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The relation between gray matter volume and the use of alcohol, tobacco, cocaine and cannabis in male polysubstance users

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ABSTRACT

Background: Neuroimaging studies have demonstrated gray matter (GM) volume abnormalities in substance users. While the majority of substance users are polysubstance users, very little is known about the relation between GM volume abnormalities and polysubstance use.

Methods: In this study we assessed the relation between GM volume, and the use of alcohol, tobacco, cocaine and cannabis as well as the total number of substances used, in a sample of 169 males: 15 non-substance users, 89 moderate drinkers, 27 moderate drinkers who also smoke tobacco, 13 moderate drinkers who also smoke tobacco and use cocaine, 10 heavy drinkers who smoke tobacco and use cocaine and 15 heavy drinkers who smoke tobacco, cannabis and use cocaine.

Results: Regression analyses showed that there was a negative relation between the number of substances used and volume of the dorsal medial prefrontal cortex (mPFC) and the ventral mPFC. Without controlling for the use of other substances, the volume of the dorsal mPFC was negatively associated with the use of alcohol, tobacco, and cocaine. After controlling for the use of other substances, a negative relation was found between tobacco and cocaine and volume of the thalami and ventrolateral PFC, respectively.

Conclusion: These findings indicate that mPFC alterations may not be substance-specific, but rather related to the number of substances used, whereas, thalamic and ventrolateral PFC pathology is specifically associated with tobacco and cocaine use, respectively. These findings are important, as the differential alterations in GM volume may underlie different cognitive deficits associated with substance use disorders.

1. Introduction

Voxel-based morphometry (VBM) studies have demonstrated large scale gray matter (GM) volume abnormalities in substance users, including tobacco users (Hanlon et al., 2016; Wetherill et al., 2015; Zhong et al., 2016), heavy alcohol users (Xiao et al., 2015; Yang et al., 2016), cannabis users (Cousijn et al., 2012; Wetherill et al., 2015) and cocaine users (Crunelle et al., 2014; Ide et al., 2014; Rando et al., 2013). However, a large portion of substance users are polysubstance users, using more than one type of drug simultaneously or at different moments close in time (Connor et al., 2014; European Monitoring Centre for Drugs and Drug Addiction, 2009). Despite the high prevalence and poor treatment outcomes associated with polysubstance use disorders (Dutra et al., 2008), little is known about neurobiological pathways associated with polysubstance use.

Importantly, most studies in this field focus on the relation between GM volume and the use of one particular substance, without controlling for the use of other substances. For instance, two recent meta-analyses demonstrated that GM volume of the prefrontal cortex (PFC) is negatively related to lifetime alcohol consumption (Yang et al., 2016) and

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lifetime tobacco consumption (Zhong et al., 2016), without taking into account other substances of abuse reported by the participants. Moreover, despite the fairly consistent finding of a negative relationship between PFC volume and lifetime exposure to alcohol or tobacco, studies in cocaine users are far less consistent: while some studies in cocaine users report a negative association between the level of cocaine exposure and PFC volumes (Alia-Klein et al., 2011; Barrós-Loscertales et al., 2011; Ersche et al., 2011; Lim et al., 2008; Makris et al., 2008; Rando et al., 2013), other studies failed to do so (Crunelle et al., 2014; Franklin et al., 2002; Hanlon et al., 2011; Kaag et al., 2014; Matochik et al., 2003; Narayana et al., 2010). Similar inconsistencies have been demonstrated in cannabis users, as some studies suggest that volume of the amygdala and hippocampus are negatively related to lifetime cannabis use (Cousijn et al., 2012; Demirakca et al., 2011b), whereas another did not find this association (Haller et al., 2013). Interactions between the use of different types of substances (Althobaiti and Sari, 2016; Jutkiewicz et al., 2008; Lopes et al., 2012; Valjent et al., 2002; Wheeler et al., 2008) may explain these inconsistent findings.

In an attempt to take polysubstance use into account, several studies applied multiple regression analyses to test for the specific relation between a certain substance and GM volume, controlling for the potential confounding effects of others substances. These studies demonstrated that GM alterations in regular cocaine users are unrelated to co-occurring amphetamine use (Mackey et al., 2014), tobacco or alcohol use (Crunelle et al., 2014; Kaag et al., 2014). In contrast, smaller thalamic volumes in opioid-dependent patients were explained by co-occurring alcohol use (Reid et al., 2008). However, multiple regression analyses only allow one to estimate the specific relation between GM volume and a certain substance, controlling for the use of other substances, but it is unable to estimate the relation between the combined use of substances (as in polysubstance use) and GM volume. One way to estimate effects of multiple substances is to use the number of different substances that were used as a regressor in the analyses, as an estimation of the relation between polysubstance use and GM volume. Using this method, we recently demonstrated that white matter integrity of the PFC is unrelated to the use of one specific substance, but is strongly (negatively) related to the number of substance used in a linear fashion (Kaag et al., 2016b). Similarly, others have demonstrated that structural abnormalities in the PFC are more profound in amphetamine users with a history of co-occurring heavy alcohol use compared to those without (Lawyer et al., 2010); in polysubstance users who smoke, compared to those who do not smoke (Pennington et al., 2015); and in polysubstance users compared to non-polysubstance using alcohol-dependent patients (Pennington et al., 2015). These studies support the hypothesis that structural abnormalities in the PFC are not substance-specific, but more generally related to the number of substances used.

