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Re-investigating the effects of chronic smoking on the pathology of alcohol-related human brain damage

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Abstract

Both pathological and neuroimaging studies have shown that chronic alcohol abuse causes generalized white matter, but limited gray matter, volume loss. Recent neuroimaging studies suggest that tobacco smoking also causes brain atrophy in both alcoholics and neurologically normal individuals. However, a recent pathological study, employing a manual technique to determine regional volumes, found no significant effects of smoking on either global or selected regional gray matter volumes in smokers or smoking alcoholics. Here a high-resolution computerized method was employed in the same cohort to evaluate four regions where neuroimaging studies have found atrophy in smokers and alcoholics: insula, thalamus, prefrontal cortex, and anterior cingulate cortex. Brain images from 44 cases comprising 16 non-smoking controls, nine smoking controls, eight non-smoking alcoholics, and 11 smoking alcoholics were quantified. No significant differences between the groups were found, although the alcoholic groups tended to have smaller volumes in most regions. Furthermore, there were no smoking or interactive effects, and no correlation between gray matter volumes and either tobacco pack-years or lifetime alcohol consumption. These results do not support the hypotheses that tobacco smoking causes gray matter loss or that smoking potentiates gray matter atrophy in chronic alcoholics.

Keywords

Alcoholism; Tobacco smoking; Human postmortem brain tissue; Brain atrophy

Introduction

Tobacco smoking and excessive alcohol consumption represent two of the biggest burdens on health systems throughout the world. The extent and severity of atrophy in alcohol-related brain damage (ARBD) has been largely consistent between pathological (de la Monte & Kril, 2014) and neuroimaging studies (Durazzo et al., 2014; Gazdzinski et al., 2006), with pathological studies, in particular, demonstrating that brain atrophy is almost exclusively due to white matter loss despite the neuronal loss in regions such as the prefrontal cortex (Krill, Halliday, Svoboda, & Cartwright, 1997; McCorkindale, Sheedy, Kril, & Sutherland, 2016).

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However, the effects of smoking-related brain damage are less clear (Pan et al., 2013; M. T.; Sutherland et al., 2016). As approximately 80% of alcoholics also smoke cigarettes (Kalman, Morissette, & George, 2005), the possibility exists that smoking or smoking and alcohol interactive effects may have been mistakenly attributed to alcohol.

Neuroimaging studies have reported smoking effects in the gray matter (GM) of each of the lobes of the brain as well as in the subcortical GM and cerebellum (Durazzo, Meyerhoff, Yoder, & Murray, 2017; Luhar, Sawyer, Gravitz, Ruiz, & Oscar-Berman, 2013; Prom-Wormley et al., 2015). A recent pathological study of 16 non-smoking controls (nsCON), nine smoking controls (sCON), eight non-smoking alcoholics (nsALC), and 11 smoking alcoholics (sALC) did not find smoking effects on regional brain volumes (McCorkindale et al., 2016). However, it was possible that the resolution of the manual volumetric technique employed could have resulted in subtle differences in volumes going undetected.

Therefore, the aim of this study was to use a higher resolution volumetric technique to re-examine four GM regions where volume loss has been reported in neuroimaging studies of smokers: the dorsolateral prefrontal cortex, insula, ACC, and thalamus.

Methods

Ethics and case collection

This work was undertaken following ethics approval from the Human Research Ethics Committee of the University of Sydney (HREC # 2014/732) using tissue obtained from the New South Wales Brain Tissue Resource Centre (NSW BTRC) following approval by the National Institute of Alcohol Abuse and Alcoholism Scientific Advisory Board. The cohort, calculation of lifetime alcohol consumption and smoking pack-years, and tissue preparation has been described previously (McCorkindale et al., 2016).

