Moderate Alcohol Consumption and the Brain: Friend or Foe?

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Abstract

Background

Alcohol consumption is widespread and increasing in some sectors of society. Whilst adverse effects on the brain of chronic heavy use are well characterised, the effects of more moderate use are poorly understood. Previous work has lacked prospectively gathered data on alcohol use. Robust evidence of harm would have important public health implications.

Methods

Associations between self-reported alcohol consumption data gathered over a thirty-year period (1985-2015), neuroimaging markers of brain structure and function, and cognition were examined in community dwelling older adults in the Whitehall II cohort (n=550). Structural, diffusion tensor, and resting state functional magnetic resonance imaging was performed in 2012 to 2015. Cognitive performance was determined cross-sectionally across multiple domains at the time of scanning and longitudinally throughout the study.

Results

No participants scored as alcohol dependent on the CAGE screening questionnaire. Higher alcohol consumption throughout the study was associated with hippocampal

atrophy (even in those drinking 14-21 units (112-<168g) weekly), lower white matter integrity of the corpus callosum, increased functional connectivity within the Default Mode Network, and faster decline of lexical fluency. No protective effects of light drinking were found. Age, sex, premorbid IQ and white matter integrity predicted better verbal memory performance in the presence of small hippocampi.

Discussion

This thesis describes three novel neuroimaging associations with moderate alcohol consumption. Alcohol, even in small quantities, may represent a modifiable risk factor for adverse brain outcomes and faster cognitive decline. Learning effects or confounding by higher premorbid intelligence or social class may explain previous findings of a protective effect of moderate alcohol consumption.

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List of abbreviations

AD	axial diffusivity
ALDH2	aldehyde dehydrogenase 2
ApoE	apolipoprotein
AUDIT	alcohol use disorders identification test
B_0	main magnetic field in MRI
BIANCA	Brain Intensity AbNormality Classification Algorithm
BMI	body mass index
BOLD	blood-oxygen-level dependent
CAGE	alcohol screening questionnaire
CC-FA	corpus callosum fractional anisotropy
CES-D	Centre for Epidemiological Studies Depression Scale
CI	confidence interval
CT	computerised tomography
DMN	default mode network
DTI	diffusion tensor imaging
DWMC	deep white matter changes
FA	fractional anisotropy
FIRST	FMRIB's automated model-based segmentation/registration tool
FIX	FMRIB's ICA-based X-noiseifier
FLAIR	fluid-attenuated inversion recovery
FLIRT	FMRIB's linear image registration tool
FMRI	functional magnetic resonance imaging
FSIQ	full-scale intelligence quotient
FSL	FMRIB software library
GABA	Gamma-Amino Butyric acid
GLM	generalized linear model
HA	hippocampal atrophy
HAROLD	hemispheric asymmetry reduction in older adults
HR	hazard ratio
HVLT	Hopkins verbal learning test
ICA	independent components analysis
ICV	intracranial volume
KS	Korsakoff's syndrome
MCI	mild cognitive impairment
MCV	mean corpuscular volume
MD	mean diffusivity
MELODIC	multivariate exploratory linear optimized decomposition into independent components
MMSE	mini-mental state examination
MoCA	Montreal cognitive assessment
MRI	magnetic resonance imaging
NMDA	N-methyl-D-aspartate
OR	odds ratio
PASA	posterior to anterior shift in aging
PET	positron emission tomography

PVWMC	periventricular white matter changes
RD	radial diffusivity
RSN	resting state networks
SCID	structured clinical interview for DSM IV disorders
SD	standard deviation
SEM	structured equation modelling
SPECT	single photon emission computed tomography
TBSS	tract-based spatial statistics
TFCE	threshold-free cluster enhancement
TMT	trail-making test
VBM	voxel-based morphometry
WKS	Wernicke-Korsakoff syndrome
WMH	white matter hyperintensity
γGT	Gamma Glutamyl Transferase

List of publications

Topiwala et al. Moderate alcohol consumption as a risk factor for adverse brain outcomes and cognitive decline. *Under review BMJ.*

Topiwala et al. Resilience and MRI correlates of clinical cognitive impairment in community dwelling elders. *BJPsych* 2015 DOI: 10.1192/bjp.bp.114.152363

Contributions statement

Professor Klaus Ebmeier, together with Professors Mika Kivimaki and Archana Singh-Manoux, conceived the Whitehall II Imaging Sub-Study.

The Whitehall II team at University College London collected longitudinal data from preceding Whitehall II phases analysed in this thesis.

Dr Anya Topiwala joined the study after the original study had been designed. Professor Ebmeier, Drs Claire Sexton and Eniko Zsoldos determined the psychometric assessment content based on knowledge of the literature and expert consensus. Dr Anya Topiwala played a role, with others in the team of researchers and assistants (especially Dr Eniko Zsoldos and Abda Mahmood) in neuropsychological testing of participants.

Pre-processing of subjects' magnetic resonance images was kindly done by two post-doctoral researchers – Drs Nico Filippini and Sana Suri.

Dr Charlotte Allan, Dr Vyara Valkonova, Peggy Fooks and Dr Anya Topiwala performed the visual ratings of the MRI scans.

Dr Ludovica Griffanti developed the Bianca tool for the autonomic extraction of white matter hyperintensity volumes.

Dr Anya Topiwala performed all subsequent analyses of MRI, alcohol and cognitive data. This doctoral manuscript is entirely the work of Dr Anya Topiwala.

Overall supervision of this work was by Professor Klaus Ebmeier and Professor Clare Mackay.

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Chapter 1 – Introduction

Alcohol use is widespread and increasing across the developed world.[1-3]
Whilst chronic heavy consumption of alcohol is known to be associated with Korsakoff's syndrome and alcoholic dementia, the effect of more moderate use on brain health is poorly understood. Light drinking is widely regarded as either harmless or even beneficial. This belief has been supported by findings in cardiovascular disease,[4,5,6] and epidemiological studies reporting lower dementia risk.[7, 8] Existing research examining cognitive outcomes has used retrospective collection of alcohol data and controlled for few potential confounding factors. Alcohol consumption is modifiable and hence robust determination of associations with poor brain outcomes would have vital public health implications, in a world under increasing social and financial pressures with an aging population.

Advanced imaging techniques, such as Magnetic Resonance Imaging (MRI), allow for detailed investigation of the aging brain in vivo. However, brain-imaging work has thus far failed to provide a convincing neural correlate that could underpin any protective effect of light drinking. Cross-sectional studies have produced conflicting results to date and there are no studies with longitudinal data on alcohol consumption together with detailed neuroimaging in the existing literature.

Chapter 2 of this thesis provides a summary of research into relationships between brain structure and function and acute and chronic alcohol intake, of heavy and moderate amounts. It surmises that the effects of moderate alcohol consumption on

the brain are poorly understood. Macrostructural grey and white matter findings are contradictory. Similarly, some studies show light drinking is protective against cognitive impairment but others have failed to replicate this. Finally, there are no published studies, to my knowledge, examining microstructural white matter indices or resting state functional connectivity in non-dependent drinkers. The overall objective of this thesis is to investigate the relationship between moderate alcohol consumption and brain structure and function. To achieve this, I aim to answer three questions, using data from the Whitehall II cohort. This study has a rich thirty-year dataset of longitudinal social, behavioural and biological variables including alcohol intake. Analyses for this thesis will use state-of-the-art neuroimaging and cognitive test performance as outcome measures. A brief synopsis of the Whitehall II cohort and of the neuroimaging methods used in this study forms Chapter 3.

1. ***Is there a relationship between non-dependent alcohol consumption and grey matter in the brain?***

Chapter 4 summarises alcohol consumption of the cohort over the study, including in relation to sociodemographic factors. The relationship between alcohol consumption over the study and grey matter is investigated. Correlations are examined initially using an unbiased voxel-wise approach across the whole brain, and post-hoc using manual and automated approaches to characterise hippocampal size.

2. ***Does alcohol intake correlate with macro- or micro-structural white matter indices?***

Chapter 5 focuses on the associations between alcohol intake and macro- and micro-structural white matter indices. Total white matter volume and white matter hyperintensities, visually rated and automatically extracted volumes, are used as measures of macrostructure. Diffusion tensor imaging indices are used to infer microstructural integrity.

3. ***What is the relationship between alcohol consumption and brain function, determined by resting state functional connectivity and cognitive performance?***

The link with brain function is assessed using resting state functional connectivity and cognitive test performance in Chapter 6. Connectivity within the Default Mode Network is examined, as previous studies have reported changes in those chronically drinking large amounts of alcohol. Correlations between alcohol consumption and functional connectivity between nodes of resting state networks are also examined. Cognitive outcomes include cross-sectional performance at the time of scanning, and additionally longitudinal trends on a more limited range of tests over a 25-year period.

Chapter 7 explores the concept of resilience, and how this may explain any mismatch between associations of alcohol with brain structure and function found in the preceding chapters. This analysis identifies sociodemographic and brain factors that are protective against the adverse effects of smaller hippocampal size on verbal

memory in this sample. Finally, Chapter 8 discusses the findings from the thesis in synthesis, including limitations, clinical implications and opportunities for further research.

Summary of main objectives

1. Is there a relationship between non-dependent alcohol consumption and brain structure?

2. What is the relationship between brain function and alcohol consumption?

Chapter 2 - Alcohol and the brain

This chapter reviews the evidence for the effects of alcohol on the brain. It includes data on current consumption patterns, government guidelines on drinking, and diagnoses relating to alcohol intake. A summary of the literature on the acute and chronic effects on brain function and structure is given. Finally, methodological limitations of existing research together with corresponding suggestions for future research are discussed.

2.1 Introduction

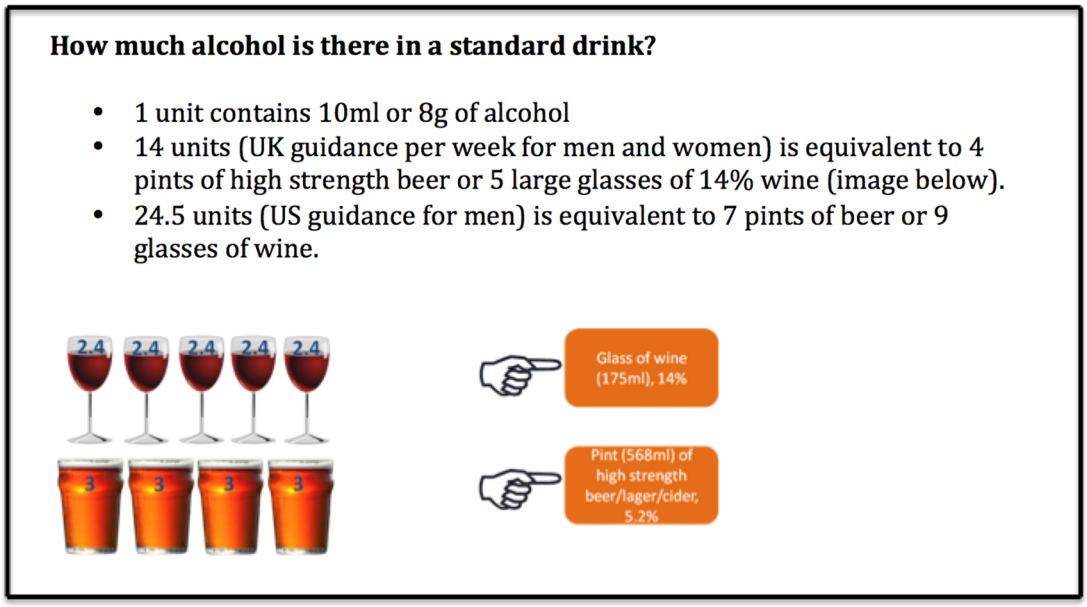
Excessive alcohol use is thought to underlie 5.9% of deaths globally.[1] Latest figures suggest that 58% of the UK population are drinking in excess of existing safe limits,[9] with 5% men and 4% of women consuming hazardous amounts (>50 units (400g) for men and >35 units (280g) for women weekly). Drinking in women and older adults of both sexes has been increasing.[10, 11] Those over 65 years of age are now more likely than any other age group to have drunk on at least five days in the preceding week.[9] Consumption also differs according to socioeconomic factors. The prevalence and frequency of drinking is highest in the least deprived areas of the UK and those in the highest income quintiles, whereas binge drinking is higher in more deprived areas. This, combined with confounding factors such as poverty and comorbid substance use, may explain the 'harm paradox', that most alcohol-related harm is in areas of deprivation, despite lower average consumption.[12] Alcohol related harm is estimated

to cost England around £21bn per year, with £3.5bn to the NHS, £11bn tackling alcohol-related crime and £7.3bn from lost work days and productivity costs.[13]

Health concerns associated with alcohol use have focused on liver dysfunction and more recently cancer.[14] The public are largely ignorant of the potential risks of drinking with regards to cognition, in contrast to widespread knowledge of effects on the liver.[15] Previous research has focused on the effects of chronic heavy drinking on the brain, and the impact of more moderate consumption is poorly understood. Robust evidence of harm would have an important impact on health public policy.

2.2 Government guidelines

Recommended drinking guidelines have remained unchanged in the UK from 1987 until 2016. Safe limits for men were set at 21 units (168g) and for women 14 units (112g) per week pre-2016. Recent evidence of associations with cancer risk[14] at lower levels prompted revision of UK government alcohol guidance to 14 units (112g) for both sexes. This has not been adopted worldwide however, with e.g. US Federal Dietary guidelines (2015-2020) allowing up to 24.5 units (196g) weekly for men.[16]



Images ©Alcohol Concern 2016

Figure 2.1 Alcohol units in standard drinks

2.3 Diagnoses related to alcohol consumption

Acute alcohol intoxication is a transient condition resulting in disturbances in conscious level, cognition, perception, affect or behaviour. Effects are closely related to dose levels, and disappear in the absence of further drinking. Definitions of pathological drinking and its sequelae differ between diagnostic classifications. ICD 10[17] divides excessive intake into harmful use, defined as a pattern of drinking that is causing damage to physical or mental health, and alcohol dependence. To meet criteria for dependence an individual has to experience ≥3 of the following symptoms for ≥1 month:

- Strong desire or sense of compulsion to drink
- Impaired capacity to control drinking

- Physiological withdrawal state
- Tolerance to the effects of alcohol
- Preoccupation with drinking to the exclusion of other interests
- Persistent drinking despite evidence of harm

In DSM IV, harmful use of alcohol is divided into alcohol abuse and alcohol dependence. DSM-5 has combined these two disorders under the umbrella term Alcohol Use Disorder. This is subdivided by severity according to the presence of symptoms from the list below (mild ≥2, moderate ≥4, severe ≥6):

- Have there been instances when you have drunk more or longer than you intended?
- More than once have you wanted to reduce your drinking but couldn't?
- Have you spent a lot of time drinking or getting over the after-effects?
- Have you wanted a drink so badly you couldn't think of anything else?
- Does drinking interfere with other aspects of your life?
- Have you continued to drink despite it causing trouble with your friends or family?
- Have you reduced other activities in order to drink?
- Have you more than once gotten into situations due to drinking that increased your chances of getting hurt?
- Have you continued to drink even though it was making you feel depressed/anxious/adding to another health problem, or after a memory blackout?
- Do you have to drink more than previously to get the same effect?