Here we extend on these previous finding, by studying GM volume in a larger sample, ranging from non-substance users to individuals who use any combination of alcohol, tobacco, cocaine and cannabis, instead of comparing only two groups of substance users as has been done in the previous studies. Structural MRI scans were collected as part of other studies (Jansen et al., 2015; Kaag et al., 2016a; Schulte et al., 2017), acquired on the same scanner and using the same MRI sequence. All subjects were classified according to the number of substances used and this number was subsequently used in a whole brain regression analyses to test the relation between the number of substances used and GM differences. In line with previous work, it was expected that the number of substances used was negatively and linearly related to GM volume, predominantly within the PFC. Additionally we used multiple regression analyses to assess the specific relation between a certain substance and GM volume, both with and without including the use of other substances as a covariate in the model.

2. Materials and methods

2.1. Participants

Structural MRI scans of 256 participants were available, collected as part of three published studies (Jansen et al., 2015; Kaag et al., 2016a; Schulte et al., 2017) between January 2012 and December 2015. In short, all participants included in the current analyses were males (aged 18–60), recruited through local advertisement in the greater Amsterdam area, The Netherlands. Study specific in- and exclusion were as followed: In the study by Schulte et al. (2017) inclusion criteria were: male; 18–55 years old; smoking at least 15 cigarettes per day; a desire to quit smoking; a Fagerström Test Nicotine Dependence (FTND; Heatherton et al., 1991) score of at least 3, indicating at least a low degree of smoking dependence, and an Alcohol Use Disorder Identification (AUDIT) score lower than 13, indicating the absence of heavy alcohol use and/or an alcohol use disorder. In the study by Kaag et al. (2016a,b) inclusion criteria were: male; 18–50 years old; non-treatment seeking; and snorting cocaine at least once per week in the last 6 months. Healthy controls were also excluded if they reported a history of substance abuse or dependence including tobacco use, and in case of any psychotropic medication use. In the study by Jansen et al. (2015), alcohol-dependent patients (DSM-IV diagnosis) were recruited from addiction treatment centers in Amsterdam and had to be abstinent from alcohol for at least three weeks. Healthy controls were recruited through internet and social media and excluded if they met any DSM-IV criteria for psychiatric disorders. Control participants had to remain abstinent from alcohol for 24 h only. While females were also included in this specific study, they were not included in the current analyses because none of the other studies included female smokers or cocaine users (Note: in the final analyses only, the male control participants are included, but none of the alcohol-dependent patients). Alcohol, tobacco, cocaine and cannabis use was quantified using the Timeline Follow-back on 1 or 6 months before the study (Sobell and Sobell, 1992) or the Composite International Diagnostic Interview (Robins et al., 1988), in all three studies. Similarly, substance use, AUDIT-scores and FTND-scores was measured in all three studies, but DSM-IV diagnosis of psychiatric disorders, including substance use disorder, were only measured in the studies by Jansen et al. (2015) and Kaag et al. (2016a,b). Motivation to change substance use was measured in substance-users only, using the motivation to change questionnaire (RCQ: Heather et al., 1991). For the current study, all these participants were combined and categorized again based on the number of substances used. The studies were approved by the local Medical Ethics Committee of the Academic Medical Center of the University of Amsterdam or the Ethics Committee of the University of Amsterdam. All participants signed the informed consent form, consistent with the declaration of Helsinki, before participating in the study.

2.2. Participant selection and subgroup categorization

Based on self-report measures of alcohol, tobacco, cocaine and cannabis use, all participants were classified as heavy drinker (>21 units of alcohol per week), light drinker (≤ 21 units of alcohol per week) or non-drinker (0 units of alcohol per week); smoker (>1 cigarette per day) or non-smoker (0 cigarettes per day); cocaine user (≥ 1 g of cocaine per week) or non-cocaine user (0 g of cocaine per week) and cannabis user (≥ 1 joint per week) or non-cannabis user (0 joints per week). Cut-off values are in accordance with previous studies (Buckner and Schmidt, 2008; Griffiths, 1996; Kaag et al., 2016a; Medina et al., 2007; Roebuck et al., 2004). The classification resulted in (3 alcohol groups × 2 smoking groups × 2 cocaine groups × 2 cannabis groups =) 24 potential subgroups of participants that varied from using either a single or no substance, to using any combination of alcohol, tobacco, cocaine and cannabis.

Because of one or more missing self-reported measures, 46

Table 1
Classification table of all participants, based on the number of substances used.

No Alcohol Use							
Non-smoking				Smoking			
No Cocaine		Cocaine		No Cocaine		Cocaine	
No Cannabis	**Cannabis**	**No Cannabis**	**Cannabis**	**No Cannabis**	**Cannabis**	**No Cannabis**	**Cannabis**
n=15	**n=0**	**n=0**	**n=0**	**n=4**	**n=1**	**n=0**	**n=0**
< 21 units of Alcohol per week							
Non-smoking				smoking			
No cocaine		cocaine		No cocaine		cocaine	
No Cannabis	**Cannabis**	**No Cannabis**	**Cannabis**	**No Cannabis**	**Cannabis**	**No Cannabis**	**Cannabis**
n=89	**n=1**	**n=1**	**n=3**	**n=27**	**n=5**	**n=13**	**n=9**
> 21 units of alcohol per week							
Non-smoking				smoking			
No cocaine		cocaine		No cocaine		cocaine	
No Cannabis	**Cannabis**	**No Cannabis**	**Cannabis**	**No Cannabis**	**Cannabis**	**No Cannabis**	**Cannabis**
n=4	**n=0**	**n=4**	**n=0**	**n=9**	**n=0**	**n=10**	**n=15**