A computerized adaptation of the manual point count volumetric technique used in the original study (McCorkindale et al., 2016) was developed in Fiji ImageJ (Schindelin et al., 2012; Schneider, Rasband, & Eliceiri, 2012). Color images of serial coronal slices were scaled in ImageJ and then counted using the 'Grid' and 'Cell Counter' plugins. The 'Grid' plugin is part of the standard ImageJ package while the 'Cell Counter' plugin is available from <https://imagej.nih.gov/ij/plugins/cell-counter.html>. The serial images were manually divided into the 4 Gray matter (GM) regions of interest (ROI): anterior cingulate cortex (ACC), insula, thalamus, and dorsolateral PFC (DLPFC), using previously described landmarks (Double et al., 1996; Halliday, Double, Macdonald, & Kril, 2003; Kril et al., 1997). The larger dorsolateral prefrontal cortex was further divided into the middle and superior frontal gyri (MFG and SFG), with the latter known to experience moderate neuronal loss in chronic alcoholics (Kril et al., 1997; McCorkindale et al., 2016). The crosses within 'ImageJ.grid' were set 1.9 mm apart giving each cross an area of 3.6 mm², or 10 greater resolution than the manual method (Fig. 1) with the regional volumes calculated as previously described (McCorkindale et al., 2016). The raw data were normalized by dividing all volumes by the mean volume of the gender controls for each ROI. Counting reliability was established by a single rater (AM) by achieving <5% variation in duplicate counts of 10% of the cases. All statistical analyses were carried out with SPSS statistical

software (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, New York, United States: IBM Corp.). The α -level was adjusted for multiple comparisons with a strict Bonferroni correction ($\alpha = 0.01$) modified to 0.02 based on the mean correlation between the five regions ($r = 0.41$) as in previous studies (Durazzo et al., 2014; McCorkindale et al., 2016). Group differences were assessed by two-way ANOVA with subsequent pair-wise analyses. Pearson correlations were used to explore relationships with smoking pack-years and lifetime alcohol consumption.

Results

This study re-examined the volumes of four ROIs in a cohort of 44 smoking and non-smoking chronic alcoholics and neurologically normal controls using a higher resolution computerized volumetric method. As previously described (McCorkindale et al., 2016), the four groups, 16 non-smoking controls (nsCON), nine smoking controls (sCON), eight non-smoking alcoholics (nsALC), and 11 smoking alcoholics (sALC) were matched for age, brain weight, brain pH, RNA integrity number (RIN), and body mass index (BMI) (Table 1). The sALC had a longer mean postmortem interval (PMI) compared with the sCON, while both mean daily intake and lifetime alcohol consumption were significantly higher in the nsALC compared with the sALC. sALC and nsALC had degrees of liver pathology with three and four individuals with cirrhosis, respectively. The causes of death were quite disparate between groups; most deaths of controls were due to ‘cardiac’ disorders, whereas the nsALC, in particular, died from ‘toxicity’ or ‘hepatic’ disorders. Similarly, diagnostic neuropathological examination showed that most controls had normal brains while half the nsALC had cerebellar vermal atrophy and 30% of sALC had Alzheimer type 2 astrocytes, consistent with hepatic encephalopathy (G. T. Sutherland, Sheedy, Sheahan, Kaplan, & Kril, 2014).

A two-way ANOVA revealed no significant alcohol, smoking, or interactive effects on the ACC, insula, thalamus, and DPFC volumes or in the MFG or SFG subregions of the latter (Table 2).

In addition, there were no differences in volume based on alcoholic or smoking status when the cohort was split into alcoholics/non-alcoholics or smokers/non-smokers (Table 3). There were no relationships between GM volumes and lifetime alcohol consumed or smoking pack-years (Table 3). The mean volumes in alcoholics were lower in five of the six regions examined while, in contrast, the volumes in the smoking group were higher in four of six regions (Table 3).