- Have you found that when the effects of alcohol were wearing off you had withdrawal symptoms?

Excessive alcohol consumption can also lead to a number of conditions with psychiatric symptoms, including psychotic disorders and delirium. One relevant disorder with regards this thesis, is amnestic syndrome (including Korsakoff's syndrome (KS)) relating to chronic (heavy) alcohol intake. This is characterised by a chronic prominent impairment in recent memory, and sometimes remote memory, with relative preservation of immediate recall, and will be discussed in further detail below.[18]

2.4 Acute intoxication

Acute intoxication with alcohol results in severe behavioural disinhibition, disrupted socio-emotional processing and impaired psychomotor performance.
The molecular basis for these actions is thought to include NMDA and GABA-A receptors.[19] High doses of alcohol reduce prefrontal and temporal lobe function, including planning, verbal fluency,[20, 21] memory[22] and complex motor control.[23] The pattern of impairment has been compared to that seen in hippocampal damage.[24] A number of neuroimaging studies have investigated the effects of acute alcohol ingestion. Interpretation of these results can be complicated as alcohol causes vasodilation, which may impact the BOLD signal. However, there is a suggestion that alcohol shifts activity away from cortical to limbic regions,[25, 26] and additionally increases prefrontal activity.[27, 28] Resting state functional connectivity studies have

reported increased connectivity in the temporal lobe, anterior cingulate, brainstem,[29] and visual cortex.[30]

2.5 Chronic alcohol consumption

2.5.1 Heavy drinking

2.5.1.1 Clinical sequelae

Chronic heavy drinking is associated with a number of serious neurological conditions, one of which is Wernicke's encephalopathy. This is an acute, often lethal, but potentially reversible neurological disorder caused by a severe deficit in thiamine (vitamin B1) aggravated by carbohydrate overload. It is characterized by the clinical triad of oculomotor disturbance, cerebellar dysfunction and altered mental state. Chronic alcoholics are at risk of this due to multiple potential factors including: poor diet, impaired absorption and altered metabolism of thiamine.[31] The estimated mortality of Wernicke's encephalopathy is 17%.[32] Untreated, 80% of survivors of Wernicke's encephalopathy will progress to the severe and usually permanent Korsakoff's syndrome.[33, 34] This is characterized by anterograde (and retrograde) amnesia, and frequently occurs together with prefrontal deficits.[35, 36] Even in 'uncomplicated' alcoholism, without Wernicke's encephalopathy or Korsakoff's syndrome, over 80% of individuals have executive deficits,[37, 38] although of a lesser severity.[39] A strong evidence base supports the association of heavy alcohol consumption over long periods with increased dementia risk and cognitive decline.[18, 40-42] There is some debate as to whether primary alcohol dementia exists, due to direct neurotoxicity, or whether cognitive deficits are entirely secondary to an additional

pathology for example due to thiamine deficiency. It is difficult to disentangle these possibilities, particularly in view of multiple confounders in dependent drinkers, such as smoking, comorbid substance abuse and increased vascular risk. Similarly, establishing the prevalence of alcohol-related dementia is difficult, due to a lack of operationally defined diagnostic criteria. Estimates differ from 9-22% of all dementia patients,[43] or even higher in nursing homes. Dementia of alcoholic aetiology may be particularly prevalent in earlier onset dementia.[44] There is no clear answer as to what level of consumption is sufficient to cause Korsakoff's syndrome. Oslin et al. have suggested a five-year intake greater than 35 units weekly for men and 28 units for women, but this needs validation.[45] Thresholds are likely to be different according to sex, comorbid conditions and genetic susceptibility.

2.5.1.2 Brain findings

Cortical atrophy

Brain atrophy in chronic alcoholism is well described (see Kril and Halliday for a review).[46] The frontal lobes are thought to be particularly vulnerable. Kril et al. found frontal cortex reductions of 23% in uncomplicated alcoholics,[47] replicating earlier findings by Harper et al.[48] Non-GABAergic pyramidal neurons are thought particularly vulnerable.[49]

Early neuroimaging studies using computerised tomography (CT) corroborated the neuropathological studies, showing cortical shrinkage and ventricular dilatation.[50-52]

Jernigan et al. compared alcoholics' brains with those of healthy controls, and found volume reductions in diencephalon, caudate, frontal and parietal cortices and the medial temporal lobe.[53] Subsequent MRI studies have also reported widespread cortical atophy,[54] which may particularly affect the frontal lobes.[55] Interestingly, longitudinal MRI has shown a degree of tissue recovery, especially of white matter, on abstinence.[56-58]

Hippocampal atrophy

The hippocampus is consistently affected in animal models of chronic alcoholism. Riley and Walker found reduced dendritic spines on hippocampal pyramidal cells and dentate granule cells in mice given an ethanol diet for four months.[59] The same group also found reduced numbers of hippocampal pyramidal (CA1) and dentate gyrus granule cells in rats with ethanol-containing diets compared to controls.[60] Cell loss may be between 20-40% depending on the region.[61] Furthermore, experiments designed to simulate binge drinking, where animals are exposed to intermittent peaking alcohol concentrations, have shown more deleterious effects on the hippocampus compared to continuous (but higher) amounts.[62]

Despite the preclinical data, evidence for hippocampal involvement in humans is less convincing. However, Bengochea and Gonzalo did describe reduced hippocampal neurons in 21 alcoholics compared to controls. Furthermore, they found no change in the size of hippocampal neuron nuclei, suggesting a lack of reaction to the insult.[63] In contrast, others have not described hippocampal cell loss.[47] Harding et al. did report reduced hippocampal volume in alcoholics, but found this was caused by a reduction in white matter not neuron loss.[64]

Two MRI studies report reductions in hippocampal volume in alcoholics. Agartz et al. found a reduction in absolute hippocampal volume, but this was proportional to global volume loss.[65] Sullivan et al., however, did report reduced anterior hippocampal volume in chronic alcoholism, after accounting for temporal lobe grey matter deficits.[66]

Memory dysfunction (particularly anterograde) in KS is thought to result from disruption primarily to the hippocampal-anterior thalamic axis, especially of white matter connections between these.[67]

Amygdala

The amygdala may also have a role.[68, 69] Rats given ethanol in their diets have reduced neural dendrites in the amygdala.[70] Reduced amygdalae volume has also been described in human alcoholics, and including those with Wernicke-Korsakoff's syndrome.[47, 70]

Cerebellum

The cerebellum, known for its importance in motor function, is now additionally thought to play a role in memory disturbance.[71, 72] A number of neuropathological studies have established cerebellar atrophy in chronic alcohol abuse.[73] 42% of alcoholic subjects were found to have atrophy of the superior vermis in an early study by Torvik and Torp.[74] The molecular layer has been suggested as particularly vulnerable to damage.[75] Imaging studies have also reported cerebellar atrophy, particularly of the vermis, which correlates with postural instability.[50, 76]

Hypothalamus and thalamus

Mamillary body atrophy is almost universal in chronic alcoholism.[77] However, animal studies suggest that other hypothalamic nuclei, particularly the supraoptic and paraventricular, may also be affected.[78, 79] Additionally, mice fed alcohol in their diets for 6-7 months have reduced volumes of medial dorsal and anterior thalamic nuclei.[80] Anterior thalamic nuclei may be affected in humans.[81]

White matter including corpus callosum

Several pre-clinical and post-mortem studies have provided evidence of prominent white matter loss following chronic heavy alcohol use, which may exceed grey matter loss.[77, 82, 83] The frontal lobes and cerebellum are particularly vulnerable.[48, 84,75] Furthermore, white matter loss in the temporal lobes has been linked to alcohol withdrawal seizures.[85] In WKS, the degree of white matter loss has been shown to inversely correlate with daily alcohol consumption.[47]

The corpus callosum, a major commissural tract, may be particularly vulnerable. In their port-mortem study, Harper and Kril reported that corpus callosum thickness in a group of alcoholics (3.19mm) was significantly reduced compared to controls (4.02mm).[86] Tarnowska-Dziduszko et al. found 57 of a total of 66 ‘chronic alcoholics’ had atrophy of the corpus callosum.[87] One study in adult rats who were fed ethanol-enriched diets with pyrithiamine or thiamine, has suggested that it is thiamine deficiency that is responsible for the corpus callosum atrophy.[88]

MRI studies have also replicated this finding of a reduction in corpus callosum volume, which does not appear to be localized to a specific region of the tract.[89, 90] More recently, with the advent of diffusion tensor imaging, microstructural disturbances of the corpus callosum have been observed in aloholics.[91, 92]

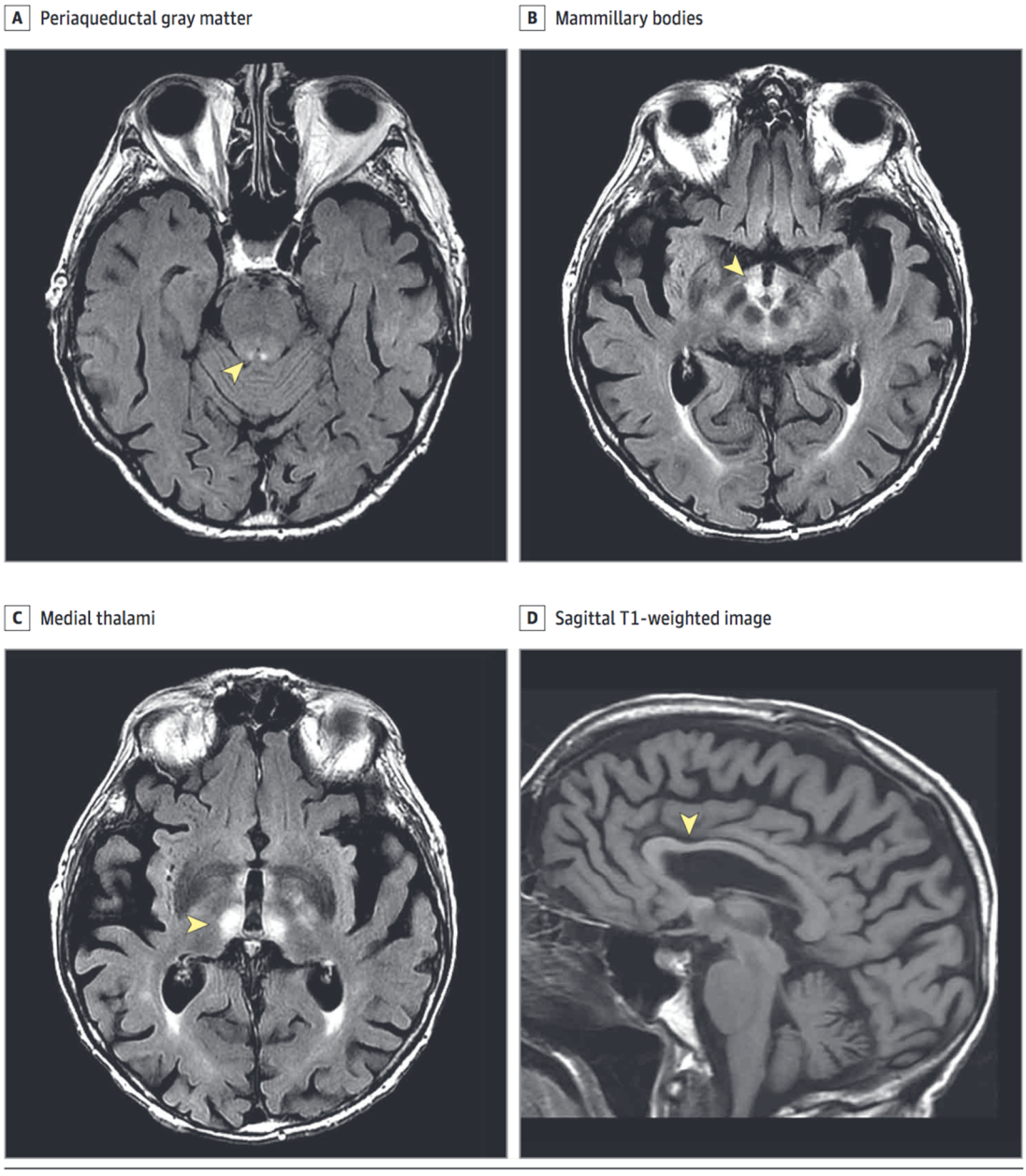


Figure 2.2 Characteristic MRI findings in Wernicke's encephalopathy/Korsakoff's syndrome.[93] Hyperintensities on FLAIR of the mammillary bodies (B), medial thalamus (C), periaqueductal grey (A) and midbrain tectum. In chronic Korsakoff's syndrome, generalized atrophy and of the corpus callosum (D) and mammillary bodies may be evident.

