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The effects of chronic smoking on the pathology of alcohol-related brain damage

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ABSTRACT

Both pathological and neuroimaging studies demonstrate that chronic alcohol abuse causes brain atrophy with widespread white matter loss limited gray matter loss. Recent neuroimaging studies suggest that tobacco smoking also causes brain atrophy in both alcoholics and neurologically normal individuals; however, this has not been confirmed pathologically. In this study, the effects of smoking and the potential additive effects of concomitant alcohol and tobacco consumption were investigated in autopsied human brains. A total of 44 cases and controls were divided into four groups: 16 non-smoking controls, nine smoking controls, eight non-smoking alcoholics, and 11 smoking alcoholics. The volumes of 26 gray and white matter regions were measured using an established point-counting technique. The results showed trends for widespread white matter loss in alcoholics ($p < 0.007$) but no effect on gray matter regions. In contrast, smoking alone had no effect on brain atrophy and the combination of smoking and alcohol showed no additional effect. Neuronal density was analyzed as a more sensitive assay of gray matter integrity. Similar to the volumetric analysis, there was a reduction in neurons (29%) in the prefrontal cortex of alcoholics, albeit this was only a trend when adjusted for potential confounders ($p < 0.06$). There were no smoking or combinatorial effects on neuronal density in any of the three regions examined. These results do not support the hypothesis that smoking exacerbates alcohol-related brain damage. The trends here support previous studies that alcohol-related brain damage is characterized by focal neuronal loss and generalized white matter atrophy. These disparate effects suggest that two different pathogenic mechanisms may be operating in the alcoholic brain. Future studies using ultrastructural or molecular techniques will be required to determine if smoking has more subtle effects on the brain and how chronic alcohol consumption leads to widespread white matter loss.

Introduction

The deleterious effects of alcohol consumption cost hundreds of billions of dollars per year at the global level with an estimated annual burden of $15 billion in Australia alone (Collins & Lapsley, 2008). Alcohol abuse is a major public health burden, with a World Health Organisation (WHO) report estimating the global mortality rate attributable to alcohol at 3.3 million per year (WHO, 2014). The majority of the mortality attributable to alcohol is from cardiovascular disease, trauma, gastrointestinal disease, and cancers. In addition, alcohol adversely affects the brain via direct and indirect mechanisms. In terms of its direct effects, amphipathic alcohol rapidly crosses the blood–brain barrier. Once inside the brain, alcohol can disrupt membrane integrity and act directly on receptors such as the N-methyl-d-aspartate (NMDA) and γ-aminobutyric acid (GABA) receptors (Kumar et al., 2009). In addition to these direct effects, comorbid liver damage and nutritional deficiencies, particularly thiamine deficiency, affect the brain and are common in individuals with alcohol use disorders (Sutherland, Sheedy, & Kril, 2014).

Deficits across a range of cognitive domains such as memory, attention (Kopera et al., 2012), and executive skills (Munro, Saxton, & Butters, 2000; Sullivan, Rosenbloom, & Pfefferbaum, 2000) have been shown to occur in alcoholics. Impairments in higher order functions such as executive skills are indicative of damage to the frontal lobe. Magnetic resonance imaging (MRI) in alcoholics has confirmed this by demonstrating that the most pronounced gray matter (GM) loss is from the frontal lobe (Pfefferbaum, Sullivan, Mathalon, & Lim, 1997; Ratti et al., 1999). In contrast, early

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pathological studies found more white matter (WM) loss than GM loss (Harper & Kril, 1985; de la Monte, 1988), and further MRI studies have since confirmed the predominance of WM atrophy (Rosenbloom & Pfefferbaum, 2008). A more recent pathological study, using unbiased stereological methods, found only subtle losses in WM volumes in chronic alcoholics that did not reach statistical significance (Kril, Halliday, Svoboda, & Cartwright, 1997). Kril and colleagues reported no changes in GM volumes but there was a significant reduction in neuron number in the prefrontal cortex.

WM atrophy has largely been thought of as secondary to neuronal loss through Wallerian degeneration. However, an alternative hypothesis is that alcohol has a primary effect on the WM with secondary loss of neurons and GM atrophy (Harper, Kril, & Daly, 1988). The reversibility of cognitive deficits following abstinence from alcohol (Kopera et al., 2012) is consistent with WM damage or a disruption of neuronal connectivity (dearborization and neuronal shrinkage) rather than widespread neuronal loss (Harper & Corbett, 1990).

In addition to nutritional deficiencies and liver dysfunction, chronic smoking is a common comorbidity in alcoholics. It has been estimated that approximately 80% of alcoholics are also chronic smokers (Kalman, Morissette, & George, 2005). There are approximately one billion active smokers in the world, with chronic smoking accounting for 9% of the global annual mortality rate or roughly double the effects of alcohol (WHO, 2012). Tobacco smoke is a complex and dynamic mixture of more than 4000 compounds, many of which can have toxic effects (Dome, Lazary, Kalapos, & Rihmer, 2010). As a result, smoking has widespread effects including cancer of multiple organs, and damage to the cardiovascular system and immune systems (WHO, 2012).