The numbers represent the number of participants in that category.
The groups in black are included in the analyses, the groups in gray are excluded from the analyses.

participants were excluded. In line with our previous research, we selected subgroups that increased in the number of substances used and had a minimal sample size of n = 10 (Kaag et al., 2016b). Doing so we ended up with 6 different subgroups that increased in the number of substance used: Non-alcohol or drug users (n = 15), light alcohol drinkers (n = 89), light drinkers who smoke tobacco (n = 27), light drinkers who smoke tobacco and use cocaine (n = 13), heavy drinkers who smoke tobacco and use cocaine (n = 10) and heavy drinkers who smoke tobacco and cannabis and use cocaine (n = 15). All other participants and subgroups were not included in the analyses (Table 1).

2.3. Magnetic resonance imaging acquisition and processing

All structural images from the three different studies were acquired on the same 3T whole body MRI 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^3.

Pre-processing was performed using the default settings of the CAT12 toolbox (http://dbm.neuro.uni-jena.de/vbm.html) within SPM12 (http://www.fil.ion.ucl.ac.uk/spm) running on MATLAB R2016a (Mathworks Inc., Natick, MA, USA). All T1- weighted images were corrected for bias and field inhomogeneities, then spatially normalized using the DARTEL algorithm (Ashburner, 2007) and segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) (Ashburner, 2007). The segmentation process was further extended by accounting for partial volume effects (Tohka et al., 2004). Subsequently, data was de-noised by applying a spatial-adaptive non-local means filter and by using adaptive maximum a posteriori estimations, which account for partial volume effects, and by applying a hidden Markov random field model, as implemented in VBM8 (Rajapakse et al., 1997). For exclusion of artifacts on the gray–white-matter border (i.e., incorrect voxel classification), we applied an internal gray matter threshold of 0.2. After pre-processing (in addition to visual checks for artifacts), all scans passed through an automated quality check protocol. Finally, the scans were smoothed with a smoothing kernel of 8 mm (FWHM).

2.4. Statistical analysis

For all participants, we calculated the number of cigarettes per day, the number of standard units of alcohol per week, the number of joints per week and the total grams of cocaine use per week. All participants were categorized based on the self-reported number of substances used, ranging from no-alcohol or drug use; light alcohol use light alcohol use + tobacco use; light alcohol use + tobacco use + cocaine use; heavy alcohol use + tobacco use + cocaine use; heavy alcohol use + tobacco use + cocaine use + cannabis use. Chi-square tests were used to test whether the four tobacco using groups were indeed matched on tobacco use; whether the three cocaine using groups were matched on cocaine use; whether the three light drinking groups were matched on alcohol use; and whether the two heavy drinking groups were matched on alcohol intake.

To assess the combined effect of substances used in GM volume, a whole brain voxel-wise regression analysis was performed in SPM12, with the number of substances used as a regressor of interest and age and intracranial volume (ICV) as covariates. All regressors were centered around the mean.

To assess the effects of substances on GM volume, without controlling for the use of other substances, four different, whole-brain voxelwise, regression analyses were performed on each substance separately, with only age and ICV as covariates.

To assess the specific effects of a certain substance on the GM volume, controlling for the use of other substances, a whole brain voxelwise multiple regression analyses were performed with the amount of alcohol, tobacco, cocaine, and cannabis used as regressors of interest and age and ICV as covariates. Results were statistically thresholded at the cluster level ($p < 0.05$, FWE corrected) with a cluster-defining threshold of $P < 0.001$, uncorrected.

3. Results

3.1. Substance use characteristics

Of all participants included in the analyses, 110 were originally scanned as part of the study by Kaag et al. (2016a), of which 38 were included as regular cocaine users and 72 as non-drug using controls. A total of 39 participants were originally scanned as part of the study by Schulte et al. (2017), of whom 24 were included as smokers and 15 as non-smoking controls. A total of 19 participants were originally scanned as part of the study by Jansen et al. (2015), all of which were included as controls. For more details see Table 2. Importantly, all participants were either non-drug or alcohol users, or non-abstinent substance users.

Subgroups did not differ in their motivation to change substance use ($F_{1,77} = 0.77$, $p = 0.58$). Age and self-reported substance use were not-normally distributed across groups; thus non-parametric tests were used to test group differences. There was no significant difference between groups in age ($\chi^2 = 8.7$, $p = 0.12$). Chi-square tests demonstrated that

Table 2
Participant characteristics of the final sample included in the analyses.