Metabolic imaging

Reduced cerebral blood flow has been described using xenon-CT,[94] SPECT,[95] and PET imaging.[96] In keeping with structural findings, hypometabolism may be frontally focused,[97] but subcortical grey matter including the hippocampi have been additionally implicated.[98]

2.5.1.3 Molecular mechanisms of brain damage

A number of different mechanisms have been proposed to account for the deleterious effects of chronically high alcohol consumption on the brain. Damage to the brain from chronically high levels of alcohol is thought to result, at least in part, from thiamine deficiency. In animal models, thiamine deficiency causes thalamic and mammillary body lesions.[99, 100] Thiamine is a co-factor, involved in the metabolism of lipids, glucose, amino acids and neurotransmitters. Deficiency of thiamine has been linked to oxidative stress, excitotoxicity, inflammatory responses, dysfunction of the blood-brain barrier, and lactic acidosis.[101] Folate deficiency is also common in alcoholics. Folate is needed for the conversion of homocysteine to methionine. Homocysteine is a full and partial agonist at NMDA and glycine receptors, and has been implicated mechanistically in alcohol-related brain damage.[102] Ethanol could be also be directly neurotoxic. Alcohol inhibits glutamate receptors. Chronic exposure induces up regulation of NMDA receptors, which leaves neurons vulnerable to excitotoxicity from massive calcium influx.[103] This may be driven especially by repeated binges and withdrawal. Cell death could also be via neuroinflammation, the induction of inflammatory mediators and microglia.[104-106] Additionally, alcohol-related liver dysfunction could mean that neurotoxic substances such as ammonia and manganese are not removed from the blood, resulting in cerebral effects.[107-109]

Samples from the frontal cortex of alcohol-dependent individuals have found differences in gene expression relating to multiple cellular functions, including cell adhesion and myelination.[110, 111] Others have found differences in protein expression according to spatial location in the brain, suggesting there may be region-specific pathological pathways.[112, 113]

2.5.2 Moderate drinking

In contrast to heavy consumption, the long-term effects of moderate alcohol consumption on cognition are poorly understood. Some authors have suggested an inverted U-shaped relationship of cognitive function with alcohol use, similar to that with cardiovascular disease.[4] Ethanol's inhibition of platelet aggregation, reduction in inflammatory markers, and alteration of plasma lipid profile have been proposed as underlying mechanisms for protection from cardiovascular disease, in addition to the antioxidant action of polyphenols present in some alcoholic drinks.[114]

2.5.2.1 Risk of dementia

Several large epidemiological studies have reported a reduced risk of dementia (of vascular and non-vascular aetiology) in light- to moderate drinkers compared with abstainers. For example, Ruitenberg et al. found those drinking 1-3 drinks per day had a reduced risk of dementia (HR 0.58 95% CI 0.38-0.9) compared with abstainers in the Rotterdam study.[8] Subjects were over 55 years old at baseline, and followed up for an average of 6 years. Dementia was excluded at baseline using a variety of screening tests, such as the MMSE, GMS and CAMCOG. In the French prospective PAQUID study, individuals who were at least 65 years old at baseline were followed

up for three years. Both 'light' wine drinkers (<1-2 drinks (16-32g) daily) and 'moderate' wine drinkers (3-4 drinks (46-64g) daily) had reduced odds of incident Alzheimer's disease.[115] Similarly, Mukamal et al. reported a lower dementia risk in those drinking 1-6 drinks (14-84g) per day in the Cardiovascular Health Study, using a nested case-control design.[7] Those thought to have dementia were excluded at baseline.

Meta-analyses of such studies have cited a protective effect of light to moderate alcohol consumption on the risk of dementia including Alzheimer's disease.[116, 117] Anstey et al. analysed 15 prospective studies and reported a pooled risk reduction of 25-8% with late life drinking. They cautioned however that it was unclear whether this represented selection bias, i.e. those still drinking in late life were likely to be those *without* dementia, or a genuine protective effect.

2.5.2.2 Risk of cognitive impairment or decline

In a large study of women in the USA, the Nurses Health Study, Stampfer et al. followed up women aged 30-55 years at baseline, using a cognitive test modelled on the MMSE and later with a verbal recall task. Those consuming one drink daily had better cognitive scores than non-drinkers at baseline, but also less cognitive decline at two year follow-up.[118] The analysis was controlled for age, education level, social integration and cardiovascular risk factors. In another US study, Ganguli et al. followed up individuals aged at least 65 years at baseline for two years on a range of cognitive tests, including the MMSE, TMT, word recall and category fluency. Both minimal (defined as <1 drink (14g) monthly) and moderate (<1 drink (14g) daily) drinkers displayed less decline on the MMSE and traits tests compared to

abstainers.[119] Interestingly, the protective effects were more pronounced when the comparison group of abstainers also included former drinkers rather than only lifelong abstainers.

However, not all studies have replicated a protective effect of light drinking on cognition. Lobo et al. did not find less MMSE decline in light drinkers than in abstainers.[120] In a Mendelian randomization study using ALDH2 genotype to determine alcohol consumption in the Guangzhou Biobank study, Yeung et al. found no association between alcohol and delayed verbal recall.[121] Others have found a protective effect of moderate alcohol use only in those not carrying the ApoE4 allele (a risk gene for late-onset Alzheimer's disease).[122, 123]

Meta-analyses have been limited by the paucity of studies. Peters et al.[116] found no protective effect of moderate alcohol consumption on cognitive decline. Similarly, Anstey et al.[117] found no significant effect of alcohol consumption on cognitive decline, but their analysis was limited to two studies and consequently had a high degree of heterogeneity**.**

2.5.2.3 Brain correlates of moderate alcohol consumption

No convincing neural correlate for a protective effect of small amounts of alcohol on human brain structure has been found, although the field is poorly studied. Reported results are inconsistent.[124, 125] Moderate alcohol consumption in older subjects has been associated with reduced total brain volume, increased ventricle size,[126] grey matter atrophy[127] and reduced frontal and parietal grey matter density,[128, 129] but others have not found such relationships,[124] or only at higher consumption levels.[130]

Associations between moderate alcohol consumption and white matter findings are also inconsistent. De Bruin reported increased white matter volume in moderate drinkers compared with abstainers,[131] whereas Anstey found the inverse relationship.[132] Similarly, increased white matter hyperintensities have been described in moderate drinkers compared with abstainers[133] but others found no association.[126, 132, 134]

2.5.2.4 Putative mechanisms for a protective effect

Possible mechanisms underlying a protective effect have been more extensively researched for cardiovascular disease. Moderate alcohol consumption has been associated with beneficial changes in blood lipid profile, platelet function, fibrinolytic activity and inflammatory markers.[135-137] Animal models have suggested a direct effect on coronary vasculature, increasing blood flow to the myocardium, via nitric oxide generation.[138] There is also some animal evidence that alcohol increases the tolerance of cardiomyocytes to ischaemic reperfusion injury by direct activation of cell survival programs.[139, 140]

Moderate alcohol consumption has also been associated with a protection from ischaemic stroke.[141, 142] There is some evidence that alcohol preconditioning reduces amyloid neurotoxicity and apoptosis in vitro by increasing protective proteins.[143]

Preclinical studies examining the effects of moderate alcohol on the brain are few. One, in adult mice exposed voluntarily to alcohol for ten weeks found an increased proliferation of cells in the dentate gyrus (hippocampus), without a corresponding

increase in apoptosis.[144] It may not be ethanol, but compounds such as polyphenols found in wine, that confer neurological beneficial effects. Wang et al. reported reduced spatial memory decline and neuropathology in a mouse model of Alzheimer's disease exposed to cabernet sauvignon (high in resveratrol, a polyphenol) compared to those drinking water or ethanol.[145] Resveratrol, found also in other foods such as peanuts, has also been reported to increase amyloid clearance.[146]

2.5.3 Methodological limitations and future directions for research

Unresolved questions about the association of moderate alcohol consumption and brain outcomes remain. There are design limits to existing studies of non-dependent drinking and brain imaging, which may explain the conflicting results reported. Most studies have been cross-sectional or with very limited prospectively gathered alcohol data. People typically underestimate their alcohol intake,[147] a problem likely to be worse in a retrospective study. Studies have also included elderly participants, in whom sub-threshold pre-symptomatic cognitive impairment may already have an impact on drinking patterns. The comparison group to moderate drinkers is usually non-drinkers. This group is likely to be heterogeneous in nature, including never-drinkers but also previously hazardous drinkers with intervening illness that has stopped their drinking. Such illnesses, either resulting from previous heavy alcohol consumption, such as liver dysfunction, or unrelated conditions such as cardiovascular disease, may negatively affect cognition. This may confound the relationship between abstinence and cognitive performance. In fact one review which grouped longitudinal studies according to how 'abstainer' was defined, found no protective effect of light drinking when former and occasional drinkers were excluded

from the abstainer category.[148] Finally, there may be confounding by other lifestyle or premorbid factors associated with moderate drinking, and existing studies have poorly controlled analyses for these.

This review of the current evidence base suggests a need for further research into the effects of moderate alcohol consumption on brain structure and function. Studies with prospective collection of data on alcohol consumption rather than retrospective recall would be likely to increase the accuracy of self-report. Collection should ideally begin at an earlier life stage than the usual appearance of cognitive symptoms, for instance at midlife, to reduce the chances of reverse causation, i.e. that cognitive impairment changes drinking patterns rather than is a consequence of them. In depth information also needs to be gathered on comorbid physical illnesses, such as cardiovascular disease. This allows analyses to be controlled for these factors, reducing the possibility that any protective effect of light drinking over abstinence is due to confounding by physical illness in the abstinent group. Finally, subjects need to be characterised in terms of socioeconomic, educational and intelligence variables, as these may be independently associated with more prevalent alcohol consumption and higher cognitive performance, and therefore could confound the relationship between the two.

Chapter 3 - Methods

In this chapter I discuss the experimental approach taken in this thesis to answer the research question, namely what is the relationship between non-dependent alcohol consumption and brain structure and function? I will consider methods of determining alcohol consumption in a cohort, and alcohol dependence. The chapter will include an overview of the Whitehall II study, from which the participants were drawn, considering potential strengths and limitations of the cohort for this investigation. Finally, I will also give an introduction to the multi-modal Magnetic Resonance Imaging techniques and the imaging protocol used in subsequent chapters of this thesis.

3.1 Determining alcohol consumption level

3.1.1 Self- and corroborative report

Perhaps the most straightforward method to estimate alcohol consumption is self-report. I am not aware of any studies evaluating the consistency between prospectively and retrospectively gathered alcohol data, but extrapolation from other data types would suggest that prospective gathering is likely to be more accurate due to reduced recall bias.[149, 150] Self-report of alcohol consumption has been subject to

methodological criticism.[147, 151, 152] Contextual and individual factors may determine its accuracy.[153]

Attempts have been made to corroborate self-report with collateral reports of consumption from family members, with varying degrees of agreement.[154, 155] Prospective daily diaries have also been proposed,[156] although these are intrusive and may influence behaviour.

Official records, such as arrests, hospital admissions secondary to alcohol, rehabilitation admissions, are likely to reflect alcohol abuse and dependence rather than moderate use, so are not an appropriate method for this research question.

3.1.2 Biochemical measurements

Alcohol absorption and metabolism varies between individuals and may be affected by age, sex, physical health comorbidity, BMI and ethnicity. Breathalysers only reflect alcohol consumption in the preceding 24 hours and are hence unsuitable to determine use over years as is likely to affect brain structure. Several blood markers have been proposed, including gamma-glutamyltranserase (γGT), liver transaminases, and mean corpuscle volume (MCV). All are markers of consistent heavy alcohol use, and are insensitive at detecting light or moderate drinking.[157] γGT is a glycoprotein enzyme found in liver, but also brain and kidney amongst others. Hepatic γGT can be raised secondary to multiple drugs (including NSAIDs – commonly taken by older adults) as well as alcohol. In laboratory experiments subjects needed to consume 60g of ethanol daily for three weeks to produce a 15% increase in γGT.[158] The same

amount of alcohol was needed for five weeks to produce slight increases in liver transaminase levels.[159] Raised MCV is similarly nonspecific and insensitive to the detection of even excessive alcohol consumption.[160]

3.2 Defining moderate drinking

There is no universal agreement about the definition of "light" or "moderate" drinking. Moderate drinking is defined variably in the literature in terms of weekly alcohol units consumed. The dictionary definition of the adjective, moderate, is "within reasonable or proper limits" or "of medium quantity". To use the former of those, together with current the recommended alcohol guidelines of less than 14 units (112g) weekly, would exclude half of this cohort. For the purposes of this thesis, we have defined moderate drinking as those individuals who are not alcohol-dependent, based on the CAGE screening questionnaire discussed below. There is a possibility that a few subjects could be drinking heavily and yet be included in the analysis. However, it is unlikely that subjects would have significant physical or mental consequences secondary to their alcohol consumption that was not highlighted from the multiple aspects of data collection.

3.2.1 CAGE

Alcohol dependence was defined in this thesis using the CAGE questionnaire[161]:

1. Have you ever felt you needed to **C**ut down on your drinking?
2. Have people **A**nnoyed you by criticizing your drinking?
3. Have you ever felt **G**uilty about drinking?
4. Have you ever felt you needed a drink first thing in the morning (**E**ye-opener) to steady your nerves or to get rid of a hangover?

Two “yes” responses indicate the possibility of alcoholism should be investigated further.

The CAGE questionnaire was first developed from a clinical study in 1968.[161] In depth interviews were conducted, and answers compared between patients with alcoholism versus those without. The minimum number of questions that could distinguish the two groups was sought. Since then the CAGE questionnaire has been validated in several different groups. Bernadt et al. found it superior to eight different laboratory tests, including the best performing, which was gamma GT (sensitivity 33%) at detecting excessive drinkers (>16 drinks (224g) daily), with a sensitivity of 93%.[162] This superiority over gamma GT has been replicated since,[163] as CAGE performs better than computer-assisted laboratory data profiles.[164]

3.2.2 AUDIT

The CAGE questionnaire was designed to identify lifetime history of abuse or dependence, and not heavy or hazardous use. Another screening questionnaire, AUDIT, was designed to identify the latter over the past year.[165] Some studies report that CAGE poorly detects heavy drinking,[166] and one review found it performed unfavourably compared to AUDIT in females.[167] However, others have reported superior performance of CAGE over AUDIT.[168, 169]

Whilst not a perfect tool, for the purposes of this thesis CAGE has been chosen as the screening questionnaire to detect alcohol dependence. First, it is briefer than AUDIT, with just four questions that can be administered in less than a minute. This is clearly

an advantage when applied to large cohort studies. Second, it is more sensitive to a lifetime history of alcohol abuse or dependence, rather than over the past year, as in AUDIT. Previous studies have shown variable protective effects of moderate alcohol consumption on the brain depending on the nature of the abstainer comparison group, i.e. whether it contains former drinkers.[119] Whilst the Whitehall II cohort has prospective alcohol data from 1985 onwards, subjects could have been alcohol dependent in their earlier lives. Lifetime history of alcohol abuse is therefore advantageous to be aware of, as it will allow fuller characterisation of especially abstainers.

3.3 The Whitehall II study

The first Whitehall cohort, the forerunner to Whitehall II, was set up in the 1960's as a British equivalent to the Framingham study. In 1985 Whitehall II commenced, with the broad research aim to investigate social and occupational influences on health and illness.[170] The included population were civil servants aged 35-55 years working in twenty Whitehall department offices in London between 1985 and 1988. The initial cohort consisted of 10,308 participants, 3413 women and 6895 men. Subjects were invited to research clinics at approximately five-year intervals, and were sent postal questionnaires between these.