Discussion

This study sought to re-examine the findings of a previous pathological study (McCorkindale et al., 2016) by applying a higher resolution volumetric technique to those areas most commonly identified by neuroimaging studies as differing between smokers and controls (Durazzo et al., 2017; Luhar et al., 2013; Prom-Wormley et al., 2015). In addition to the DLPFC, the study evaluated areas with relatively small volumes where subtle volume changes could have previously gone undetected. The findings here are similar to the

previous pathological study with no smoking or alcohol effects in the DLPFC, insula, ACC, or thalamus. There were also no effects when the SFG, known to experience neuronal loss in chronic alcoholics (Krill et al., 1997; McCorkindale et al., 2016) or MFG, the two subregions of the DLPFC, were considered separately. Furthermore, there were no correlations between lifetime alcohol or tobacco consumption and regional volumes. Corrected volumes tended to be smaller among the alcoholics compared to non-alcoholics for most regions, while smokers tended to have larger values when compared to non-smokers. The latter result casts further doubts on tobacco smoking acting as an independent risk factor for brain atrophy. These trends in alcoholics may represent the direct effects of alcohol but are more likely a complex interaction between nutritional deficiencies (particularly thiamine), liver disease, and chronic alcohol intoxication (Butterworth, 1995). Interestingly, four nsALC had cerebellar vermal atrophy consistent with thiamine deficiency (Baker, Harding, Halliday, Krill, & Harper, 1999), as opposed to one sALC, whereas three sALC had astrocyte changes consistent with hepatic encephalopathy (G. T. Sutherland et al., 2014). It is not clear why sALC and nsALC differed in their neuropathological presentation, as the proportion of liver cirrhosis was similar between the groups, while a potential association between tobacco smoking and hepatic encephalopathy has not been described.

These results appear inconsistent with previous neuroimaging studies, yet among those MRI studies no one region was identified in more than 50% of the reports. Indeed, a meta-analysis (~200 smokers) in 2013 found significant loss in the ACC (Pan et al., 2013), while a larger meta-analysis (~750 smokers) in 2016, including the majority of studies in the 2013 report, found significant loss in multiple GM regions but not the ACC (M. T. Sutherland et al., 2016). A possible methodological explanation for the differences between pathological and MRI studies is that regional boundaries are defined differently. Automated parcellation techniques are used in MRI studies, while in pathological studies, each brain is manually parcellated using established anatomical landmarks. In addition, some MRI techniques such as voxel-based morphometry are whole-brain analyses rather than analyses within ROIs. It follows that if smoking-related GM changes are localized, then one technique may detect them but not the other, due to differences in how regions were defined.

An alternative explanation is that cigarette smoke affects individuals in subtly different ways. It is a complex composition of over 4000 different compounds (Dome, Lazary, Kalapos, & Rihmer, 2010) and it affects multiple organs including the liver and vasculature, which in turn can affect the brain. Furthermore, the composition of tobacco smoke varies between both brands and countries, leading to heterogeneous levels of compounds in the body following smoking (Ashraf, 2012; Benowitz et al., 2015; Gray et al., 2000). Future volumetric studies could examine this issue through the self-reporting of cigarette preferences and stratified analyses on individual compounds where data are available. The nitrosamine NNK has been shown to have additive effects with alcohol in rats, albeit at levels exceeding those estimated to arise from tobacco smoking (Papp-Peka, Tong, Krill, De La Monte, & Sutherland, 2016). These studies are being increasingly combined with new molecular techniques such as MALDI imaging to understand the mechanisms by which alcohol and perhaps tobacco smoking damages white matter in animal models (Yalcin, Nunez, Tong, & de la Monte, 2015) and humans (de la Monte et al., 2018).

In summary, the results support a previous global pathological study that found no alcohol, smoking, or interactive effects on regional GM volumes. Furthermore, although the GM volumes in alcoholics tended to be smaller, the volumes in smokers tended to be larger than controls. Therefore, the hypothesis that chronic tobacco smoking results in localized GM atrophy is not supported. Future pathological studies in larger cohorts using this improved volumetric technique are needed to confirm these results.

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Fig. 1. Comparison of volumetric techniques.

