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DOI:

[10.1016/j.euroneuro.2011.06.004](https://doi.org/10.1016/j.euroneuro.2011.06.004)

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Citation for published version (APA):

Stone, J., Bhattacharyya, S., Barker, G. J., McGuire, P. K., & Bhattacharyya, S. (2012). Substance use and regional gray matter volume in individuals at high risk of psychosis. *European Neuropsychopharmacology*, 22(2), 114-122. <https://doi.org/10.1016/j.euroneuro.2011.06.004>

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Substance use and regional gray matter volume in individuals at high risk of psychosis

Running title: Substance use and gray matter volume

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Key Words: Psychosis, Alcohol, Nicotine, Tobacco, Cannabis, Gray Matter, MRI

Individuals with an at risk mental state (ARMS) are at greatly increased risk of developing a psychotic illness. Risk of transition to psychosis is associated with regionally reduced cortical gray matter volume. There has been considerable interest in the interaction between psychosis risk and substance use. In this study we investigate the relationship between alcohol, cannabis and nicotine use with gray matter volume in ARMS subjects and healthy volunteers. Twenty seven ARMS subjects and 27 healthy volunteers took part in the study. All subjects underwent volumetric MRI imaging. The relationship between regional gray matter volume and cannabis use, smoking, and alcohol use in controls and ARMS subjects was analysed using voxel-based morphometry. In any region where a significant relationship with drug was present, data were analysed to determine if there was any group difference in this relationship. Alcohol intake was inversely correlated with gray matter volume in cerebellum, cannabis intake was use was inversely correlated with gray matter volume in prefrontal cortex and tobacco intake was inversely correlated with gray matter volume in left temporal cortex. There were no significant interactions by group in any region. There is no evidence to support the hypothesis of increased susceptibility to harmful effects of drugs and alcohol on regional gray matter in ARMS subjects. However, alcohol, tobacco and cannabis at low to moderate intake may be associated with lower gray matter in both ARMS subjects and healthy volunteers – possibly representing low-level cortical damage or change in neural plasticity.

Introduction

Individuals with an At Risk Mental State (ARMS) are at increased risk of developing psychosis (Phillips, Yung & McGorry 2000). They have also been shown to have reduced gray matter compared to healthy volunteers (Pantelis et al. 2003, Borgwardt et al. 2007), and, with transition to psychosis, show a further reduction in gray matter volume (Pantelis et al. 2003, Takahashi et al. 2009), findings which have been confirmed by recent meta-analyses (Smieskova et al. 2010, Fusar-Poli et al. 2011). The cause of these changes is not known, but it has been suggested that reductions in gray matter volume might occur, in part, because of increased sensitivity in ARMS subjects to the potentially toxic effects of drugs and alcohol (Welch et al. 2010). Cannabis, tobacco and alcohol have been implicated in brain volume changes in patients with schizophrenia (Sullivan et al. 2000, Nesvag et al. 2007, Szeszko et al. 2007, Tregellas et al. 2007, Bangalore et al. 2008, Rais et al. 2008a, McClernon 2009, Van Haren et al. 2010), and there is some evidence that moderate levels of intake may be associated with reduced gray matter volume in individuals at genetic risk of schizophrenia, and even in healthy volunteers (McClernon 2009, Gallinat et al. 2006, Sasaki et al. 2009, Verbaten 2009, Lorenzetti et al. 2010). Here, using data acquired during an earlier study (Stone et al. 2009), we investigate the hypothesis that the degree of recent cannabis, alcohol and tobacco use is negatively associated with gray matter volume in a dose-dependent manner, and that ARMS subjects show a stronger negative association between substance use and gray matter volume than healthy volunteers due to increased sensitivity to neurotoxic effects.

Methods

Twenty seven ARMS subjects were recruited from OASIS (Outreach and Support in South London), part of the South London and Maudsley NHS Trust. Twenty seven age- and sex-

matched controls were recruited from the same geographical area through advertisements that outlined the study. Subjects with an ARMS were identified using the Comprehensive Assessment of At Risk Mental State (CAARMS) (Yung et al. 2005). All subjects who expressed interest in participation were offered a face-to-face interview, where the full details of the study, including its possible risks and benefits were explained. Controls had no personal history of psychiatric symptoms, psychotropic medication or medical illness, and no family history of schizophrenia. For both groups, exclusion criteria included history of severe head injury (loss of consciousness for over 5 minutes), drug or alcohol dependence, metallic implants, and pregnancy.