	Phase 1 1985-88	Phase 3 91-93	Phase 5 97-99	Phase 7 03-04	Phase 9 07-09	Phase 11 2011
Ages (yrs.)	35-55	39-64	45-69	50-74	55-79	60-85
Participants	10308	8637	7830	6967	6755	-
Social circumstances & Behaviour, Smoking, alcohol, exercise, sleep diet (FFQ)	X	X	X	X	X	X
Biological measures: BP, BMI, waist, lipids, glucose, insulin, stored blood (-80C)	X	X	X	X	X	X
2-h OGTT		X	X	X	X	X
Inflammatory markers		X		X	X	X
Autonomic (HRV)			X	X	X	X
Genetic material				X	X	X
Psychosocial factors: Work, social support, - participation, care provision	X	X	X	X	X	X
Health outcomes: CHD, stroke, diabetes, cancer, mortality, medications	X	X	X	X	X	X
Function: Physical, social & mental	X	X	X	X	X	X
Cognitive tests, physical/lung function tests			X	X	X	X

Table 3.1 Data collected on participants in the Whitehall II study

Eight hundred subjects were randomly selected from Phase 11 of the Whitehall II cohort, and invited to Oxford to participate in the imaging sub-study. Figure 3.1 summarises the sequence of steps involved. The analysis comprising this thesis includes the first 550 participants. This sample size was chosen partly for reasons of optimal timing for completion of the work, and additionally due to FMRIB upgrading, as the SiemensVerio was replaced by a Prisma MRI scanner

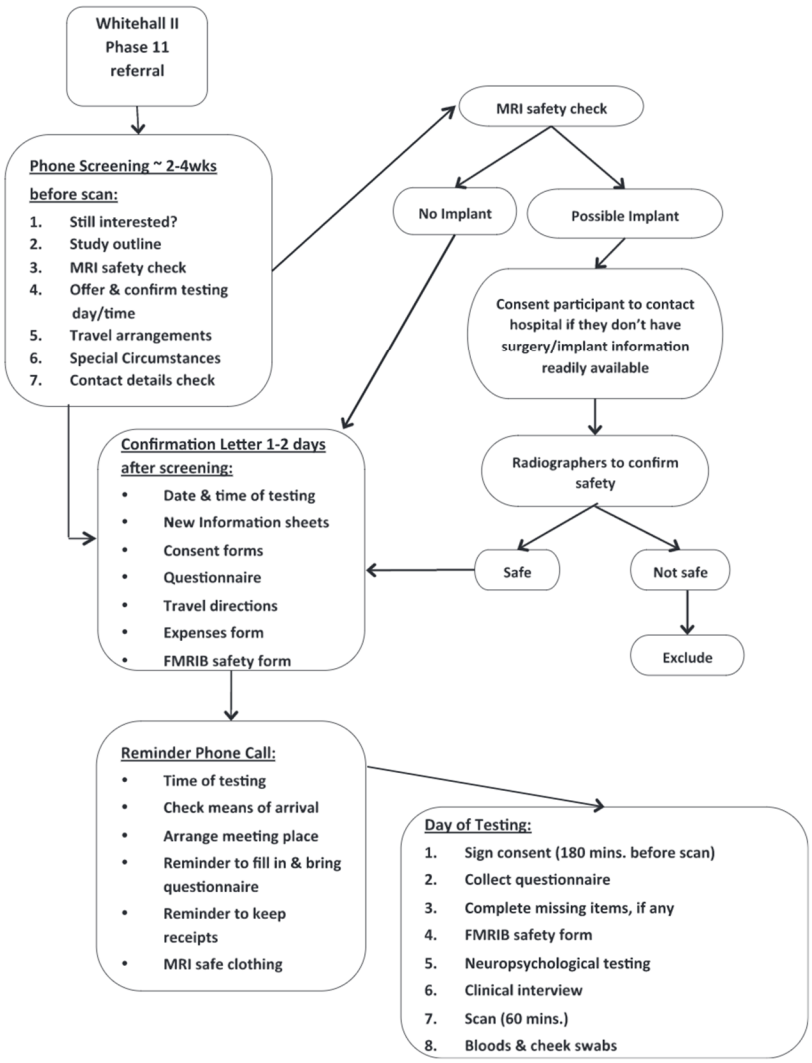


Figure 3.1 Flow-chart summarising the stages of the Whitehall II imaging sub-study.[171]

3.3.1 Why is Whitehall II a suitable cohort to answer the research question?

There are several longitudinal cohort studies with magnetic resonance imaging of participants. As with any sample, the Whitehall II cohort has unique strengths and limitations. Table 3.3 summarises those studies, which might be the biggest 'competitors' to Whitehall II for the purposes of this thesis. This is not a comprehensive list, for example it excludes the Sao Paolo Aging Study and the Maastricht Aging Study, which both have MRI, but are of much shorter duration.[172, 173] Whitehall II has the longest period of prospective data collection on alcohol consumption (30 years) of any of the studies, and importantly this begins at midlife before cognitive impairment may induce changes in drinking habits. Many participants are drinking moderately (66% of men 0.1-19.9g/day),[41] but few very heavily. The cross-sectional neuropsychological assessment at the time of scan is the most comprehensive, and includes assessment of estimated premorbid IQ. The endpoint imaging, although on fewer subjects than the Rotterdam study, uses a magnet of higher strength (3T versus 1.5T) and has more imaging sequences. Of note, it includes resting state fMRI, unique amongst cohort studies of this duration. There are now thirty years of detailed data available for subjects, which is important to control for multiple potential confounders of cognition. Attrition rates are relatively low for a study of such duration, with 61% of those at entered baseline still participating in the latest phase (11). However, the group demographics reflect the composition of the civil service in the 1980's – namely a paucity of women (and of those employed half in clerical or support roles), a lack of ethnic minorities in higher occupational grades, and no manual workers (Table 3.2).

Civil Service employment grade	Salary range (£)	Whitehall II study grade	Sample size at baseline (Phase 1)		
			Men	Women	Total
Unified Grade 1–6	28 904–87 620	1	1015	118	1133
Unified Grade 7	25 330–36 019	2	1632	263	1895
Senior Executive Officer	18 082–25 554	3	1228	198	1426
Higher Executive Officer	14 456–20 850	4	1498	478	1976
Executive Officer	8 517–16 668	5	881	660	1541
Clerical and Support Staff, e.g. messengers, porters, telephonists, typists	7 387–11 917	6	641	1696	2337

Table 3.2 Salary range (01.08.92) and sample size at baseline in the Whitehall study[170]

	Whitehall II	Rotterdam (RS)	Lothian Birth Cohorts (LBC)	PROSPER	Honolulu-Asia Aging study
Location	London	Rotterdam	Scotland	UK and Holland	Hawaii
Recruitment method	Civil service	Geographical	Those born 1921/1936 attending school in Scotland June 1932/1947	RCT pravastatin, those with history or CVD risk	Geographical, Japanese-American men
Started	1985	1990 RSI, 2000 RSII, 2006 RSIII	1932/1947		1965 (as Honolulu Heart program) 1991 80% HAAS
Follow up	5 yearly clinic and postal questionnaire in between	RSI: 1994-5, 1997-2000, 2002-04, 2009-11 RSII: 2004-05, 2011-12 RSIII: 2012	Wave 1: 2004-07, 2: 2008-10, 3:2011-13 *LBC1921*: mean age 79, 83, 89, 90 *LBC1936*: 70, 73, 76	Mean 3.2 years	1994 1997
Number	10,308 initial	10,994 current	87498 initial	5804	3734
Age at baseline	35-55 years at start (mean 44)	RSI&II: >55 years, RSIII: >45 years	11 years at start	70-82 years	34-65 years at start (1965)
Sex distribution	67% men	59% men original cohort	51% male start	48% male	100% men
Occupation	Civil service	Mixed	Mixed	Mixed	Mixed
Morbidity	Community dwelling	897 in care homes at start		Mean 28 (but <24 excluded)	
Cognitive tests	*Cross-sectional*: MoCA, TMT A and B, Lexical and Semantic Fluency, RCF, HVLT-R, BNT, Digit Span and Digit Coding, TOPF, CANTAB *Longitudinal*: Verbal recall, AH4, Mill Hill,	*Longitudinal:* MMSE, executive and memory battery added. *Subsequently:* Stroop, memory, letter digit substitution, verbal fluency	11years: Moray House Test No.12 1932: Binet 1000 (IQ) Wave 1+2: WAIS III (symbol search, matrix reasoning, letter-number sequencing, digit span, block design; reaction times/ Wechsler memory scale III – logical, spatial, verbal paired.	MMSE, picture-learning, Stroop, letter digit coding,	Dementia assessment (CASI screen, if + neuropsychological assessment, interview, neuroimaging, clinician structured diagnosis)

	category and letter fluency, MMSE				
MRI protocol	3T T1, T2, T2*, FLAIR, DTI, resting state fMRI	1.5T T1, T2* GRE, FLAIR, DTI 1995-99 – scan study, random subsets 2005 on core protocol	1.5T T1, T2, T2*, FLAIR, DTI	1.5T Structural and FLAIR	1.5T T1, T2, proton-density weighted
Number scanned	800	5886 up until 2011	730 wave 2	554 (baseline and follow up after 33 months)	599

Table 3.3 Comparison of several major longitudinal cohort studies with Magnetic Resonance Imaging.

3.4 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a non-invasive in vivo neuroimaging technique that, in contrast to x-ray computed tomography and emission tomography, carries no radiation load. Depending on the tissue focus of investigation, different imaging sequences are used as explained in detail below. These features make it suitable for large research studies on non-clinical populations.

3.4.1 Structural imaging

Protons in water molecules in the body have a charge and appear to rotate around an axis. Under normal conditions these spins are randomly orientated. In a MRI scanner is a strong magnet that exerts a powerful field (B_0). This aligns the proton spins, which are "precessing" at a frequency determined by the strength of $B_{0,}$ creating a net magnetisation ('excitation'). Application of a radio-frequency pulse tilts this net magnetisation briefly, but then the protons drift back to $B_{0.}$ The rate of recovery of the magnetisation in longitudinal (T_1) and transverse (T_2) planes is measured using a radio-frequency coil. Varying the repetition time (between successive pulses) and echo time (at which signal is measured) allows differing relaxation times in different tissues to be exploited to distinguish tissue types. To differentiate spatial positions of protons for image generation, a second magnetic gradient field is applied, varying the precession frequency proportionally to the field, and allowing specific slice selection and spatial localisation.

T1-weighted MRI is particularly good at identifying the contrast between grey and white matter, making it an ideal modality to study structural grey matter changes. Grey matter across whole brain can be examined using these images on a voxel-by-voxel with no a priori hypothesis (voxel-based morphometry, VBM).[174, 175] Additionally it can be used to identify subcortical volumes such as the hippocampi, for visual rating or automated volume extraction using shape analysis.[176]

3.4.2 Fluid Attenuation Inversion Recovery

Fluid attenuation inversion recovery (FLAIR) uses a special inversion recovery sequence with two pulses, to 'erase' the fluid signal by adjusting the inversion time. FLAIR images are particularly helpful when wanting to visualise white matter pathology, for example white matter hyperintensities.

3.4.3 Diffusion tensor imaging

Diffusion tensor imaging (DTI) measures the diffusion of water molecules within body tissues in multiple different directions. At each voxel, the diffusion tensor is modelled as an ellipsoid, with its orientation characterised by three eigenvectors and their strength by three eigenvalues. In circumstances where there are no restrictions to diffusion, movement of water molecules is isotropic (equal in all directions). Diffusion is limited to a greater extent orthogonally to the axon (anisotropic) by the presence of an intact myelin sheath. Based on this notion, several diffusion indices are used to make inferences about white matter fibre integrity. For example, fractional anisotropy (FA) explains the ratio of diffusion parallel to the axon compared to in other directions. The higher the value, the better the inferred fibre integrity. Axial

diffusivity (AD) describes diffusion parallel to, and radial diffusivity (RD) perpendicular to the to the principal fibre direction. Mean diffusivity (MD) is the apparent diffusion coefficient averaged over all directions. Further details about DTI and the analysis pipeline used (TBSS) are in Chapter 5.

3.4.4 Resting state functional MRI

Functional MRI makes inferences about neural activity in the brain from changes in blood oxygenation (BOLD). Haemoglobin in blood is diamagnetic when oxygenated but paramagnetic when deoxygenated. The theory behind this imaging modality is that neural activity induces increased cerebral blood flow to the region, which 'oversupplies' the region with oxygenated haemoglobin, paradoxically increasing the oxygen concentration and hence increasing the BOLD signal. At rest, the BOLD signal has characteristic low-frequency fluctuations, which are temporally and spatially correlated and consistent across individuals (RSNs).[177] Independent components analysis (ICA) can be used to decompose the dataset into a set of statistically independent spatial component maps and their associated time courses.

3.4.5 Imaging protocol in the Whitehall II imaging sub-study

The sequences and imaging parameters used in this study are detailed in Figure 3.2. For the purposes of this thesis, analysis includes MEMPR, FLAIR, dMRI and multiband resting images.

	Structural				Functional	
Sequence	MEMPR	FLAIR	T2*	dMRI	Multiband	Standard
Condition	———	———	———	———	Resting	Resting
TR in ms	2530	9000	36	8900	1300	3000
TE in ms	1.79/3.65/5.51/7.37	73	30	91.2	40	30
Flip angle	7°	150°	15°	———	66°	90°
Voxel in mm^3	1x1x1	0.9x0.9x3	0.7x0.7x1.5	2x2x2	2x2x2	3x3x3
FoV read	256	220	220	192	212	192
FoV phase	100%	100%	81.3%	100%	100%	100%
Base resolution	256	256	320	96	106	64
Phase resolution	100%	100%	100%	100%	100%	100%
TI in ms	1380	2500	———	———	———	———
Bandwidth	651 Hz/Px	283 Hz/Px	170 Hz/Px	1680 Hz/Px	1814 Hz/Px	2368 Hz/Px
Orientation	S	T	T	T	T	T
b-value	———	———	———	1500 s/mm^2	———	———
N. of volumes	———	———	———	———	460	200
N. of directions	———	———	———	60 + 5 b = 0 s	———	———
Acquisition time	6 m 12 s	4 m 14 s	4 m 17 s	9 m 56 s	10 m 10 s	10 m

Figure 3.2 MRI sequences and parameters used in the study. Abbreviations: S, Sagittal orientation; T, transverse orientation.[171]

3.4.6 Visual rating of MRI scans

To complement more advanced imaging analysis techniques, which are used exclusively in research settings; work in the subsequent chapters also includes visual ratings scales. These are used clinically, as they can be implemented quickly after training of raters, and require no specialist software or imaging analysis. In fact they can even be applied to CT images. In this thesis we use the Scheltens scale for medial temporal lobe (hippocampal) atrophy (Figures 3.3 and 3.4), and the Fazekas scale for deep and periventricular white matter hyperintensities (Figure 3.5). The Scheltens scale has shown fair to good inter-observer reliability in previous studies.[178] On a random subset of this cohort, inter-rater reliability (intraclass coefficient) was 0.8-0.9 and intra-rater reliability 0.7-0.9.[179] It correlates well with hippocampal volumetry.[180] In terms of clinical correlates, it can distinguish Alzheimer's patients from controls fairly reliably,[181] with a similar prediction accuracy to manual hippocampal volumetry and multivariate classification.[182] The Fazekas visual rating scale for white matter hyperintensities has a fair to good inter-rater agreement,[183, 184] but is easy to apply

even to poor quality scans. Scores predict cognitive decline in mild cognitive impairment,[185] and correlate with histopathological findings.[186]

Score	*Width of choroid fissure*	*Width of temporal horn*	*Height of hippocampal formation*
0	N	N	N
1	↑	N	N
2	↑↑	↑	↓
3	↑↑↑	↑↑	↓↓
4	↑↑↑	↑↑↑	↓↓↓

↑ = increase, ↓ = decrease. N = normal.