Images of the same brain slice of a right cerebral hemisphere at the anterior aspect of the hippocampus show the relative resolution of (A) manual grid technique (0.36 cm^2 per point) and (B) 'ImageJgrid'. The latter utilizes color images, a higher resolution counting grid (0.036 cm^2), and the flexibility of 'zooming in' to facilitate accurate feature and boundary identification

Table 1

Demographics and clinical characteristics.[#]

Parameter	Mean (S.D.)	nsCON	sCON	nsALC	sALC
Group size		16	9	8	11
Males/Females		8/8	5/4	5/3	5/6
Mean age (y)		54 (9)	56 (5)	52 (8)	56 (6)
Cause of Death ^a		13,2,1,0,0	9,0,0,0,0	0,1,4,1,2	5,3,1,1,1
Brain weight (g)		1408 (165)	1401 (121)	1293 (120)	1332 (180)
Postmortem interval (h)		29 (11)	26 [*] (11)	36 (14)	38 [*] (16)
Brain pH		6.6 (0.3)	6.5 (0.3)	6.7 (0.1)	6.6 (0.2)
Brain Pathology ^b		14,2,0,0,0	6,2,0,0,1 ^c	3,1,4,0,0	6,1,1,3,0
RNA integrity number		6.8 (1.6)	7.4 (0.5)	7.4 (1.0)	7.3 (1.3)
Body mass index		29.9 (5.7)	29.9 (4.4)	31.9 (18.0)	25.1 (6.4)
Liver score (0,1,2) ^d		8:7:0	4:5:0	0:4:4	4:4:3
Mean daily alcohol (g)		11 (13)	13 (11)	317 ^{**} (204)	189 ^{**} (104)
Lifetime alcohol (kg)		120 (135)	149 (146)	3503 ^{**} (2228)	2314 ^{**} (1478)
DSM-IV (Abuse:Dependence)				2:6	5:6
Pack-years			35 ^{**} (18)		39 ^{**} (18)

[#] = adapted from McCorkindale et al. (McCorkindale et al., 2016).^{*} = $p < 0.05$,^{**} = $p < 0.01$; compared to nsCON or for all alcoholics compared to all controls.^a = Cause of death of 0 = cardiac, 1 = respiratory, 2 = toxicity, 3 = hepatic, and 4 = other.^b = Brain pathology of 0 = normal, 1 = cerebrovascular disease, 2 = cerebellar vermal atrophy, 3 = hepatic encephalopathy (presence of type 2 Alzheimer astrocytes [G. T. Sutherland et al., 2014]), 4 = other.^c = Liver pathology score of 0 = normal, 1 = steatosis, and 2 = cirrhosis.^d = No liver score was available for one nsCON.

Table 2

Group-wise comparison of smoking and alcohol on regional volume.

Region	Normalized group volume				Two-way ANOVA (p value)			
	nsCON	sCON	nsALC	sALC	Overall	Alcohol	Smoking	Alcohol × smoking
ACC	1.00	1.09	1.01	0.97	0.66	0.44	0.70	0.32
Insula	1.00	1.07	0.88	1.03	0.14	0.13	0.05	0.48
Thalamus	1.00	1.04	0.93	0.96	0.34	0.08	0.40	0.86
SFG	1.00	1.05	1.06	1.04	0.90	0.68	0.89	0.59
MFG	1.00	0.92	0.90	0.88	0.37	0.25	0.42	0.55
DLPFC	1.00	0.98	0.98	0.96	0.95	0.73	0.71	0.99

Table 3

Comparisons of alcoholics and smokers and associations with lifetime consumption.

Region	Normalized group volume		Pearson correlation (p value)		
	Alcoholic	Non-alcoholic	Smoker	Non-smoker	Lifetime alcohol ^a Pack-years ^b
ACC	0.99	1.05	1.03	1.01	0.47 0.68
Insula	0.95	1.04	1.05	0.94	0.63 0.39
Thalamus	0.95	1.02	1.00	0.97	0.35 0.42
SFG	1.05	1.02	1.04	1.03	0.80 0.63
MFG	0.89	0.96	0.90	0.95	0.77 0.48
DLPFC	0.97	0.99	0.97	0.99	0.99 0.51

^aCorrelation between lifetime alcohol consumption and volume in the alcoholic group.

^bCorrelation between smoking pack-years and volume in the smoking group.