Prior to scanning, all subjects were interviewed about their family and personal psychiatric history, current and past medication use and current and past use of alcohol, tobacco and illicit drugs. Alcohol use was recorded as the average number of units per week taken in the previous month. Tobacco use was recorded as the number of cigarettes taken per day. Cannabis use was recorded as the number of times that cannabis was taken in the previous year. As the cannabis intake for one ARMS subject was 3 times that of the next heaviest user, data on cannabis use were log transformed.

All subjects underwent volumetric MRI scanning. Scanning took place on a General Electric (Milwaukee, USA) 3 Tesla HDx Magnetic Resonance system. After positioning the subject in the scanner with a foam rest under their knees, an initial localizer scan was performed to measure the interhemispheric angle and the AC-PC line (the line passing through the upper part of the anterior commissure and the lower part of the posterior commissure – approximated from the anterior and posterior corpus callosum).

Structural images were acquired using an axial 2D T2-weighted Fast Spin Echo scan and an axial fast FLAIR scan (total scan time 5 minutes), both prescribed parallel to the AC-PC line. These were followed by a whole brain 3D coronal IR-SPGR (inversion recovery prepared spoiled gradient echo) scan, prescribed from the midline sagittal localizer, giving isotropic 1.1mm voxel size in a scan time of approximately 6 minutes (TE = 2.82ms; TR = 6.96ms; TI = 450ms; flip angle = 20°).

Segmentation was performed using Statistical Parametric Mapping software (SPM5, Wellcome Department of Imaging Neurosciences, University College London, UK). Gray matter probability images were then “modulated” (to compensate for the effect of spatial normalisation) by multiplying each voxel value by its relative volume before and after warping. The segmented images were then smoothed with an 8mm x 8mm x 8mm Gaussian kernel to reduce noise, and also allow for the effects of small residual mis-registrations.

In order to reduce multiple comparisons, and to correct for the the fact there was a high degree of overlap between the use of all three substances, correlations of gray matter volume difference with alcohol, cannabis and tobacco intake were analysed in a single model by fitting a GLM at each intracerebral voxel in standard space using the BAMB package. Given that structural brain changes are likely to extend over a number of contiguous voxels, test statistics incorporating spatial information such as 3D cluster mass (the sum of suprathreshold voxel statistics) are generally more powerful than other possible test statistics, which are informed only by data at a single voxel (Bullmore et al. 1999). A relatively lenient p-value ($p=0.05$) was initially set to detect voxels putatively demonstrating differences between groups; spatial clusters of such voxels were then searched for and the “mass” of each cluster found (the sum of suprathreshold voxel statistics it comprises) was tested for

significance. As no parametric distribution is known for cluster mass, permutation based testing, implemented in the BAMM package, was used to assess statistical significance at both the voxel and cluster levels (Bullmore et al. 1999). At the cluster level, rather than set a single a priori p-value below which findings are regarded as significant, the number of clusters which would be expected by chance alone for a range of p-values was calculated. The statistical threshold for cluster significance for each analysis was then set such that the expected number of false positive clusters by chance alone would be less than one (Bullmore et al. 1999). Group differences in the relationship between gray matter volume and substance use were analysed using a separate GLM implemented in R (Ihaka, Gentleman 1996).

As antipsychotic and antidepressant medication have been reported to affect brain volume (Ho et al. 2011, Navari, Dazzan 2009, Smieskova et al. 2009, Moore et al. 2009, Warner-Schmidt, Duman 2006), we reanalysed the group differences in R excluding ARMS subjects who were taking antidepressant or antipsychotic medication at the time of scanning. Lastly, to investigate the possibility that the multivariate analysis may have masked some drug effects shared by two or more of the substances, we then performed separate post-hoc BAMM analyses examining the relationship between gray matter volume and cannabis, alcohol and tobacco intake without covariates.