Figure 3.3 Scheltens visual atrophy rating scoring.[187]

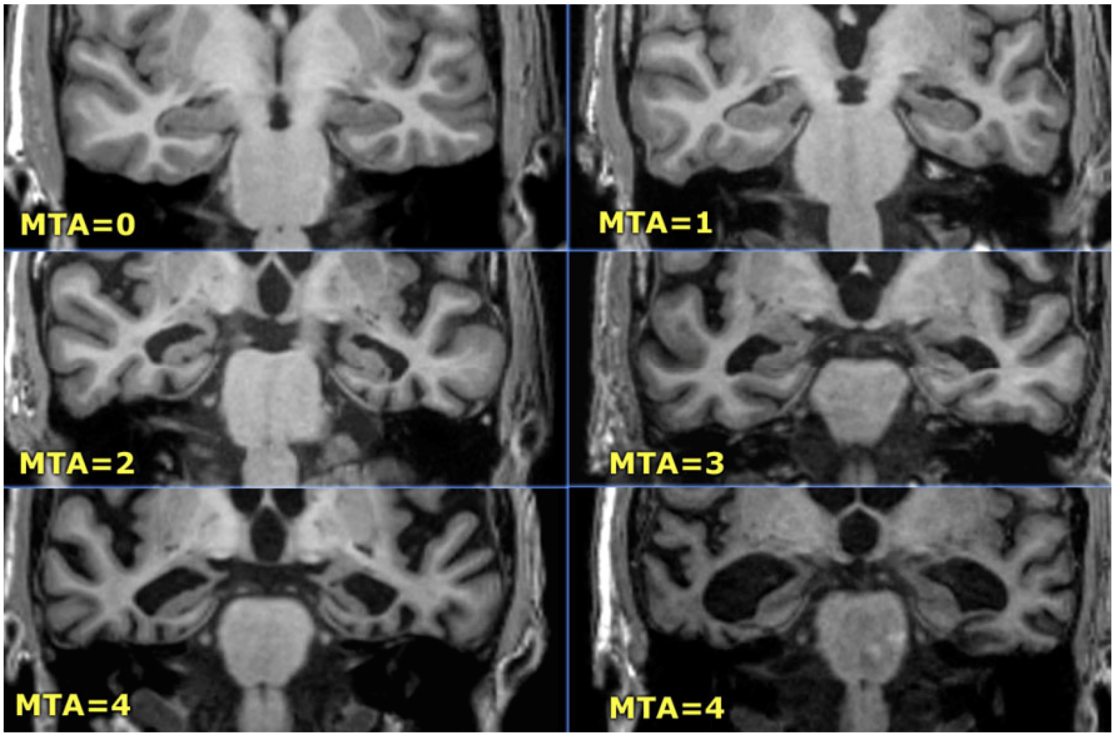


Figure 3.4 Examples of ratings for Scheltens scale for medial temporal lobe atrophy. Image reproduced with permission from Radiology Assistant.[188]

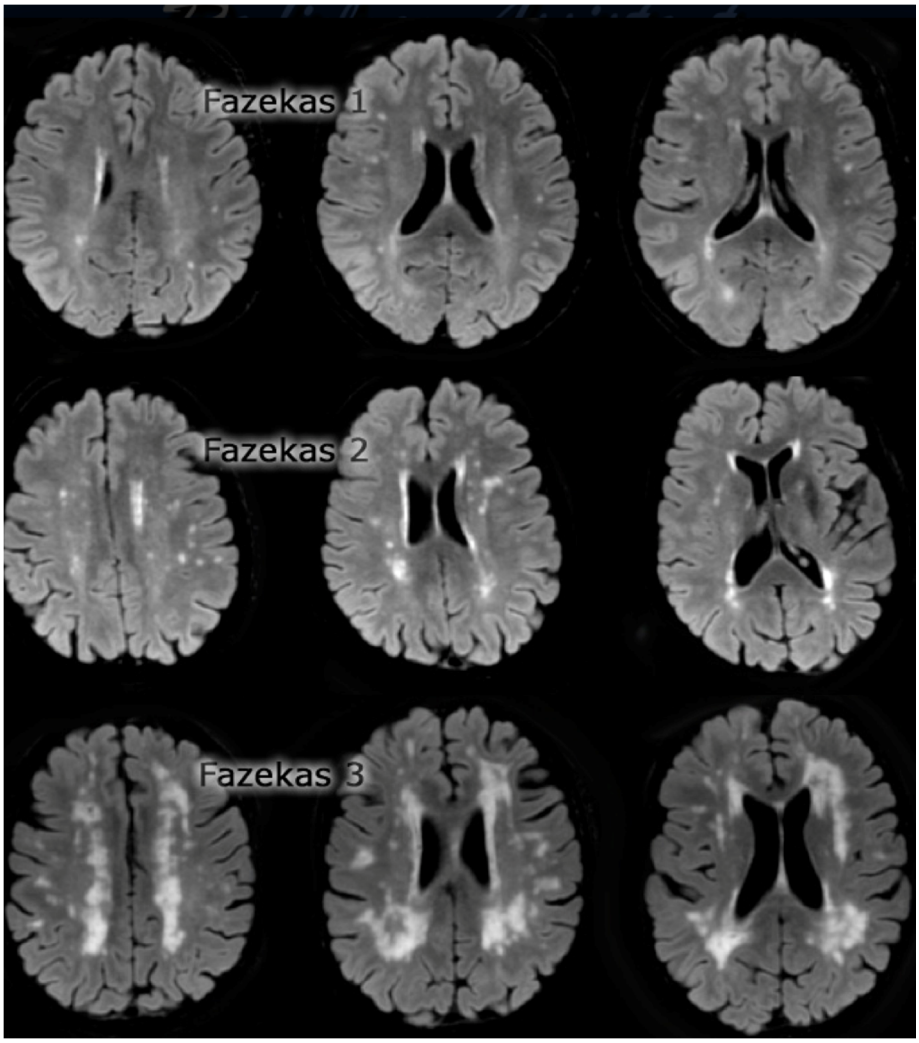


Figure 3.5 Fazekas visual rating of white matter hyperintensities. Image reproduced with permission from Radiology Assistant.[188]

3.5 Cognitive testing

Cognitive function was assessed at Phases 3, 5, 7, 9, 11 and again at the time of the MRI using lexical[189] (how many words beginning with a letter can be generated in one minute) and semantic[190] (how many words in a category can be named in one minute) fluency tests. Recall of a list of 20 words was tested at Phases 3, 5, 7, 9 and 11. The Mini-Mental State Examination (MMSE)[191] was performed at Phases 5, 7, 9, and 11.

The following psychometric tests were used at the time of scanning (study end):

- SCID[192]
- Montreal Cognitive Assessment (MoCA)[193]
- Trail Making Test (TMT A and B)[194, 195]
- Rey-Osterrieth Complex Figure (RCF) copy, immediate, delay, recognition[196]
- Hopkins Verbal Learning Test (HVLT-R) immediate, delay, recognition[197]
- Boston Naming Test (BNT)[198]
- Digit span[199]
- Digit coding[200]
- Test of Premorbid Function (TOPF)[201]
- Dot counting and letter naming (from ACE III) (for the latter participants)

The MoCA is a 30-point cognitive screening test with subtests for verbal recall, clock drawing, cube copying, phonemic fluency, attention task, naming and orientation, amongst others. The TMT requires subjects to 'connect the dots' of twenty-five consecutive targets on a sheet of paper as fast as possible. In TMT A the targets are numbers, and in TMT B alternating numbers and letters. The RCF involves initially copying and then recalling a complex geometric diagram at increasing time intervals. In the HVLT-R task the subject must recall a list of twelve words over the course of three trials immediately and after a delay. The BNT examines semantic memory and requires naming of a series of images shown to the participant. Digit Span includes recall of a lengthening list of digits forwards, backwards, and rearranged in ascending order (DSF, DSB, DSS). In Digit Coding, participants have to write the appropriate novel symbol for each number under time pressure. The TOPF consists of a list of

written words, which must be read aloud and is marked for correct pronunciation. Premorbid IQ can be calculated from the raw score, adjusted for sex and years of education. Dot counting involves a subject stating the number of dots in an image, and letter naming the letter from a partially obliterated image.

Chapter 4 - Alcohol consumption and grey matter measures

4.1 Introduction

Brain atrophy in chronic alcoholism is well described (see Kril and Halliday for a review).[46] This is at least in part due to grey matter shrinkage. Volume reductions have been described in the diencephalon, caudate, frontal and parietal cortices and the medial temporal lobe.[53] Reduced grey matter in alcohol addiction has now been replicated several times,[202, 203] also using voxel-based morphometry methods.[204] Up to 5% cortical atrophy has been described,[54] and may selectively affect the frontal lobes, at least in older alcoholics.[205]

What effects, if any, can be caused by non-dependent alcohol consumption is unclear. A few studies have reported negative associations between moderate alcohol and brain size. Fukuda et al. found non-drinkers to have larger brains (assessed as % of intracranial volume) compared to moderate drinkers (>7 drinks (98g) per week). Others have reported increased ventricular and sulcal size.[126, 132] Moderate drinking (defined as >15 units (120g) / week) in older subjects has been associated with grey matter atrophy and reduced frontal and parietal grey matter density.[127, 130, 131] In contrast, others have described increased grey matter in light to moderate drinkers (< 16 units (128g) / week) in similar brain regions (frontal, parietal, temporal and occipital lobes).[128, 129] Unresolved questions persist because of design limits to existing studies of non-dependent drinking and brain imaging. Decades of alcohol consumption cannot be randomised. Most studies to date have been cross-sectional or

with very limited prospectively gathered alcohol data. People typically underestimate their alcohol intake,[147] a problem likely to be worse in a retrospective study. Studies have also included elderly participants, in whom sub-threshold pre-symptomatic cognitive impairment may already have an impact on drinking patterns. Definitions of 'moderate' drinking vary, with some studies potentially including harmful drinkers, as they do not specifically exclude heavy or dependent drinkers. For example, in Fukuda et al., the authors state that 50% of 'moderate' drinkers were drinking more than 14 units (112g) weekly.

4.2 Objectives and hypotheses

We aimed to investigate the relationship between prospectively gathered data on alcohol consumption, and grey matter density, in non-dependent (<2 CAGE) drinkers. Our hypotheses were two-fold:

1. Light drinking (<7 units (56g) weekly) is protective against a reduction in grey matter density in comparison to abstinence.
2. Heavier drinking (above contemporaneous recommended guidelines (>112g for women and >168g for men) is associated with reduced grey matter density, particularly of the frontal regions.

4.3 Methods

4.3.1 Study design and participants

Five hundred and fifty subjects were randomly selected for the current Whitehall II imaging sub-study (2012-2015) from the Whitehall II cohort study.[206] The Whitehall II study was established in 1985 at University College London, with the aim of investigating the relationship between socioeconomic status, stress and cardiovascular health. Further details of the cohort are in Chapter 3 of this thesis. In order to make the sample as representative as possible of the cohort at baseline, a random sample of 800 Whitehall II participants who participated in Phase 11 clinical examination or Phase 10 pilot examination and were willing and able to give informed consent to attend the imaging sub-study at the Oxford Centre for Functional MRI of the Brain (FMRIB) was selected. A list of 1380 participants to be invited to FMRIB was drawn randomly from the consenting men and women at high, intermediate and low socioeconomic groups (including 30 participants with depressive symptoms based on Phase 10 pilot and previous WHII clinical examinations). The purpose of oversampling 580 participants was to reach the targeted 800 participants after allowing for those who declined to participate, or were unable to participate because safety reasons precluded participation in the MRI scan. The scanning was on-going at the time of this study; the 550 participants included in the present analysis are those who had their MRI 2012-5. Sociodemographic, health and lifestyle variables including alcohol consumption were measured over a follow-up period of approximately thirty years, at approximately five-year intervals (Phase 1: 1985-8,

Phase 3: 1991-3, Phase 5: 1997-9, Phase 7: 2003-4, Phase 9: 2007-9, Phase 11: 2011-12). Information about personality traits was determined by questionnaire at Phase 1 and included trait impulsivity (question: "Are you hot-headed?") coded as a binary outcome (yes (1)/no (0)).

Alcohol variables collected at each phase included: units drunk per week, frequency of drinking per week over the previous year, as well as the CAGE screening questionnaire.[207] Weekly consumption was used in this analysis, as there is less likelihood of a ceiling effect in comparison to drinking frequency. Average alcohol use across the study was calculated as mean consumption per week averaged across all study phases. Subjects were deemed 'abstinent' if they consumed less than 1 unit (8g) of alcohol per week. 'Light' drinking was defined as between 1 and <7 units (8-<56g) per week and 'moderate' drinking as 7 to <14 units (56-<112g) per week for women and 7-<21 (56-<168g) units for men, based on use in the existing literature and government guidelines. "Unsafe drinking" was defined according to pre-2016 (21 units (168 g) per week for men and 14 units (112 g) for women) and newly revised UK Department of Health guidelines (>14 units (112 g) for men and women), and further categorised (14-<21, 21-<30, >30 units (112-<168, 168-<240g) weekly) for the purposes of the logistic regression analysis (see statistical analysis).[208] Non-dependent drinkers were defined as those scoring less than 2 on the CAGE questionnaire.