	Study-specific inclusion criteria	0.Non-substance users	1.low-moderate drinkers	2.low-moderate drinkers + Tobacco users	3.low-moderate drinkers + Tobacco + Cocaine users	4. Heavy drinkers + Tobacco + cocaine users	5. Heavy drinkers+ Tobacco+ Cocaine+ Cannabis users
Sample size		**n=15**	**n=89**	**n=27**	**n=13**	**n=10**	**n=15**
Data from Kaag et al., (2015)	Age: 18-50 years						
Controls	no substance use; abuse or dependence	n=11	n=61	n=0	n=13	n=10	n=15
Regular cocaine users	Snorting cocaine, ≥ 1x/wk. for 6 months	n=0	n=0	n=0	n=0	n=0	n=0
Data from Jansen et al., (2015)							
Controls	No DSM-4 diagnosis	n=3	n=14	n=3	n=0	n=0	n=0
Alcohol dependent patients (abstinent)	3 weeks abstinent In treatment	n=0	n=0	n=0	n=0	n=0	n=0
Data from Schulte et al., (2017)	Age: 18-55 yrs. AUDIT < 13						
Controls	Non-smoking	n=1	n=14	n=0	n=0	n=0	n=0
Regular smokers	≥ cigarettes per day Desire to quit smoking FTND ≥ 3	n=0	n=0	n=24	n=0	n=0	n=0
Readiness to change-score			44.64±4.5	43.87±5.2	41.23±7.1	43.30 ±6.3	40.9
FTND-score		0[a]	0[a]	5,9 (11%=unknown)	4,3	6,4	6,4
AUDIT-score		1±2	**5±3**	**6±8**	**14±7**	**17.5±7**	**14,5±5**
Age		38,6	34,2	38,8	31,4	33,1	35,6
Alcohol (glasses / week)		**0**	**3.50±6**	**5.08±6.4**	**12.05±13.9**	**46.15±29.7**	**34.70±28.7**
Tobacco (cigarettes / day)		**0**	**0**	**20±5**	**15.00±17**	**20±12**	**20±20**
Cocaine (gram / months)		**0**	**0**	**0**	**6.83±6.8**	**8.33±15.4**	**9.33±5.6**
Cannabis (joints / week)		**0**	**0**	**0**	**0**	**0**	**3±8.8**
% Lifetime Alcohol abuse[b]		0% (7% = unknown)	15% (7%=unknown)	0% (89%=unknown)	84%	90%	87%
% Lifetime Alcohol dependence[b]		0% (7% = unknown)	0% (7% = unknown)	0% (89% =unknown)	23%	70%	33%
% Lifetime cocaine abuse[b]		0% (7% = unknown)	0% (16% =unknown)	0% (89% unknown)	85%	90%	87%
% Lifetime Cocaine dependence[b]		0% (7% =unknown)	0% (16% =unknown)	0% (89% unknown)	85%	90%	81%
% Lifetime cannabis abuse[b]		0% (7% = unknown)	0% (16% =unknown)	0% (89% unknown)	15%	30%	47%
% Lifetime Cannabis dependence[b]		0% (7%=unknown)	0% (16%=unknown)	0% (89% unknown)	8%	10%	50%

[a]All participants were non-smokers, so the FTND was not assessed
[b]Lifetime substance use or dependence was assessed using the DSM-IV criteria. This was not assessed in Schulte et al., (in preparation).
Cells in light gray indicate that groups were supposed to be matched on that specific variable, but in fact differed.

there was a significant difference in alcohol intake between the three light-drinking groups ($\chi^2 = 18.72$, $p < 0.001$). Follow-up tests revealed that light drinkers who use tobacco and cocaine drank significantly more compared to light drinkers who use tobacco but no cocaine ($\chi^2 = 7.77$, $p = 0.005$) while this latter group drank significantly more compared to light drinkers who did not use tobacco or cocaine ($\chi^2 = 4.07$, $p = 0.04$). The heavy drinking groups and smoking groups were well-matched on alcohol ($\chi^2 = 0.89$, $p = 0.35$) and the tobacco use ($\chi^2 = 5.64$, $p = 0.13$), respectively.

The only measures of substance use severity that was taken in all three studies were smoking severity (FTND) and alcohol use severity (AUDIT). Chi-square tests demonstrated that smoking severity was similar in all four smoking groups ($\chi^2 = 2.88$, $p = 0.41$). However, similar to alcohol intake, the light drinking groups differed significantly in alcohol-use severity ($\chi^2 = 22.82$, $p < 0.001$), as light drinkers who use tobacco and cocaine reported a significantly higher AUDIT-score compared to light drinkers who use tobacco but no cocaine ($\chi^2 = 15.91$, $p < 0.001$) and light drinkers who do not use tobacco or cocaine ($\chi^2 = 21.29$, $p < 0.001$) whereas these latter two groups did not differ in terms of alcohol use severity. These results suggest that, despite our efforts, the light drinking groups were not perfectly matched on alcohol intake or alcohol use severity.

It should be noted that despite the fact that light drinkers who also used tobacco and cocaine reported a 'low to moderate' level of alcohol use (equal or less than 21 units per week), a substantial proportion of these participants met DSMIV criteria for lifetime alcohol abuse (84%) or dependence (23%). Similarly, a relatively large proportion of the cocaine users who did not report the use of cannabis in the past 6 months met DSMIV criteria for lifetime cannabis abuse (45%) or dependence (18%) (see Table 2). Unfortunately, DSMIV diagnosis of substance use disorder was not assessed in all three studies; hence, the DSMIV diagnoses were absent for 7% of the non-substance users and light drinkers, as well as for 89% of the light drinkers who used tobacco, making statistical comparisons on this variable impossible.

3.2. The relation between GM volume and the use of alcohol, tobacco, cocaine, and cannabis

To assess the combined effect of substances used on GM volume, multiple regression analysis was performed with the number of substances used as a regressor of interest and age and ICV as covariates. This analysis revealed a significant negative relation between the number of substances used and gray matter volume of the dmPFC and vmPFC (Fig. 1 in red; Table 3).