Results

Demographic details have been reported in an earlier study (Stone et al. 2009). There were no significant differences in age, sex, ethnicity, social class or IQ (table 1). Eight ARMS individuals but none of the controls had previously taken psychiatric medication and five of these were still taking medication (four had taken quetiapine, but only one was taking it at the time of the first scan, one was taking aripiprazole, two were taking citalopram and one was

taking sertraline) (Stone et al. 2009). ARMS subjects had significantly greater levels of psychopathology – including clinically significant anxiety and depressive symptoms (table 1). All ARMS subjects were offered cognitive behaviour therapy as part of their standard care. There were no significant differences in alcohol, cannabis or other illegal substance use between healthy volunteers and ARMS subjects, but ARMS subjects smoked more cigarettes per day (table 2). In addition to having attenuated psychotic symptoms, three of the ARMS subjects also had a first degree relative with schizophrenia, and one had a first degree relative with bipolar affective disorder. Four of the ARMS subjects went on to develop a schizophreniform psychosis. They had non-significantly higher alcohol (14 compared to 7 units per week) and tobacco intake (7.5 compared to 5 cigarettes per day) at baseline than the non-transition subjects, but did not report any cannabis use in the year prior to their baseline assessment.

Alcohol intake was negatively correlated with gray matter volume in cerebellum ($\beta=-0.03$, $SE=0.01$, $t=-2.99$; $p=0.005$; fig. 1), cannabis intake was negatively correlated with gray matter volume in prefrontal cortex ($\beta=-1.89$, $SE=0.59$, $t=-3.23$; $p<0.005$; fig. 2), and number of cigarettes smoked was negatively correlated with gray matter in left temporal cortex, parietal cortex and occipital cortex ($\beta=-0.018$, $SE=0.005$, $t=-3.361$; $p<0.005$; fig. 3). These relationships were present in healthy volunteers and ARMS subjects, with no significant group interactions. There was one outlier in the alcohol group regression, but after removal of this subject, the relationship between alcohol intake and cerebellar gray matter was still significant ($\beta=-0.027$, $SE=0.020$, $t=-0.6$; $p<0.05$). Reanalysis of all data excluding ARMS subjects who were taking medication at the time of scanning did not significantly affect any results: alcohol, cannabis and tobacco use were still significantly related to gray matter volumes and there were no significant group interactions.

Post-hoc regression analyses excluding covariates revealed significant negative relationships with substance use in the same brain regions as reported in the multivariate analysis. In addition, alcohol was negatively associated with gray matter volume in parahippocampal gyrus, superior temporal gyrus, hippocampus and inferior frontal gyrus; cannabis was negatively associated with gray matter volume in cingulate and left insula; and smoking was negatively associated with gray matter volume in anterior cingulate, dorsolateral prefrontal cortex, insula, temporal cortex and caudate nucleus (see supplementary material). There were no positive associations of gray matter volume with substance use in any brain region. There were no significant interactions with group in any of the regions showing a significant relationship with substance intake.

Discussion

We found that heavier use of tobacco, cannabis and alcohol was associated with lower gray matter volume in distinct brain regions, but we found no evidence that this relationship was more marked in ARMS subjects. This finding contrasts with an earlier study of individuals with a genetic risk for schizophrenia, which found that alcohol and cannabis use were both associated with gray matter volume reductions in these subjects, but not in controls (Welch et al. 2010). Possible reasons for the differences in findings may be that the subjects in the present study had a clinical rather than a genetic risk of psychosis and had a lower level of substance intake.

In patients with schizophrenia, the relationship between reduced gray matter volume and cannabis or alcohol has only been reported in subjects with heavy use or dependence (Sullivan et al. 2000, Mathalon et al. 2003), so there may be a threshold above which the

combined effect of alcohol or cannabis use with psychosis risk becomes associated with lower gray matter volume. Alternatively, the differential effects of cannabis use in patients with psychosis may only emerge over time. A well designed longitudinal study of patients with schizophrenia showed a marked difference in gray matter reductions in patients who continued to take cannabis compared to those who did not (Rais et al. 2008b), whereas this finding was not apparent in an earlier cross-sectional study (Cahn et al. 2004).

