groups compared with the other groups, suggesting that these smaller groups did not result in less reliable WM measures. Fifth, visual inspection of the data seems to suggest that there is a large difference between non-alcohol and drug users, and moderate users of alcohol (3.8 units per week). However, the observed difference is not significant, and we should be cautious with interpretations. Thus, although Fig. 2 seems to suggest that even moderate alcohol is strongly associated with a loss of WM integrity, it should not be interpreted as a consequence of moderate alcohol use, and it is also difficult to interpret it as some predisposing factors for moderate alcohol us. Future research with larger samples are needed to explain this finding. Finally, because this study was aimed to investigate WM alterations in cocaine users, we did not include non-cocaine using heavy alcohol users. Further research is needed to investigate whether the relation between the number of substance used and WM alterations also holds in non-cocaine users.

In summary, this study demonstrated that there is a negative relationship between the number of substances

used and WM alterations, mainly within the prefrontal lobe. Because this region is involved in drug-craving, impulsive–compulsive behavior and decision-making, these results may explain some of the difficulties encountered in the treatment of polysubstance users. In addition this study is an important first step in understanding how certain substances interact on a neural level. Further research is needed on the pathophysiology of these changes and the potential relevance of these findings in the treatment of polysubstance use.

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Author Contribution

Data were obtained by AMK, who prepared the first draft of the manuscript. AMK, MC and GvW analyzed the data. JRH, LR and WvdB actively participated in writing and revising the manuscript for publication.

References

Beaulieu C (2002) The basis of anisotropic water diffusion in the nervous system - a technical review. NMR Biomed 15:435–455.

Buckner JD, Schmidt NB (2008) Marijuana effect expectancies: relations to social anxiety and marijuana use problems. Addict Behav 33:1477–1483.

Caan M, Khedoe G, Poot D, Den Dekker A, Olabarriaga S, Grimbergen K, Van Vliet L, Vos F (2010) Adaptive noise filtering for accurate and precise diffusion estimation in fiber crossings. Lect Notes Comput Sci (including Subser Lect Notes Artif Intell Lect Notes Bioinformatics) 6361 LNCS:167–174.

Chang L, Alicata D, Ernst T, Volkow N (2007) Structural and metabolic brain changes in the striatum associated with methamphetamine abuse. Addiction 102:16–32.

Connor JP, Gullo MJ, White A, Kelly AB (2014) Polysubstance use. Curr Opin Psychiatry 27:269–275 Available at: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00001504-201407000-00006.

Crews FT, Boettiger CA (2009) Impulsivity, frontal lobes and risk for addiction. Pharmacol Biochem Behav 93:237–247. DOI: 10.1016/j.pbb.2009.04.018.

Dutra L, Dutra L, Stathopoulou G, Stathopoulou G, Basden SL, Basden SL, Leyro TM, Leyro TM, Powers MB, Powers MB, Otto MW, Otto MW (2008) A meta-analytic review of psychosocial interventions for substance use disorders. Am J Psychiatry 165:179–187 Available at: http://www.ncbi.nlm.nih.gov/pubmed/18198270.

Ersche KD, Simon Jones P, Williams GB, Turton AJ, Robbins TW, Bullmore ET (2012) Abnormal brain structure implicated in stimulant drug addiction. Science 335:601–605.

European Monitoring Centre for Drugs and Drug Addiction (2009) Polydrug use: patterns and responses.

Griffiths E (1996) Sensible drinking: doctors should stick with the independent medical advice. Br Med J 312:1 Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2349701/pdf/bmj00523-0005.pdf.

Gulani V, Webb a G, Duncan ID, Lauterbur PC (2001) Apparent diffusion tensor measurements in myelin-deficient rat spinal cords. Magn Reson Med 45:191–195.

Hamelink C, Hampson A, Wink D a, Eiden LE, Eskay RL (2005) Comparison of cannabidiol, antioxidants, and diuretics in reversing binge ethanol-induced neurotoxicity. J Pharmacol Exp Ther 314:780–788.

Hasan KM, Alexander AL, Narayana P a (2004) Does fractional anisotropy have better noise immunity characteristics than relative anisotropy in diffusion tensor MRI? An analytical approach. Magn Reson Med 51:413–417.

Herting MM, Schwartz D, Mitchell SH, Nagel BJ (2010) Abnormalities in youth with a family history of alcoholism. Alcohol Clin Exp Res 34:1590–1602.

Hua K, Zhang J, Wakana S, Jiang H, Li X, Reich DS, Calabresi P a, Pekar JJ, van Zijl PCM, Mori S (2008) Tract probability maps in stereotaxic spaces: analyses of white matter anatomy and tract-specific quantification. Neuroimage 39:336–347.