To assess the specific effects of substances used on GM volume, when controlling for the use of the other substances, multiple regression analysis was performed with the amount of alcohol, tobacco, cocaine, and cannabis used as a regressor of interest and age and ICV as regressors of no-interest. This analysis revealed a negative correlation between monthly cocaine use and GM volume of the vlPFC (including the orbital frontal cortex) and right insula (Fig. 1 in blue; Table 4) whereas daily tobacco use was negatively correlated with GM volume of the thalami (Fig. 1 in green, Table 4). Importantly, GM volume reductions related to the number of substances used do not overlap with the GM regions that showed a dose-response relationship with cocaine or tobacco use. That is, for none of these substances we demonstrated a negative dose-response relationship with dmPFC or vmPFC volume. There were no positive or negative associations between gray matter volume and monthly cannabis use or weekly alcohol use.

To assess the effects of substances on GM volume, uncorrected for the use of other substances, four whole brains, FWE-cluster corrected, regression analyses were performed with the amount of alcohol or tobacco or cocaine or cannabis use as a regressor of interest, and age and ICV as regressors of no-interest. These analyses demonstrated that there

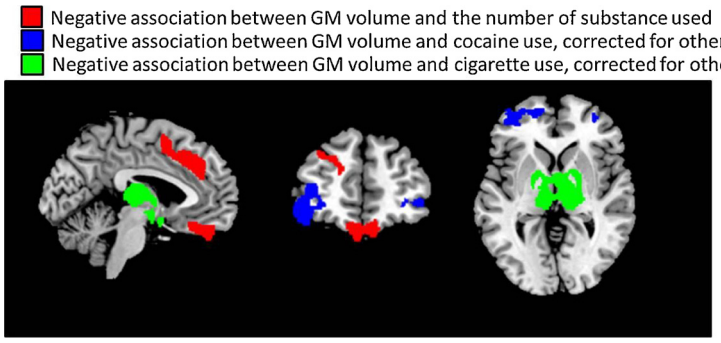

Fig. 1. Regional alterations in gray matter volume related to the number of substances used do not overlap with the volume of gray matter regions that show a substance dose-response relationship. The number of substances used is negatively correlated to gray matter volume in the dorsomedial prefrontal cortex and the orbital medial prefrontal cortex. These regions do not overlap with the regions that display a dose-response relationship with cocaine, tobacco and cannabis. That is, cocaine use is negatively related to regions in the more lateral regions of the orbital frontal cortex, and tobacco use is negatively correlated to volume of the thalamus. (For interpretation of the references to colour in the text, the reader is referred to the web version of this article.)

was no significant dose-response relation between weekly cannabis use and GM volume. However, alcohol, tobacco and cocaine all showed a negative dose-response relation to dmPFC volume, the same region where the volume was negatively related to the total number of substances used (Fig. 2, Table 5) In addition, for cocaine there was a negative dose-response relation with GM volume of the vmPFC and insula, whereas daily tobacco use was also negatively correlated to GM of the thalamus.

4. Discussion

The study aimed to investigate the relation between gray matter volume and the use of alcohol, tobacco, cocaine, and cannabis in male (poly)substance users. By categorizing participants on the number of substances used, ranging from nothing to the combination of alcohol, tobacco, cocaine, and cannabis, we demonstrated a negative relationship between the number of substances used and volume of the dmPFC and vmPFC. While previous studies have demonstrated smaller mPFC volume in cocaine users (Ersche et al., 2011; Fein et al., 2002), cannabis users (Lopez-Larson et al., 2011), heavy alcohol users (Xiao et al., 2015; Yang et al., 2016) and smokers (Zhong et al., 2016), the current study suggest that volume of the mPFC is in reality not (dose-dependently) related to these specific substances, but rather to the number of substance used. This is in line with our previous study that demonstrated that the number of substances used was negatively related to white matter integrity, specifically within the prefrontal cortex (Kaag et al., 2016b). In general, the mPFC has been implicated in a wide range of cognitive processes and behavior, including cognitive control, emotion regulation, decision making and goal-directed behavior (Bechara, 2005; Etkin et al., 2011; Ridderinkhof, 2004). However, the dorsal part (dmPFC) has been mainly implicated in the expression of (drug) conditioned behavior, whereas the ventral part (vmPFC) has been mainly implicated in the inhibition of (drug) conditioned behavior (Gourley and Taylor, 2016; Moorman et al., 2015). Hence the negative relation between the number of substances used and GM volume of the dmPFC and vmPFC may underlie an imbalance between this 'Go vs. Stop' system, resulting in impaired control of drug-taking behavior (Moorman et al., 2015). Interestingly, smaller dmPFC volumes have also associated with poorer treatment outcome in alcohol-dependent patients (Rando et al., 2011). Therefore, smaller dmPFC volumes in polysubstance users may be related to the often observed relative treatment-resistance within this population (Dutra et al., 2008).