An increased susceptibility of gray matter volume to tobacco intake has been hypothesised to underlie some of the gray matter changes in patients with schizophrenia (Tregellas et al. 2007). One study supported this hypothesis (Tregellas et al. 2007), but a recent study found no association between longitudinal changes in gray matter volume and tobacco intake (Van Haren et al. 2010). In individuals at risk of psychosis, neither the present study, nor the earlier study of individuals at genetic risk of schizophrenia (Welch et al. 2010), found any evidence for any differential effect of tobacco intake on gray matter volume compared to healthy volunteers.

The present finding that low to moderate intake of alcohol and tobacco use are associated with gray matter changes in healthy volunteers has been reported in earlier studies, although not all reports are in agreement – particularly regarding the brain regions affected. A recent systematic review suggests that low levels of alcohol may be associated with reduced gray matter volume, particularly affecting frontal brain regions (Verbaten 2009). In the present study, the cerebellum was the main region showing reduction in gray matter volume with increasing alcohol intake after covarying for smoking and cannabis use, as reported in patients with schizophrenia dependent on alcohol (Sullivan et al. 2000). However, in the post-

hoc analysis excluding covariates, reductions in frontal and temporal cortex gray matter were also related to increasing levels of alcohol intake.

Our finding of an association between cigarette smoking and reduced gray matter volume in parietal and temporal cortices is in keeping with an earlier report that chronic cigarette smoking was associated with reduced gray matter volume in these regions in alcohol dependent subjects and light drinkers (Gazdzinski et al. 2005). Other studies have reported that smokers have reduced gray matter volume in frontal cortex (Brody et al. 2004), as found in the current analysis excluding substance use covariates.

The present finding of a prefrontal cortex gray matter reduction associated with cannabis use in healthy volunteers is relatively novel. Using a region of interest approach, gray matter reductions in medial temporal cortex associated with moderate to heavy cannabis use have been reported in healthy volunteers, but individuals studied generally had much higher intake of cannabis than in the present study, and negative findings employing the same method have also been reported (Lorenzetti et al. 2010, Demirakca et al. 2010). In keeping with the present finding, a reduction in N-acetylaspartate (NAA), a marker of neuronal integrity (Moffett et al. 2007), has been reported in frontal cortex in association with cannabis use in two separate studies (Hermann et al. 2007, Cowan, Joers & Dietrich 2009). Reduced prefrontal blood flow has also been reported in cannabis users (Martin-Santos et al. 2010). Reduced prefrontal blood flow and reduced frontal cortex NAA have also been reported in patients with schizophrenia (Hill et al. 2004, Brugger et al. 2010), suggesting that cannabis use may lead to similar deficits in neuronal integrity and brain function in prefrontal cortex to those occurring in schizophrenia.

The neuropathological basis of the gray matter change detected using MRI, and the clinical significance, is unclear, but the reversibility of brain volume changes in patients with alcohol dependence has led to suggestions that some of the effects of alcohol on gray matter as measured by MRI may be due to non-specific dehydration (Zipursky, Lim & Pfefferbaum 1989), and it has been demonstrated that dehydration is associated with brain volume reductions (Dickson et al. 2005, Kempton et al. 2009). The fact that T2 did not change with abstinence (as would be expected with rehydration) argues against this hypothesis, however (Schroth et al. 1988). Furthermore, acute administration of alcohol has not been shown to increase MRI measures of dehydration (Rooney et al. 2000). Alternatively, gray matter differences may reflect regional loss of neurons or changes in neural plasticity, or may be due to differences in personality type rather than a direct effect of the drug on brain volume (Gardini, Cloninger & Venneri 2009).

There are a number of potential weaknesses of this study that we acknowledge – firstly, the study was relatively small, and the range of substance use, particularly for cannabis, was fairly limited. Nonetheless, the levels of use reported in this study closely reflect those that are common in the population with moderate “social” use, and so any relationship between substance use and brain volume suggested by this study may be of significant public health interest. Secondly, the questions used to rate substance use did not take into account lifetime use, and so associations reported by the present study will reflect recent use. It is unclear whether abstinence would lead to an increase in gray matter volume in the affected regions. Lastly, a longitudinal design would assist in determining whether continued substance use is associated with further changes in gray matter volume in each group.

Conclusions

Alcohol, tobacco and cannabis at low to moderate intake may be associated with lower gray matter in both ARMS subjects and healthy volunteers – possibly representing low-level cortical damage or change in neural plasticity. The present study does not support the hypothesis that ARMS subjects are at greater risk of gray matter changes associated with substance intake, although it is possible that at higher levels of intake, or with prolonged use, a differential effect may emerge.