Participants were included in the imaging sub-study if they were safe to have MRI and able to give informed consent. Exclusions were due to incomplete or poor quality

imaging data or gross structural abnormality (e.g. a brain cyst), incomplete alcohol use data (>2 phases missing), sociodemographic, health data (Figure 4.1).

4.3.2 MRI analysis

All MRI scans were acquired at the University of Oxford Functional Magnetic Resonance Imaging of the Brain (FMRIB) Centre, using a 3 Tesla Siemens Verio scanner (2012-15). T1-weighted and diffusion tensor (DTI) 3T MRI sequences were used for analyses.[171]

4.3.3 Tissue segmentation

T1-weighted images were processed using FSL tools (FMRIB Software Library, www.fmrib.ox.ac.uk/fsl) and 'fsl_anat (Beta version)' (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/fsl_anat). This reorients images to standard (MNI) space, corrects for bias field, registers the images to standard space (using linear FLIRT[209, 210] and non-linear FNIRT[211] registration), and extracts whole brain volumes ('BET').[212] FMRIB's Automated Segmentation Tool (FAST) allowed extraction of measures of total grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF). GM and WM volumes were adjusted for total intracranial volume.

4.3.4 Voxel-based morphometry (VBM)

Relationships between cognitive function and grey matter were examined initially using voxel-based morphometry, an objective method to compare grey matter density between individuals in each voxel (smallest distinguishable image volume) of the

structural image. Structural data were analysed with FSL-VBM [213] http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM), an optimised VBM protocol [214] carried out with FSL tools.[215] First, structural images were brain-extracted and grey matter-segmented before being registered to the MNI 152 standard space using non-linear registration.[211] The resulting images were averaged and flipped along the x-axis to create a left-right symmetric, study-specific grey matter template. Second, all native grey matter images were non-linearly registered to this study-specific template and "modulated" to correct for local expansion (or contraction) due to the non-linear component of the spatial transformation. The modulated grey matter images were then smoothed with an isotropic Gaussian kernel with a sigma of 3 mm. Finally, voxelwise, a Generalised Linear Model (GLM) was applied using permutation-based non-parametric testing, correcting for multiple comparisons across space (threshold-free cluster enhancement, tfce).

4.3.5 Hippocampal volumes

Hippocampal volumes were calculated using FIRST (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIRST),[176] an automated model-based segmentation/registration tool, in a two-stage process – first using all subcortical masks, and second a hippocampal mask only. Both were visually inspected to check optimal segmentation. Extracted hippocampal volumes were corrected for intracranial volume and averaged across left and right sides.

4.3.6 Statistical analysis

All analyses were done with R,[216] unless otherwise stated. Differences between included and excluded subjects, i.e. representativeness of included participants, were examined using t-tests of means (continuous variables) or chi-square tests of independence (categorical variables). According to variable type, means (standard deviations), medians (interquartile ranges) or numbers (percentages) were used to summarise sociodemographic and clinical measures for included subjects who were split by safe vs. unsafe average alcohol use averaged over all phases, on the basis of UK contemporary (pre-2016) guidelines. Significant differences between safe and unsafe drinkers in continuous variables were tested using t-tests of means (normally distributed) or Wilcoxon Rank Sum Tests (non-normally distributed) and in binary categorical variables (and MMSE, MoCA and FRS which have lower and upper bounds) using Fisher's exact test of proportions. In view of small group numbers (<5) for social class, a simulation test was performed to estimate group differences. Weekly consumption of alcohol (units and grams) was described using means, standard deviations, medians and interquartile ranges.

Alcohol trends over time were examined using mixed effects modelling, with time from study baseline (Phase 1) as the independent variable and alcohol consumption (units per week) as the dependent variable. This method accounts for missing data, and correlation of repeated measures (in this case alcohol use). Intercepts (baseline consumption) and slopes (trends over study) were calculated for each participant. The ability of other variables to predict longitudinal trends of alcohol consumption was

tested by inclusion of the following in the mixed effects model: age, sex, education, premorbid IQ, social class, Framingham Risk Score (a composite measure including smoking, cardiovascular disease or diabetes, cardiovascular medication), exercise frequency, club attendance, voluntary work, visits with friends and family, lifetime history of Major Depressive Disorder on SCID binarised yes (2)/no (1), and current psychotropic medication binarised yes (2)/no (1).

Mean alcohol consumption (units per week) across all study phases was included as an independent variable in voxel-based morphometry (grey matter density as dependent variable) and TBSS analyses (FA/MD/RD/AD as dependent variable). Voxelwise, a Generalised Linear Model (GLM) was applied using permutation-based non-parametric testing (randomise),[217] correcting for multiple comparisons across space (threshold-free cluster enhancement, tfce). Results were judged significant, if adjusted $p<0.05$.

Two post-hoc tests were used to confirm the associations between alcohol consumption and hippocampal size following the VBM analysis. First, logistic regression was used to calculate odds ratios for left and right *hippocampal atrophy* versus no atrophy (visual atrophy ratings based on a cut-off of 0/1 on the Scheltens' scale),[187] given average alcohol consumption across study phases. The latter was categorised as abstinent (<1 unit (8g), reference group), 1 to <7 units (8-<56g), 7-14 units (56-<112g), 14 to <21 units (112-<168g), 21 to <30 units (168-<240g) and >30 units (>240g) per week. Second, multiple linear regression with *hippocampal volume* (extracted from FIRST, ICV-adjusted and transformed by squaring in order to

normalize the residuals) as the dependent variable and alcohol consumption as an independent variable was performed.

In all analyses, the following potential confounding variables (identified from knowledge of the literature) were included as independent variables: age, sex, premorbid IQ, education, social class, Framingham Risk Score, current psychotropic medications (number), lifetime history of Major Depressive Disorder (SCID, binarised as yes (2)/no (1)), exercise frequency, club attendance, voluntary work, visits with friends and family. The choice of MDD over CES-D scores was based on a more robust evidence base for an association between brain structure and major depression than sub-threshold depressive symptoms. In the subset with data on personality traits (n=179), post-hoc analyses of hippocampal volume/atrophy were additionally adjusted for impulsiveness (binarised (yes (1)/no (0)).[218]

4.4 Results

4.4.1 Participants and descriptive data

Twenty-three subjects were excluded from the VBM and visual ratings analyses based on structural brain abnormalities, poor quality images, missing alcohol (n=5) or confounder data (Figure 4.1).

Excluded subjects did not significantly differ from included subjects on any of the reported characteristics. There were a higher proportion of males, and subjects were slightly less educated, with higher blood pressure and lower depressive symptoms compared to the larger Whitehall II cohort (see Table 4.1), but no significant differences from the entire imaging sub-study sample to date. Sociodemographic, health and lifestyle data are reported for the 527 included participants, separated into alcohol consumption groups (Table 4.2). Mean age was 43.0 years (S.D.=5.4) at the study start. Unsafe drinkers differed from safe drinkers by having a higher premorbid IQ, a higher percentage of men and smokers, and higher Framingham Risk Scores. No subjects reported current illicit substance use.

Median alcohol consumption across study phases (Figure 4.2 and Table 4.3) was 10.0 units (80.0 g) per week (IQR=14.0 (112.0g)) for men, and 5.0 units (40.0 g) per week (IQR=8.0 (64.0g)) for women. Baseline consumption (intercept in mixed effects model) was significantly different between men and women (mean difference=5.0, 95% CI=3.1 to 6.9, $p<0.001$). Weekly alcohol intake did not significantly increase over the phases of the study for the group as a whole (change in weekly alcohol units

for every ten year increase from study baseline=0.15, 95% CI=-0.21 to 0.51, p=0.4), but trends over time correlated with baseline intake (intercepts and slopes correlated negatively (r= -0.43, 95%CI: -0.50 to -0.36), i.e. those drinking more at baseline tended to lower their consumption more over the course of the study - regression to the mean. Male sex (change in weekly alcohol units compared to females=4.89, 95%CI=2.54 to 7.19, p<0.001) and higher premorbid FSIQ (change in weekly alcohol units for every one IQ point=0.18, 95%CI: 0.06 to 0.30, p=0.004) predicted higher baseline consumption but not consumption changes with time. Other sociodemographic and clinical factors were not related to consumption. Average alcohol use over the study was over "safe limits" in 13.6% women and 20.0% men, as judged by pre-2016 UK guidelines (>21 units (168g)/week for men, >14 units (112g)/week for women), and 40.3 % as judged by the 2016 revised UK guidelines (>14 units (112g) per week for men and women). Scores on the CAGE questionnaire, were below the sensitive screening cut-off of 2^{161} for all participants at all Whitehall II phases (Table 4.4).

4.4.2 Alcohol and grey matter

Average alcohol consumption over the study (units per week) was negatively correlated with grey matter density in the VBM analyses, especially in hippocampi (Figure 4.3), even after adjustment for multiple potential confounders. Frontal regions were unaffected. There were no positive correlations between alcohol intake and grey matter density.

Increased alcohol consumption compared to abstinence was also associated with increased odds of visually rated hippocampal atrophy (defined as score >0 on Scheltens visual rating scale; Table 4.5 and Figure 4.4). This was a dose-dependent effect. The highest odds were in those drinking more than 30 units (>240g) per week (OR 5.8 95% CI: 1.8 to 18.6 p=<0.0001), but odds of atrophy were higher compared to abstinence even in those drinking at moderate levels of 14- <21 units (112-<168g) per week (OR 3.4 95% CI: 1.4 to 8.1 p=0.007). There was no protective effect (i.e. reduced odds of atrophy) of light drinking (1- <7 units (8-<56g) per week) over abstinence. Findings were similar in sub-analyses of men alone, but not in women.

Mean hippocampal volumes (raw and ICV-adjusted) were within the range cited in the literature (Table 4.6) and correlated with visual ratings of hippocampal atrophy (Spearman's rho= -0.4 p<0.001).[219-221] Consistent with VBM and visual ratings findings, alcohol consumption independently predicted FIRST-extracted hippocampal volume (%ICV) (Figure 4.5 and Table 4.7). Excluding the three highest drinkers (>60 units (>480g) weekly) did not substantially change the results (Table 4.8). On the subset, for whom personality trait data were available from Phase 1 (n=179), additionally adjusting the analysis for trait impulsivity did not alter the findings.

4.5 Discussion

We have found a previously uncharacterised dose-dependent relationship between alcohol consumption over 30 years of follow-up and visually rated hippocampal atrophy. The hippocampal findings were consistent between the brain-wide voxel-based approach, automatically extracted volumes, and clinical visual ratings of

hippocampal atrophy. The effect was dose dependent, and increased odds of visual hippocampal atrophy were found even in moderate drinkers (14- <21 units (112-<168g) weekly in men).

Strengths of this study are the 30-year longitudinal alcohol data and the detail of available confounder data. Additional strengths include the availability of large N and the advanced imaging analysis methods. Grey matter findings were replicated using a voxel-based approach, automated hippocampal volumes, and visual ratings. Visual atrophy ratings are known to correlate closely with automated methods (own data) and are more applicable to clinical settings.[222] In large neuroimaging studies automatic segmentation is widespread.[223, 224] The automated approach we use (FIRST) has been shown to give accurate and robust results.[176]

Reported alcohol consumption increased for men and women between Phase 11 and the time of scanning, despite this being a relatively short time interval. One hypothesis for this discrepancy is differences in data acquisition. At the time of the scan subjects spent a longer duration with the researcher, which may have resulted in more accurate reporting of their alcohol consumption.

The hippocampal visual atrophy associations we found in the total sample were replicated in men alone, but not in women. This may reflect a lower power to detect an effect in women, in part due to the sample being dominated by men (a reflection of the civil service gender disparity in 1980s), and in part because few of the included women drank heavily.

Hippocampal atrophy is a sensitive and relatively specific marker of Alzheimer's disease,[225] although it has been reported in chronic alcoholics.[65, 66, 129] The brain regions most vulnerable to alcohol abuse are said to be the frontal lobes.[130] Higher but non-dependent alcohol use was not associated with reduced grey matter density in frontal regions or impaired cognition in our sample. However, VBM used in this analysis examined for a linear association in each voxel between grey matter density and alcohol consumption. This method may not be sensitive to non-linear relationships, or overall frontal lobe volume differences with alcohol. Region of interest analyses could be used to further investigate this. Only one study has reported hippocampal findings in non-dependent drinkers. This used a manual tracing rather than voxel-based or visual rating approach to estimate hippocampal size. They reported a protective effect of moderate alcohol in comparison with abstinence, which conflicts with our results.[129] However, alcohol consumption was determined cross-sectionally, making it difficult to exclude reverse causation. Additionally, a number of known hippocampal size confounders, such as depression, were not controlled for in Den Heijer et al.[226] Other studies in non-dependent drinkers have reported either no effect,[132, 147, 227] or a negative correlation with global grey matter, but not hippocampal atrophy.[126, 127] In contrast with our first hypothesis, we observed no protective effect of light drinking compared with abstinence on grey matter in contrast to some other studies.[128, 129] Neither of these controlled for (premorbid) IQ or socioeconomic class. The reported protective effect may be due to confounding: we found a positive association between alcohol intake and IQ, as have others.[228] These factors separately predict better performance on cognitive tests, and hence less brain atrophy. In line with our second hypothesis, we did find adverse associations with heavier alcohol consumption. The mechanism for this is unclear. Ethanol and acetaldehyde (a

metabolite) are neurotoxic[229] and cause reduced numbers[230, 231] and morphological changes in hippocampal neurones in animal models.[232] Associated thiamine and folate deficiency,[233] repeated head trauma, cerebrovascular events, liver damage, and repeated intoxication and withdrawal have also been implicated. The risk of right-sided hippocampal atrophy was significantly greater at >14 alcohol units weekly compared to abstinence, and left-sided atrophy at >30 units weekly. However, claims of laterality cannot be made as 95% confidence intervals for the odds ratios were overlapping. More significant hippocampal atrophy on the right has been described in those at higher risk of Alzheimer's disease (asymptomatic ApoE4 homozygotes),[234] as well as in those with mild cognitive impairment or Alzheimer's disease.[235] A higher proportion of unsafe compared to safe drinkers were smokers (Table 4.2), raising the possibility that smoking could be confounding the results. However, smoking is a component of the Framingham Risk Score, which all analyses were controlled for. Family history of alcoholism was not considered in these analyses. Previous studies have reported associations with family history and cognitive measures and brain structure.[236-239]

The VBM analysis also showed associations between increased alcohol consumption and reduced grey matter density in the amygdalae. This result could not be confirmed using other methods, as automated segmentation of these regions was unreliable, and we are unaware of any reliable visual atrophy rating scales. Amygdala atrophy has been described in Alzheimer's disease,[240] and is implicated in preclinical models of alcohol misuse,[241] alcohol abuse relapse[242] and in abstinent alcoholics,[243] although others have found no association with lower levels of consumption.[132]

Recommended guidelines for drinking have remained unchanged in the UK from 1987 until 2016. Our findings support the recent reduction in UK safe limits and call into question the current US guidelines which suggest that up to 24.5 units (196g) weekly is safe for men, as we found increased odds of hippocampal visual atrophy at just 14-21 units (112-168g) weekly, and we found no support for a protective effect of light consumption.