We also demonstrated a specific relation (controlling for the use of other substances) between tobacco use and thalamic volumes, as well as a specific relation between cocaine use and volume of the vlPFC and insula. This is not the first study to demonstrate a negative relation between smoking and thalamus volume (Hanlon et al., 2016; Liao et al., 2012; Peng et al., 2015; Yu et al., 2017). The thalamus is critically involved in the motivation, emotional drive, and planning of goal-directed behavior (Haber and Calzavara, 2009). Moreover, the thalamus has a very high density of acetylcholine (nicotine) receptors, making this region the primary target for nicotine binding (Wonnacott, 1997; Zubieta et al., 2001). Because of this, the thalamus may be specifically vulnerable for the neurotoxic effects of nicotine (and not other substances) and/or may play a critical role in the development and persistence of nicotine dependence (Hanlon et al., 2016).

The specific, negative (dose-dependent) relation between cocaine use and volume of the vlPFC is also in line with previous studies (Franklin et al., 2002; Matochik et al., 2003; Rando et al., 2013). The vlPFC (including the OFC) has been suggested to be involved in higher-order emotional control (Shiba et al., 2016) and automatic response tendencies and response inhibition (Goldstein and Volkow, 2011). This could be related to impaired decision making (Fernandez-Serrano et al., 2011) and (neural) hyperresponsiveness to negative emotional stimuli (Crunelle et al., 2015; Kaag et al., 2016a) in regular cocaine users.

In line with another study (Bullock et al., 2017) we did not find a

Table 3
The combined effect of substance use on gray matter volume.

	Cluster size *# voxels*	Cluster *P-value*	Voxel *z-value*	Peak voxel *MNI-coordinates*			Voxel region (AAL atlas/broadmann area)
# of substances used: negative correlation	4065	< 0.001	4,77	6	15	42	R midcingulate cortex
			4,6	−27	40	32	L middle frontal cortex
			4,51	0	24	38	L medial frontal cortex
			4,46	−16	44	26	L superior frontal cortex
			4,44	−2	8	52	L supplementary motor area/BA 32
			3,75	−6	28	33	L midcingulate cortex/BA 9
			3,66	12	0	48	R supplementary motor area
			3,52	−6	38	26	L anterior cingulate cortex/BA32
	2315	< 0.001	4,72	−3	45	−24	L rectus/BA 11
			4,14	9	50	−22	R superior frontal cortex, orbital part
			3,85	9	28	−21	R rectus
			3,38	10	12	−20	R olfactory cortex
# of substances used: positive correlation	*No significant positive correlations*						

All results were $p < 0.05$, cluster level family-wise error corrected with an initial height threshold of $p = 0.001$ uncorrected.

Table 4
The specific effect substance use on gray matter volume, controlled for the use of other substances

	Cluster size *# voxels*	Cluster *P-value*	Voxel *z-value*	Peak voxel *MNI-coordinates*			Voxel region (AAL atlas/broadmann area)
Monthly cocaine use: Negative correlation	2426	< 0.001	4,34	−30	48	−6	L medial orbital frontal cortex
			4,25	−14	57	−2	Frontal_Sup_Medial_L
			3,81	−21	51	−9	Frontal_Sup_Orb_L
			3,75	−38	50	4	Frontal_Mid_L/BA 10
			3,59	−45	56	9	Frontal_Mid_L/BA 46
			3,49	−51	34	−12	Frontal_Inf_Orb_L
	883	0.003	3,84	57	30	−9	Frontal_Inf_Orb_R/BA47
			3,76	34	51	0	Frontal_Mid_R
			3,48	46	21	−4	Insula_R
Monthly cocaine use: positive correlation	*No significant positive correlations*						
Daily cigarette use: negative correlation	7686	< 0.001	5,74	10	−18	14	Thalamus_R
			5,42	20	−4	0	Pallidum_R
			5,39	10	−2	0	Thalamus_L
Daily cigarette use: positive correlation	*No significant positive correlations*						
Monthly cannabis use	*No significant positive or negative correlations*						
Weekly alcohol use	*No significant positive or negative correlations*						

All results were $p < 0.05$, cluster level family-wise error corrected with an initial height threshold of $p = 0.001$ uncorrected.

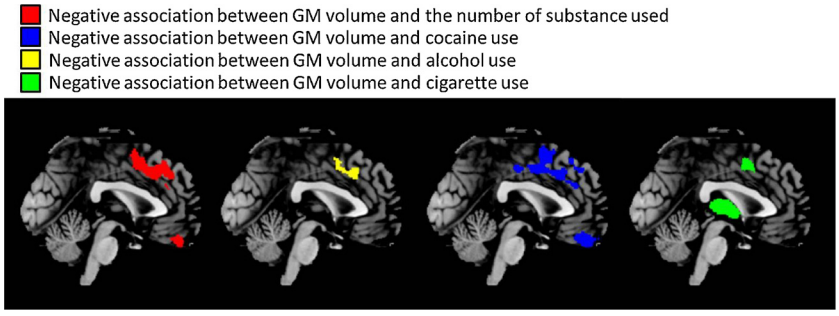

Fig. 2. The relation between GM volume and alcohol, tobacco, tobacco and alcohol use, uncorrected for the use of other substances. Alcohol, cocaine and tobacco use were all negatively related to dorsomedial prefrontal cortex volume, the same region that showed a negative relation with the number of substances used. Additionally, cocaine use was negatively related to ventromedial prefrontal cortex volume and tobacco use was negatively related to volume of the thalami.

Table 5
The general effect substance use on gray matter volume, uncontrolled for the use of other substances.