Conflict of interests

The authors report no biomedical financial interests or potential conflicts of interest.

Role of Funding Source

This study was funded by a Medical Research Council Clinical Training Fellowship (Grant No. [G0500477](#)) awarded to JS. The MRC had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Acknowledgements

We acknowledge infrastructure support from the National Institute for Health Research Biomedical Research Centre for Mental Health at the South London and Maudsley National Health Service Foundation Trust and Institute of Psychiatry, King's College London. We thank members of the Outreach And Support In South London team who were involved in the recruitment, management, and clinical follow-up of the at-risk mental state subjects in this study.

Table 1: Demographic and clinical details

	ARMS	Controls	Significance
Age mean(SD)	25(5)	25(4)	P=0.883
Sex f/m	13/14	14/13	Fisher's=1.0
Ethnicity Caucasian/African-Caribbean/ se Asian/ African/ Asian/ other	15/2/1/1/2/6	12/7/2/2/3/1	Chi sq=0.426
Social class (parent's occupation) ab,c1,c2,de, unknown	2/6/9/3/7	2/14/6/1/4	Chi sq=0.23
National Adult Reading Test mean(SD)	28.69(12.24)	32.89(7.96)	P=0.147
Current antipsychotic or antidepressant medication y/n	5/22	0/27	Fisher's=0.051
Education no qualifications/ GCSE/A-level/ degree/higher degree	2/13/5/7/0	0/3/16/6/2	Chi sq=0.003
Hamilton Anxiety Scale mean(SD)	12.52(11.37)	1.85(2.63)	P=0.00005
Hamilton Depression Rating Scale mean(SD)	10.51(8.85)	1.63(2.71)	P=0.00002
PANSS positive mean(SD)	11.67(3.33)	7.26(0.81)	P=0.0000002
PANSS negative mean(SD)	8.93(3.05)	7.19(0.79)	P=0.007
PANSS general mean(SD)	22.22(4.46)	16.81(1.18)	P=0.000001

Table 2: Intake of drugs and alcohol in ARMS subjects (n=27) and controls (n=27). Alcohol use is presented as units per week, tobacco as cigarettes per day, all other drugs as occasions per year.

	ARMS mean(SD)	Controls mean(SD)	Significance (p)
Alcohol (units per week)	6.96(10.58) range=0-45	7.74(8.07) range=0-30	p=n.s.
Tobacco (cigarettes per day)	5.2(5.46) range=0-15	1.59(3.73) range=0-16	p<0.01
Cannabis (occasions per year)	67.11(223.75) range=0-1095	13.03(44.51) range=0-178	p=n.s.
Amphetamine (occasions per year)	0.07(0.266) range=0-1	0.48(2.31) range=0-12	p=n.s.
Cocaine (occasions per year)	0.22(0.64) range=0-3	1(4.61) range=0-24	p=n.s.
LSD (occasions per year)	0.04(0.19) range=0-1	0(0) range=0-0	p=n.s.
Heroin (occasions per year)	0(0) range=0-0	0(0) range=0-0	p=n.s.
Ketamine (occasions per year)	0.37(0.19) range=0-1	0.37(0.19) range=0-1	p=n.s.
Ecstasy (occasions per year)	0.44(1.60) range=0-8	0.66(1.69) range=0-6	p=n.s.
Other Drugs (occasions per year)	0.22(0.97) range=0-5	0(0) range=0-0	p=n.s.

Figure 1: inverse correlation between gray matter volume and alcohol intake (corrected for cannabis and tobacco intake; $p=0.005$). There was no significant difference between the relationship in controls (open circles) and ARMS subjects (closed triangles).

Figure 2: inverse correlation between gray matter volume and cannabis use (log transformed and corrected for alcohol and tobacco intake; $p < 0.005$). There was no significant difference between the relationship in controls (open circles) and ARMS subjects (closed triangles).

Figure 3: inverse correlation between gray matter volume and tobacco smoking (corrected for cannabis and alcohol intake; $p < 0.005$). There was no significant difference between the relationship in controls (open circles) and ARMS subjects (closed triangles).

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