Jacobus J, McQueeny T, Bava S, Schweinsburg BC, Frank LR, Yang TT, Tapert SF (2009) White matter integrity in adolescents with histories of marijuana use and binge drinking. Neurotoxicol Teratol 31:349–355. DOI: 10.1016/j.ntt.2009.07.006.

Kaag AM, Crunelle CL, van Wingen G, Homberg J, van den Brink W, Reneman L (2014) Relationship between trait impulsivity and cortical volume, thickness and surface area in male cocaine users and non-drug using controls. Drug Alcohol Depend Available at: http://www.ncbi.nlm.nih.gov/pubmed/25278147 [Accessed November 3, 2014].

Lane SD, Steinberg JL, Ma L, Hasan KM, Kramer LA, Zuniga EA, Narayana PA, Moeller FG (2010) Diffusion tensor imaging and decision making in cocaine dependence. PLoS One 5:e11591.

Lawyer G, Bjerkan PS, Hammarberg A, Jayaram-Lindström N, Franck J, Agartz I (2010) Amphetamine dependence and comorbid alcohol abuse: associations to brain cortical thickness. BMC Pharmacol 10:5.

Lim KO, Choi SJ, Pomara N, Wolkin A, Rotrosen JP (2002) Reduced frontal white matter integrity in cocaine dependence: a controlled diffusion tensor imaging study. Psychiatry Interpers Biol Process 51:890–895. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12022962.

Lim KO, Helpern J a (2002) Neuropsychiatric applications of DTI - a review. NMR Biomed 15:587–593.

Lim KO, Wozniak JR, Mueller B a, Franc DT, Specker SM, Rodriguez CP, Silverman AB, Rotrosen JP (2008) Brain macrostructural and microstructural abnormalities in cocaine dependence. Drug Alcohol Depend 92:164–172. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2693223&tool=pmcentrez&rendertype=abstract [Accessed October 10, 2011].

Ma L, Hasan KM, Steinberg JL, Narayana PA, Lane SD, Zuniga EA, Kramer LA, Moeller FG (2009) Diffusion tensor imaging in cocaine dependence: regional effects of cocaine on corpus callosum and effect of cocaine administration route. Haut 104:262–267. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?.

Medina KL, Schweinsburg AD, Cohen-Zion M, Nagel BJ, Tapert SF (2007) Effects of alcohol and combined marijuana and alcohol use during adolescence on hippocampal volume and asymmetry. Neurotoxicol Teratol 29:141–152. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1821342&tool=pmcentrez&rendertype=abstract

Moeller FG, Hasan KM, Steinberg JL, Kramer L a, Valdes I, Lai LY, Swann AC, Narayana P a (2007) Diffusion tensor imaging

eigenvalues: preliminary evidence for altered myelin in cocaine dependence. Psychiatry Res 154:253–258 Available at: http://www.ncbi.nlm.nih.gov/pubmed/17321725 [Accessed October 28, 2014].

Moeller FG, Hasan KM, Steinberg JL, Kramer LA, Dougherty DM, Santos RM, Valdes I, Swann AC, Barratt ES, Narayana PA (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.

Mon A, Durazzo TC, Abe C, Gazdzinski S, Pennington D, Schmidt T, Meyerhoff DJ (2014) Structural brain differences in alcohol-dependent individuals with and without comorbid substance dependence. Drug Alcohol Depend 144:170–177. DOI: 10.1016/j.drugalcdep.2014.09.010.

Mori S, Zhang J (2006) Principles of diffusion tensor imaging and its applications to basic neuroscience research. Neuron 51:527–539.

Narayana P a, Ahobila-Vajjula P, Ramu J, Herrera J, Steinberg JL, Moeller FG (2009) Diffusion tensor imaging of cocaine-treated rodents. Psychiatry Res 171:242–251 Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2677684&tool=pmcentrez&rendertype=abstract [Accessed March 22, 2013].

Narayana P a, Herrera JJ, Bockhorst KH, Esparza-Coss E, Xia Y, Steinberg JL, Moeller FG (2014) Chronic cocaine administration causes extensive white matter damage in brain: diffusion tensor imaging and immunohistochemistry studies. Psychiatry Res 221:220–230 Available at: http://www.ncbi.nlm.nih.gov/pubmed/24507117 [Accessed October 28, 2014].

Nichols T, Ph D (2010) Mixed effects and group modeling for fMRI data.