	Cluster size *# voxels*	Cluster *P-value*	Voxel *z-value*	Peak voxel *MNI-coordinates*			Voxel region (AAL atlas/broadmann area)
Monthly cocaine use: Negative correlation	5975	< 0.001	5,27	−34	44	32	Frontal_Mid_L/BA9
			4,81	−4	32	30	Cingulum_Ant_L/BA32
			4,45	−42	44	28	Frontal_Mid_L
			4,26	−14	57	−2	Frontal_Med_Orb_L
			4,14	−2	8	54	Supp_Motor_Area_L/BA6
			4,09	−15	34	27	Frontal_Sup_Medial_L/B32
			4,07	−2	4	44	Cingulum_Mid_L
	3569	< 0.001	4,62	9	52	−22	Frontal_Sup_Orb_R
			4,42	−14	32	−16	Rectus_L/BA47
			4,28	3	60	−21	Rectus_R/BA11
			3,86	16	60	−2	Frontal_Sup_Medial_R
			3,43	26	20	−18	Insula_R
			3,29	10	12	−20	Olfactory_R
	1990	< 0.001	3,39	27	40	−14	Frontal_Mid_Orb_R/BA11
Monthly cocaine use: positive correlation	*No significant positive correlations*						
Weekly alcohol use: negative correlation	1006	0.002	4,39	9	9	44	Cingulum_Mid_R
			3,69	0	22	38	Frontal_Sup_Medial_L
			3,42	−4	28	32	Cingulum_Mid_L
			3,37	0	8	51	Supp_Motor_Area_L
Weekly alcohol use: positive correlation	*No significant positive correlations*						
Monthly cannabis use:	*No significant negative or positive correlations*						
Daily cigarette use: negative correlation	4816	< 0.001	4,96	−9	−20	9	Thalamus_L
			4,92	15	−2	4	Medial Dorsal Nucleus
			4,69	6	−12	8	Thalamus_R
			3,62	−20	−8	−3	Pallidum_L
Daily cigarette use: positive correlation	*No significant positive correlations*						

All results were $p < 0.05$, cluster level family-wise error corrected with an initial height threshold of $p = 0.001$ uncorrected.

specific relation between alcohol use and mPFC volume, after controlling for the use of other substance. This may come as a surprise as mPFC volume reductions have consistently been demonstrated in alcohol-abusing populations (Bühler and Mann, 2011; Yang et al., 2016), and could indicate that poly-substance use may have played a role in these findings too (which should be investigated further in future studies).

However, because many previous studies did not account for the use of other substances, it may very well be that the negative relation between alcohol use and mPFC volume, at least partly, reflects a negative relation between the mPFC volume and the number of other substances used. Indeed, it has been suggested that alcohol use is more frequent, in polydrug users (Barrett et al., 2006). Finally, we did not demonstrate a negative relation between cannabis use and GM volume. While, the current finding does replicate some other negative studies (Cousijn et al., 2012; Haller et al., 2013), we may have had too little statistical power to demonstrate a significant relation between cannabis use and cortical GM volume (as only 6% of the current sample actually smoked cannabis).

Because of the cross-sectional nature of the current study, we cannot draw any conclusions on the causal relation between [1] the smaller mPFC volume and the number of substances used, [2] the smaller thalami volume and tobacco use and [3] the smaller vlPFC volume and cocaine use. Nicotine, alcohol, cocaine, and cannabis all have different mechanisms of action (Pierce and Kumaresan, 2006): cocaine directly acts on the dopamine transporter in the ventral tegmental area (VTA), alcohol positively modulates GABAergic interneurons in the VTA, cannabinoids inhibit GABA release from the VTA; nicotine activates acetylcholine receptors, increase glutamate and decreases GABA transmission in the VTA. Because of these different (sometimes opposite) working mechanisms, it is unlikely that the negative relation between mPFC volume and the number of substances used is the result of the neurotoxic effects of these substances on the brain. Therefore, smaller mPFC volumes may predispose an individual to develop a (poly) substance use disorder, rather than being a consequence of (poly) substance use. This hypothesis is supported by a previous study that demonstrated that smaller dmPFC volumes predict escalating substance use (Becker et al., 2015). Alternatively, the smaller dmPFC volume may be related to substance use severity in general. Moreover, the current study provides important evidence that thalamic pathology is specifically associated with tobacco use (and not another substance of abuse), whereas vlPFC pathology is specifically related to cocaine use (and not another substance of abuse), which could reflect specific neurotoxic effects of these substances. Importantly, these hypothesized causal relationships between substance use and mPFC, vlPFC, and thalami volume should be confirmed using animal studies or longitudinal human studies.