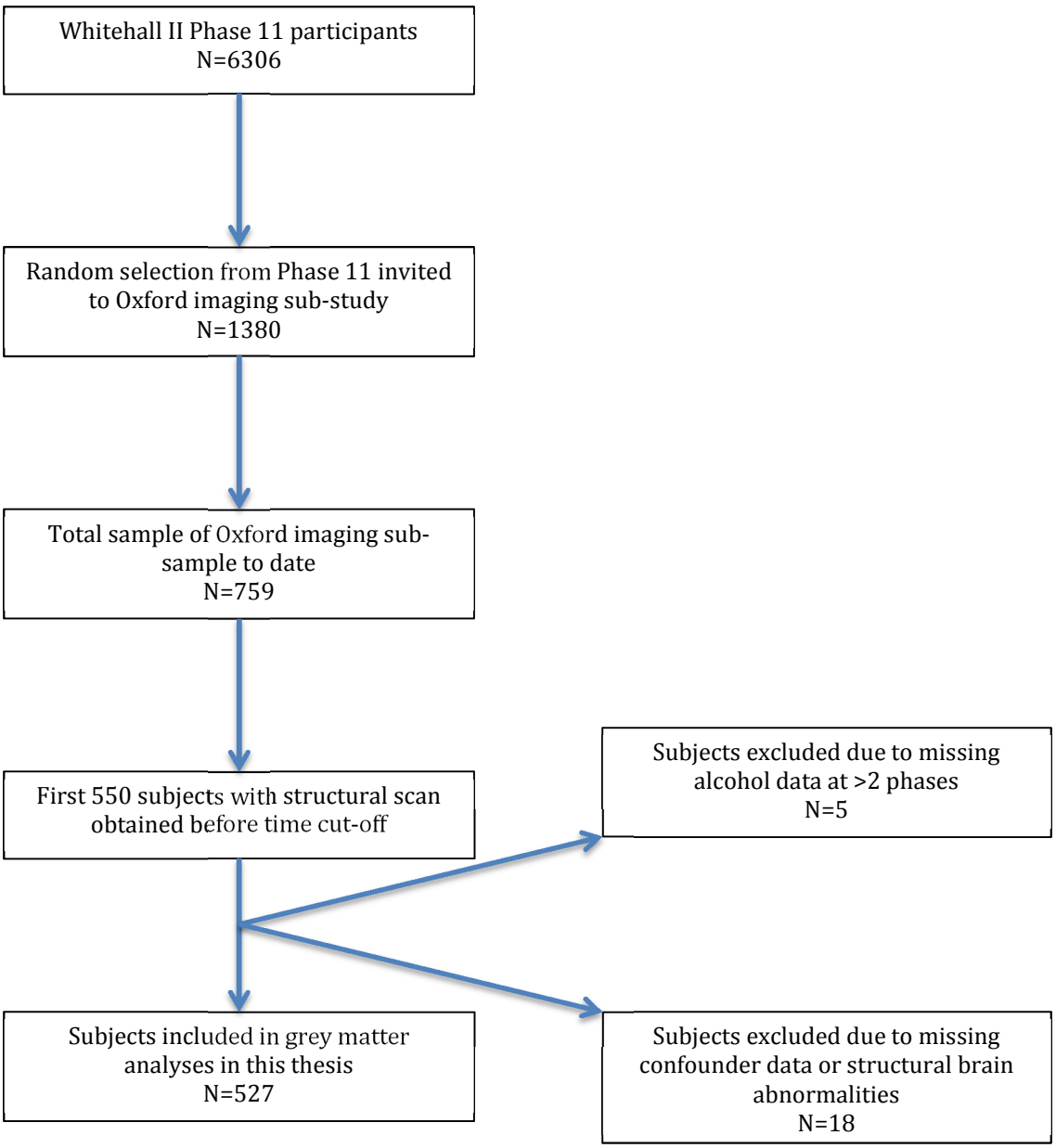


Figure 4.1 Flow chart of participants included in analysis.

Variable	MRI Sample N=527			Total imaging sub-study sample to date N=759			Phase 11 Participants N=6306			Difference in means or proportions (95% CI), p values	
	N	Mean/ %	S.D.	N	Mean/ %	S.D.	N	Mean/%	S.D.	Analysed sample vs. Phase 11	Analysed sample vs. total imaging study
Age [years]	527	69.6	5.3	752	69.8	5.2	6306	69.8	5.9	-0.2 (-0.7 to 0.3), p=0.5	-0.2 (-0.8 to 0.4), p=0.5
Sex	527			759			6306			-0.1 (-0.2 to -0.1), p<0.001	0.3 (-4.3 to 4.8), p=0.9
Female	103	19.5%		150	19.8%		1947	29.3%			
Male	424	80.5%		609	80.2%		4459	70.7%			
Full time education [years]	527	14.6	3.2	756	14.6	3.3	5101	15.1	4.2	-0.5 (-0.9 to -0.1), p=0.008	0.0 (-0.4 to 0.4) p=1.0
CES-D	527	5.0	5.8	755	5.3	6.2	5855	7.3	7.6	-2.3 (-3.0 to -1.6), p<0.001	-0.3 (-1.0 to 0.4), p=0.4
Systolic BP [mmHg]	527	140.8	17.6	753	141.2	17.9	5652	127.8	16.5	13.0 (11.5 to 14.5), p<0.001	-0.4 (-2.4 to 1.6), p=0.7
Diastolic BP [mmHg]	527	76.5	10.6	753	77.4	10.5	5652	70.8	9.9	5.7 (4.8 to 6.6), p<0.001	-0.9 (-2.1 to 0.3), p=0.1

Table 4.1: Comparison of the sample analysed in this thesis with the total imaging sample to date (n=759) and with the Whitehall II cohort at Phase 11 (n=6306).

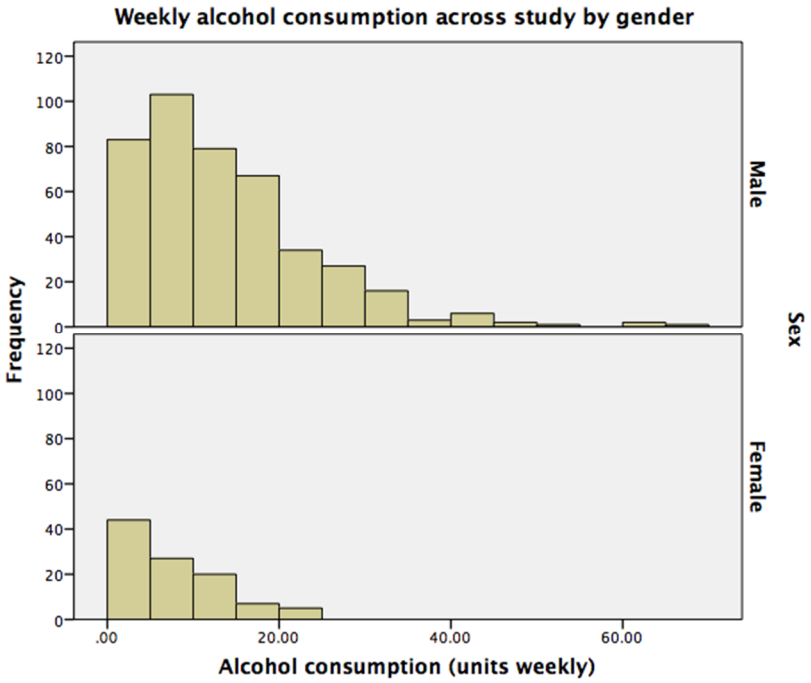


Figure 4.2 Average alcohol units weekly by gender.

	Mean (S.D.),[1] median (IQR)[2] or n (%)[3]		Difference between groups: mean (95% CI),[4] W statistic,[5] OR (95% CI)[6] or Pearson statistic,[7] p values
	Drinking within guidelines N=428	**Drinking above guidelines N=99**	
Age at start (years)	43.0 (5.4)[1]	42.8 (5.1)[1]	0.2 (-1.0 to 1.4), p=0.7[4]
Sex, n male (%)	339 (79.2%)[3]	85 (85.9%)[3]	3.4 (1.8 to 6.6), p<0.001[6]
Marital status (% married)*	308 (72.0%)[3]	81 (81.8%)[3]	1.8 (1.0 to 3.2), p=0.06[6]
Full-time education (years)	14.0 (5.0)[2]	15.0 (4.4)[2]	19448, p=0.2[5]
Full Scale Intelligence Quotient (estimated from TOPF)*[8]	117.4 (10.6)[1]	120.0 (8.3)[1]	-2.6 (-4.5 to -0.7), p=0.009[4]
Social class 1, 2, 3 & 4 (%)[9]	62, 328, 34, 4 (14.5%, 76.6%, 7.9%, 0.9%)[3]	21, 76, 2, 0 (21.2%, 76.8%, 2.0%, 0%)[3]	7.4 (0.4)[7]
Smoker (%)*	11 (2.6%)[3]	11 (11.1%)[3]	4.7 (1.8 to 12.4), p<0.001[6]
Systolic blood pressure*	140.3 (17.8)[1]	143.3 (16.7)[1]	-3.0 (-6.9 to 0.8), p=0.1[4]
Diastolic blood pressure*	76.3 (10.5)[1]	77.7 (10.9)[1]	-1.4 (-3.7 to 0.9), p=0.2[4]
Framingham Stroke Risk total score (%)*	9 (9)[2]	11 (10)[2]	0.9 (0.8 to 0.9), p<0.001[6]
History of Major Depressive disorder (%)*[10]	79 (18.5%)[3]	16 (16.2%)[3]	0.9 (0.4 to 1.6), p=0.7[6]
Social visits (weekly)*	4.4 (3.2)[1]	3.9 (3.1)[1]	0.5 (-0.2 to 1.2), p=0.2[4]
Psychotropic medication (%)*	62 (14.5%)[3]	12 (12.1%)[3]	0.8 (0.4 to 1.6), p=0.6[6]
MoCA total (/30)*[11]	28 (3)[2]	28 (3)[2]	1.0 (0.9 to 1.2), p=0.9[6]
MMSE baseline total (/30)[12]	29 (2)[2]	29 (3)[2]	1.2 (1.0 to 1.5), p=0.08[6]

[1] Mean (standard deviation).
[2] Median (interquartile range).
[3] Number (percentage).
[4] Mean difference (95% confidence interval), p value.
[5] W statistic and p value.
[6] Odds ratio (95% confidence intervals), p value.
[7] Pearson statistic and p value.
[8] Test of Premorbid Function.
[9] Social class based on occupation at phase 3: 1=professional, 2=managerial, 3=skilled non-manual, 4=skilled manual.
[10] Structured Clinical Interview for DSM IV (SCID).
[11] Montreal Cognitive Assessment.
[12] Phase 7 (n=389).
[*] Time of Scan.

Table 4.2 Baseline (Phase 1 unless otherwise indicated) summary characteristics of 527 participants (unless marked) included in analysis by drinking within (<14 units (112g) weekly women, <21 units (168g) weekly men) and above contemporaneous (pre-2016) UK Department of Health guidelines, on average over study duration.

	Men							Women					
	Weekly alcohol consumption Units (grams)							Weekly alcohol consumption Units (grams)					
Phase	**Age (Mean, S.D.)**	**Mean**	**Standard deviation**	**Median**	**Interquartile range**	**N (%) 'Unsafe' drinkers (Pre-2016 guidelines)**	**N (%) 'Unsafe' drinkers (Post-2016 guidelines)**	**Age (Mean, S.D.)**	**Mean**	**Standard deviation**	**Median**	**Interquartile range**	**N (%) 'Unsafe' (Pre- = Post-2016 guidelines)**
1	43.2 (5.4)	13.1 (104.4)	13.1 (104.6)	10.0 (80.0)	14.0 (112.0)	72 (17.1)	135 (32.1)	42.8 (5.3)	7.3 (58.4)	8.0 (64.4)	5.0 (40.0)	8.0 (64.0)	12(11.9)
3	48.3 (5.4)	12.7 (101.5)	12.0 (95.7)	10.0 (80.0)	13.0 (104.0)	78 (18.4)	124 (32.1)	48.0 (5.3)	7.6 (60.6)	8.3 (66.7)	50 (40.0)	10.3 (82.0)	13(14.4)
5	53.7 (7.5)	16.4 (130.8)	15.2 (121.3)	12.0 (96.0)	17.0 (136.0)	113 (28.5)	177 (44.6)	52.5 (9.1)	9.3 (74.5)	9.7 (77.4)	6.5 (52.0)	10.0 (80.0)	17(17.2)
7	59.6 (5.4)	14.3 (114.8)	12.2 (97.6)	12.0 (96.0)	15.0 (112.0)	90 (22.0)	164 (40.0)	59.1 (5.2)	8.1 (64.7)	7.5 (59.6)	6.0 (48.0)	12.0 (96.0)	16(16.5)
9	64.6 (5.4)	12.6 (101.1)	11.0 (88.6)	10.0 (80.0)	16.0 (128.0)	76 (18.4)	140 (34.0)	64.0 (5.2)	7.2 (57.5)	7.3 (58.1)	6.0 (48.0)	9.3 (74.0)	14(13.7)
11	68.7 (5.4)	11.7 (94.3)	10.6 (85.5)	10.0 (80.0)	13.0 (104.0)	62 (14.6)	121 (28.5)	68.2 (5.2)	5.6 (45.1)	5.7 (45.2)	4.0 (32.0)	8.3 (66.0)	8(7.8)
Time of Scan	69.2 (5.4)	16.8 (112.3)	15.9 (122.9)	13.8 (101.7)	17.6 (134.8)	113 (26.7)	206 (48.6)	69.3 (5.3)	9.6 (77.1)	10.5 (84.2)	6.6 (52.8)	13.6 (108.8)	9(9.0)
Average		14.0 (111.8)	10.7 (85.8)	11.5 (92.3)	12.8 (102.5)	85 (20.0)	171 (40.3)		7.8 (62.7)	6.2 (49.6)	6.4 (51.4)	10.1 (80.9)	14(13.6)

Table 4.3 Summary of alcohol consumption by gender at each study phase (n=527) including: weekly units (grams) consumed; percent drinking over safe weekly limits, as defined by pre-2016 (>14 units/112g women, >21 units/168g men), and post-2016 guidelines

		CAGE score n (valid %)		
Phase	Total with CAGE completed	0	1	2/3
3	525	468 (88.6)	57 (11.4)	0 (0)
5	494	439 (89.2)	55 (10.8)	0 (0)
7	527	470 (88.7)	57 (11.3)	0 (0)
9	499	444 (88.5)	55 (11.5)	0 (0)
11	509	453 (88.8)	56 (11.2)	0 (0)

Table 4.4 Summary of CAGE (screen for alcohol dependence) scores across study phases.