O'Neill J (2015) Interaction of methamphetamine abuse, tobacco abuse, and gender in the brain. Am J Drug Alcohol Abuse 00:1–3. DOI: 10.3109/00952990.2015.1043439.

Olabarriaga SD, Glatard T, de Boer PT (2010) A virtual laboratory for medical image analysis. IEEE Trans Inf Technol Biomed 14:979–985.

Pereira RB, Andrade PB, Valentão P (2015) A comprehensive view of the neurotoxicity mechanisms of cocaine and ethanol. Neurotox Res 28:253–267. Available at: http://link.springer.com/10.1007/s12640-015-9536-x.

Romero MJ, Asensio S, Palau C, Sanchez A, Romero FJ (2010) Cocaine addiction: diffusion tensor imaging study of the inferior frontal and anterior cingulate white matter. Psychiatry Res 181:57–63 Available at: http://www.ncbi.nlm.nih.gov/pubmed/19959341 [Accessed October 5, 2011].

Rueckert D, Sonoda LI, Hayes C, Hill DL, Leach MO, Hawkes DJ (1999) Nonrigid registration using free-form deformations: application to breast MR images. IEEE Trans Med Imaging 18:712–721.

Savjani RR, Velasquez KM, Thompson-Lake DGY, Baldwin PR, Eagleman DM, De La Garza II R, Salas R (2014) Characterizing white matter changes in cigarette smokers via diffusion tensor imaging. Drug Alcohol Depend 145:134–142 Available at: http://linkinghub.elsevier.com/retrieve/pii/S0376871614018687.

Shahand S, Santcroos M, Mohammed Y, Korkhov V, Luyf A, van Kampen AHC, Olabarriaga SD (2011) Front-ends to biomedical data analysis on grids. In Proceedings of HealthGrid. Available at: http://amc-app1.amc.sara.nl/twiki/pub/EBioScience/EBioinfraGateUserDoc/ebioinfragate.pdf.

Sheehan D V, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar GC (1998) The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 59 (Suppl 2):22–33;quiz 34–57 Available at: http://www.ncbi.nlm.nih.gov/pubmed/9881538 [Accessed September 19, 2013].

Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, Watkins KE, Ciccarelli O, Cader MZ, Matthews PM, Behrens TEJ (2006) Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. Neuroimage 31:1487–1505.

Sobell L, Sobell MB (1992) Timeline follow-back: a technique for assessing self-reported alcohol consumption. In: Allen J, Litten RZ, eds. *Measuring Alcohol Consumption: Psycosocial and Biological Methods*, pp. 41–72. New Jersey: Humana Press.

Song S-K, Sun S-W, Ramsbottom MJ, Chang C, Russell J, Cross AH (2002) Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. Neuroimage 17:1429–1436.

Song SK, Yoshino J, Le TQ, Lin SJ, Sun SW, Cross AH, Armstrong RC (2005) Demyelination increases radial diffusivity in corpus callosum of mouse brain. Neuroimage 26:132–140.

Sun SW, Liang HF, Trinkaus K, Cross AH, Armstrong RC, Song SK (2006) Noninvasive detection of cuprizone induced axonal damage and demyelination in the mouse corpus callosum. Magn Reson Med 55:302–308.

Wakana S, Caprihan A, Panzenboeck MM, Fallon JH, Perry M, Gollub RL, Hua K, Zhang J, Jiang H, Dubey P, Blitz A, van Zijl P, Mori S (2007) Reproducibility of quantitative tractography methods applied to cerebral white matter. Neuroimage 36:630–644. DOI: 10.1016/j.neuroimage.2007.02.049.

Wheeler-Kingshott CAM, Cercignani M (2009) About "axial" and "radial" diffusivities. Magn Reson Med 61:1255–1260. DOI: 10.1002/mrm.21965.

Wozniak JR, Lim KO (2006) Advances in white matter imaging: a review of in vivo magnetic resonance methodologies and their applicability to the study of development and aging. Neurosci Biobehav Rev 30:762–774.

Xu J, DeVito EE, Worhunsky PD, Carroll KM, Rounsaville BJ, Potenza MN (2010) White matter integrity is associated with treatment outcome measures in cocaine dependence. Neuropsychopharmacology 35:1541–1549 Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2965037&tool=pmcentrez&rendertype=abstract [Accessed June 26, 2011].

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 Bioli:n/a – n/a DOI: 10.1111/adb.12237.

Zalesky A (2011) Moderating registration misalignment in voxelwise comparisons of DTI data: a performance evaluation of skeleton projection. Magn Reson Imaging 29:111–125. DOI: 10.1016/j.mri.2010.06.027.