In the current study, we combined VBM data from three different studies, which resulted in the inclusion of 169 substance using and non-substance using males. This large population enabled us to investigate the effect of polysubstance use in a general population sample. Because we assessed the relation between the number of substances used and GM volume in addition to applying multiple regression analyses, we demonstrated structural alterations in the mPFC that would otherwise have been missed. These findings contribute to the existing literature because currently very little is known about the relation between gray matter volume and polysubstance use, despite the high prevalence of polysubstance use and the severity of mental health issues associated with polysubstance use (Connor et al., 2014; Dutra et al., 2008). Moreover, our study provides an alternative and additional method to address the issue of polysubstance use among substance dependent populations, which could be applied in future studies as well. However, the current (naturalistic) study also has some limitations: First, because we wanted to include subgroups that increased in the number of substance used, by adding the use of one extra substance in each subgroup, and because some other subgroups were considered too small to be included in the analyses, some potentially important groups, were excluded. Future studies should be performed to establish whether the relation between dmPFC volume and the number of substance used, also holds for other combination of substances. Another result of our categorization method is that not all 'groups' had an equal sample size and some were relatively small compared to the other groups; however, we used our categorization to label participants with the number of substance used, that was subsequently used a regressor in the analyses. Doing so, the unequal sample sizes do not yield a statistical issue. Moreover, while we aimed to end up with subgroups of substances users that differed from each other only in the number of substances used, alcohol use in smokers was significantly higher compared to non-smokers, and alcohol use in cocaine users was significantly higher compared to non-cocaine users. Therefore, we cannot exclude the possibility that the negative relation between the number of substances used and the mPFC volume is mainly driven by alcohol use. Through collapsing even more datasets, we may be able to address these issues in future studies. It should be noted that, in the current study, we used a cut-off of 21 units 10 g of alcohol per week (total of 210 g of alcohol). While this is in line with the current guidelines used in the Netherlands to define excessive drinking in men (Netherlands Institute of Mental Health and Addiction, 2016), it is slightly higher than the novel guidelines by the NIAAA that defines and a moderate alcohol use as a maximum of 14 units of alcohol per week containing 14 g of alcohol per unit (a total of 196 g of alcohol per week). Note that the current guidelines in the Netherlands were also adjusted to: best not drink, and when you drink maximally one glass per day.

Another limitation of this study is that we did not address the specific interactions between the different substances. While the interaction between different substances is a very important issue (Althobaiti and Sari, 2016; Jutkiewicz et al., 2008; Lopes et al., 2012; Valjent et al., 2002; Wheeler et al., 2008) testing these four-way interactions would have been highly complex in the current sample. In addition, we have focused our research on the relation between the number of substances used and brain volume, thereby seemingly ignoring a lot of other important factors involved in the polysubstance use, including the age of onset and whether the substances are used simultaneously. Unfortunately, we did not have this data because of the exploratory nature of the study and could therefore not assess these important questions. In this study only, males were included because this leads to more homogenous groups and therefore strengthens the results of the study. Moreover, while the prevalence of smoking in the Netherlands is relatively similar among males and females (22% and 17% respectively), the prevalence of cocaine use is three times as high among males than females (Netherlands Institute of Mental Health and Addiction, 2016). It may, therefore, be more relevant to assess the relation between substance use and gray matter volume in males first. It is important to note, however, that the few studies that have included both males and females, provided limited evidence for relevant sex differences in gray matter volume in alcohol use disorder (Demirakca et al., 2011a; Thayer et al., 2016) and cocaine use disorder (Ide et al., 2014). Nonetheless, it remains to be investigated how the current findings translate to a female population. Another potential concern of this study could be that the difference in- and exclusion criteria of the studies, resulted in populations that are too different to collapse in one analysis. However, due to our categorization none of the abstinent alcohol-dependent patients originally included as part of the study by Jansen et al. (2015), were included in the final analyses. Therefore, only active users of cocaine (included as part of the study by Kaag et al., 2016a), cigarettes (included as part of the study by Schulte et al., 2017), and matched non-drug using controls, were included in our final analyses. A potentially relevant difference between these populations could be that the cigarette smokers were included in an intervention study and therefore motivated to change their smoking behavior, whereas the cocaine users were not specifically included based on their motivation to change their cocaine use behavior. Based on the total score of the RCQ, it became evident, however, that there were no significant differences between

smokers and cocaine users in their motivation to change smoking and cocaine use, respectively or between the subgroups as included in the analyses. Hence, we are confident that potential differences between the different study populations with respect to motivation to change, did not affect our results.

While we acknowledge these limitations that are related to the naturalistic design used in the current study, we strongly feel that this study does provide relevant and important information that contributes to our neurobiological understanding of polysubstance use.

In sum, we demonstrated a negative effect of the number of substances used on the GM vmPFC and dmPFC volumes as well as a substance non-specific dose-response relationship between substance use and dmPFC volume. In addition, we showed a substance-specific negative association between tobacco use and thalamic GM volume as well as a substance-specific negative association between cocaine use and vlPFC GM volume. These results suggest that a smaller GM mPFC volume either reflects substance use severity or may predispose to (poly) substance use whereas smaller GM thalamic volume and smaller GM vlPFC volume are more likely to result from the neurotoxic effects of tobacco and cocaine on the brain. However, only animal or longitudinal studies can establish this causal relationship. Because the dmPFC, vmPFC, vlPFC and thalamus have all been implicated in different neurocognitive processes (Bechara, 2005; Etkin et al., 2011; Goldstein and Volkow, 2011; Gourley and Taylor, 2016; Haber and Calzavara, 2009; Moorman et al., 2015; Ridderinkhof, 2004; Shiba et al., 2016), the combined and specific effects of alcohol tobacco, cocaine and cannabis on GM volume may reflect differential cognitive deficits among substance users.

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Contributions

AMK collected a part of the data, analyzed all the data and perpetrated the first draft of the manuscript. MHJS and JMJ collected parts of the data. GvW assisted with the data analyses. GvW, JH, WvdB, RWW, LS, AEG and LR actively participated in writing and revising the manuscript for publication. All authors have read and approved the final manuscript.

Conflicts of interest

No conflict declared.

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