The broad effects of tobacco smoke throughout the body along with known (such as nicotine) and suspected neuroactive components raise the possibility that chronic smoking may cause brain atrophy. Evidence has been accumulating that this is the case, with a number of neuroimaging and cognitive studies reporting findings of brain dysfunction in chronic smokers. While studies into the acute effects of nicotine show increased cognitive performance in smokers (Heishman, Kleykamp, & Singleton, 2010), studies in chronic smokers have revealed cognitive deficits across a range of domains (Durazzo, Cardenas, Studholme, Weiner, & Meyerhoff, 2007) and in a range of different age groups (Deary et al., 2003; Durazzo, Meyerhoff, & Nixon, 2012; Jacobsen et al., 2005). These deficits in brain function are indicative of structural damage, with evidence from MRI studies of both WM and GM loss in chronic smokers. Unlike alcohol, where there is a relatively well-defined pattern of GM loss, there is not a clear picture of GM loss in smokers. While a number of MRI studies have reported GM loss in the frontal lobe (Brody et al., 2004; Fritz et al., 2014; Gallinat et al., 2006), other studies have found GM loss in the temporal lobe, occipital lobe, parietal lobe, cerebellum, and in some subcortical structures (Das, Cherbuin, Anstey, Sachdev, & Easteal, 2012; Gallinat et al., 2006; Gazdzinski et al., 2005; Hanlon et al., 2014; Kühn et al., 2012). A recent meta-analysis of seven MRI studies (213 smokers and 205 non-smokers) found that the only common site with significant GM volume loss was the anterior cingulate cortex (ACC) (Pan et al., 2013).

The inconsistency in results extends to MRI studies of WM with reports of increases, decreases, and no change in regional WM volumes (Fritz et al., 2014; Gallinat et al., 2006; Yu, Zhao, & Lu, 2011; Zhang et al., 2011). Increases in WM have been found in the temporal lobe (Gazdzinski et al., 2005) and in the cingulate cortex (Yu et al., 2011), while decreases in WM have been reported in frontal regions of the brain (Luhar, Sawyer, Gravitz, Ruiz, & Oscar-Berman, 2013; Zhang et al., 2011).

The majority of past studies into the effects of alcohol on the brain have not taken smoking status into account. This raises the possibility that some of the effects of smoking may have been mistakenly attributed to alcohol. A study in alcoholics by Gazdzinski and colleagues found significant GM losses from the temporal, parietal, and occipital lobes (Gazdzinski et al., 2005). These are not regions typically associated with alcohol-related brain damage (ARBD) and the authors postulated that participant smoking status may account for some of the differences. They retrospectively analyzed their results and found significant reductions in temporal, parietal, and occipital lobe volumes in smoking alcoholics (sALC) compared with non-smoking alcoholics (nsALC) (Durazzo et al., 2007). Following on from this, other studies have found evidence of a greater loss of GM and/or WM in sALC compared to nsALC (Durazzo et al., 2014; Luhar et al., 2013).

A mix of both pathological and MRI studies has been used to assess volume changes in ARBD; however, no pathological investigations of smokers have been published. The current study explores the effects of chronic alcohol consumption and smoking in autopsied brain tissue.

Methods

Ethics and case collection

This study was undertaken following ethics approval from the Human Research Ethics Committee of the University of Sydney (HREC #2014/732). Tissue was 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 study included 16 non-smoking controls (nsCON), nine non-smoking controls (nsCON), 11 smoking alcoholics (sALC), and eight non-smoking alcoholics (nsALC).

Classification

All cases were classified retrospectively based on the donor's clinical and pathological records as previously described (Sutherland et al., 2016). Briefly, the NSW BTRC classifies a subject as 'Alcoholic' when there has been the consumption of greater than 80 g of ethanol per day for the majority of adult life. Lifetime alcohol was calculated using the following formula: number of years drinking $\times$ mean pure ethanol consumed per day (g) $\times$ 365 (days). Any recorded periods of abstinence exceeding 6 months in duration were subtracted from the number of drinking years. Smokers were defined as individuals who had been current smokers who smoked every day (no pack-year threshold). A non-smoker was an individual who had no reported smoking history. One pack-year is equivalent to smoking one pack of cigarettes (20 cigarettes) per day for one year.

Autopsy procedure

The detailed protocol for the processing of the brain tissue has been published previously (Sutherland et al., 2016). Briefly, the right and left hemispheres of each brain were randomly assigned as fixed or frozen. One hemisphere was fixed in 10% formalin for 3 weeks. The length of the hemisphere was determined prior to embedding in agar, and sectioning was done at 3 mm intervals in the coronal plane using a rotary meat slicer. The digital images of serial coronal sections were used for the volumetric aspect of the study. Following imaging, standardized tissue blocks were removed and embedded in paraffin.