Figure 4.3 Voxel-based morphometry results (threshold-free cluster enhancement (tfce) corrected): significant (p<0·05, red) negative correlation between weekly alcohol units (average of all phases across study) and grey matter density. N=527. Adjusted for age, sex, education, premorbid FSIQ, social class, physical exercise, club attendance, social activity, Framingham Stroke Risk Score, psychotropic medication, and history of Major Depressive Disorder.

	Men						Women						All					
	R Hippocampal atrophy (vs. none)			L Hippocampal atrophy (vs. none)			R Hippocampal atrophy (vs. none)			L Hippocampal atrophy (vs. none			R Hippocampal atrophy (vs. none)			L Hippocampal atrophy (vs. none)		
Alcohol (units weekly)	**N (Total)**	**OR (95% CI)**	**P**	**N (Total)**	**OR (95% CI)**	**P**	**N (Total)**	**OR (95% CI)**	**P**	**N (Total)**	**OR (95% CI)**	**P**	**N (Total)**	**OR (95% CI)**	**P**	**N (Total)**	**OR (95% CI)**	**P**
0-<1	9 (22)			12 (22)			4 (15)			7 (15)			13 (37)			19 (37)		
1 to <7	55 (99)	1.6 (0.6-4.3)	0.4	69 (99)	1.7 (0.6-4.8)	0.3	12 (41)	1.1 (0.2-5.6)	0.9	20 (41)	0.8 (0.2-3.6)	0.8	67 (140)	1.5 (0.7-3.4)	0.3	89 (140)	1.3 (0.6-3.0)	0.5
7 to <14	68 (132)	1.7 (0.6-4.5)	0.3	85 (132)	1.5 (0.6-4.2)	0.4	19 (33)	3.1 (0.6-16.6)	0.2	22 (33)	2.0 (0.4-10.2)	0.4	87 (165)	2.0 (0.9-4.4)	0.1	107 (165)	1.4 (0.6-3.2)	0.4
14 to <21	57 (86)	**3.2 (1.1-9.3)**	**0.028**	59 (86)	2.0 (0.7-5.7)	0.2	6 (11)	4.2 (0.6-28.8)	0.1	9 (11)	6.2 (0.7-55.2)	0.1	**63 (97)**	**3.4 (1.4-8.1)**	**0.007**	68 (97)	1.9 (0.8-4.6)	0.1
21 to <30	38 (54)	**3.9 (1.3-12.0)**	**0.017**	39 (54)	2.2 (0.7-6.8)	0.2	1 (3)	1.1 (0.04-26.9)	0.97	2 (3)	0.4 (0.4-10.2)	0.4	**39 (57)**	**3.6 (1.4-9.6)**	**0.009**	41 (57)	1.9 (0.7-4.9)	0.2
≥30	24 (31)	**5.2 (1.4-19.0)**	**0.012**	27 (31)	**6.3 (1.5-27.0)**	**0.013**							**24 (31)**	**5.8 (1.8-18.6)**	**<0.0001**	**27 (31)**	**5.7 (1.5-21.6)**	**0.01**

Table 4.5 Adjusted ORs for left and right sided hippocampal atrophy on Scheltens visual rating score (reference based on abstainers), with average alcohol consumption (weekly units) 1- <7 (8-<56g), 7- <14 (56-112g), 14- <21 (112-<168g) and 21- <30 (168-<240g), ≥30 units (>240g) (abstinence <1 units as reference category). Significant (p<0.05) results in bold. N=527. Analyses adjusted for age, sex, premorbid IQ, education, social class, Framingham Risk Score, history of Major Depressive Disorder (SCID), exercise frequency, club attendance, social visits, current psychotropic medication. Numbers with hippocampal atrophy (N) and total numbers in drinking category (Total N) are also given.

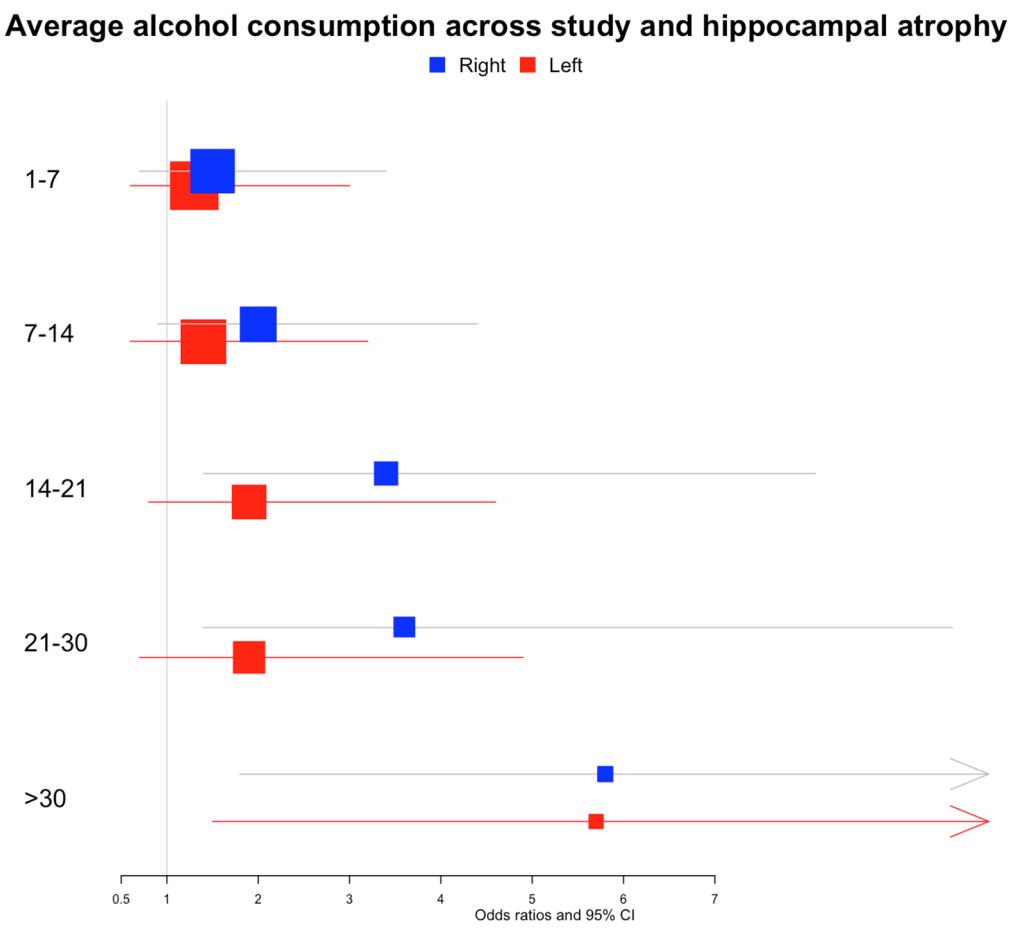


Figure 4.4 Adjusted odds ratios (box – ORs, size reflects precision of estimate, whiskers - 95% CI, clipped at 10 (arrow)) for left and right sided hippocampal atrophy on Scheltens visual rating score (against abstainers) with average alcohol consumption (weekly units) 1- <7; 7- <14; 14- <21; and 21- <30, and ≥30 units (abstinence <1 units weekly as reference category). N=527. Adjusted for age, sex, education, premorbid FSIQ, social class, physical exercise, club attendance, social activity, Framingham Stroke Risk Score psychotropic medication, and history of Major Depressive Disorder.

	Mean (S.D.)
Right hippocampal volume (unadjusted, mm^3)	3474 (433)
Left hippocampal volume (unadjusted, mm^3)	3368 (444)
Right hippocampal volume (adjusted as % of ICV)	2.42 (0.30)
Left hippocampal volume (adjusted as % of ICV)	2.35 (0.32)

Table 4.6 Mean (S.D.) raw and adjusted (%ICV) hippocampal volumes extracted using FIRST for the sample.

	Change in hippocampal volume[2] (% intracranial volume) for every 10 unit increase in weekly alcohol consumption	P value	95% CI
Unadjusted alcohol	-0.26	<0.001	-0.37 to -0.15
Adjusted alcohol[1]	-0.19	<0.001	-0.30 to -0.08

[1] Analyses adjusted for age, sex, premorbid IQ, education, social class, marital status, Framingham Risk Score, history of Major Depressive Disorder (SCID), exercise frequency, club attendance, social visits, current psychotropic medication.
[2]Hippocampal volume squared.

Table 4.7 Multiple linear regression results, with squared hippocampal volume (% of ICV) as the dependent variable and average alcohol consumption across study as an independent variable.

	Change in hippocampal volume[2] (% intracranial volume) for every 10 unit increase in weekly alcohol consumption	P value	95% CI
Alcohol adjusted[1] (all cases)	-0.19	<0.001	-0.30 to -0.08
Alcohol adjusted[1] (excluding highest 3 drinkers)	-0.18	0.003	-0.29 to -0.06

[*] For every 10 unit increase in alcohol weekly
[1] Analyses adjusted for age, sex, premorbid IQ, education, social class, marital status, Framingham Risk Score, smoking, history of Major Depressive Disorder (SCID), exercise frequency, club attendance, social visits, current psychotropic medication.
[2]Hippocampal volume squared.

Table 4.8: Outlier analysis of multiple linear regression results, with squared hippocampal volume (% of ICV) as the dependent variable and average alcohol consumption as an independent variable.

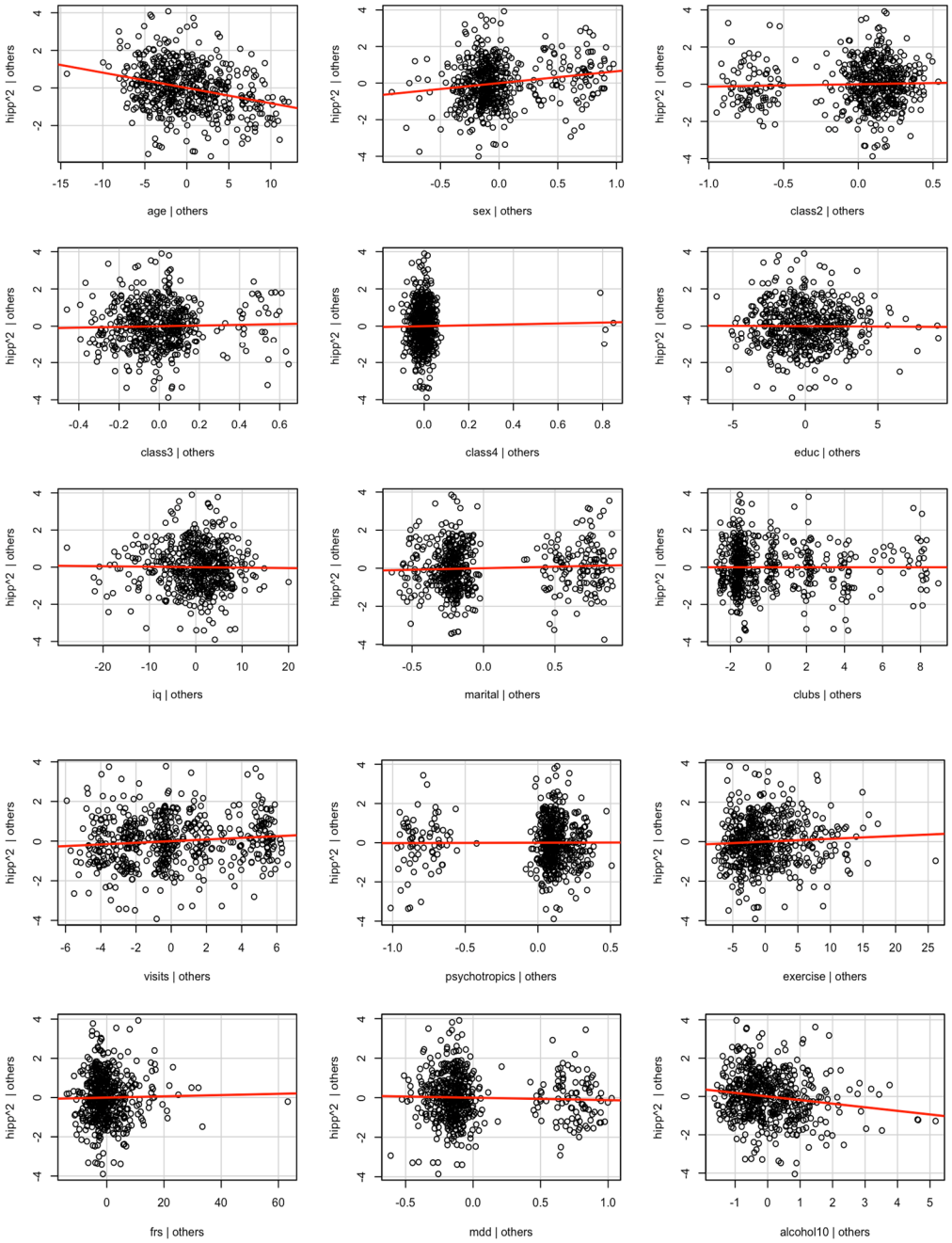


Figure 4.5 Partial regression plots showing the relationships between a number of variables and hippocampal volume (extracted from FIRST, as %ICV, squared), controlling for other variables in the model. For example, the last plot visualises the relationship between alcohol and hippocampal size, after controlling for: age, sex, social class, education, FSIQ, marital status, club participation, social visits, psychotropic medication, exercise, Framingham Risk Score and history of Major Depressive Disorder. The x-axis units are residuals for a regression model omitting alcohol. The y-axis units are residuals from a regression of alcohol against the remainder of the independent variables. The slope of the red line represents the partial regression coefficients.

Chapter 5: Alcohol consumption and white matter

5.1 Introduction

Post-mortem studies have provided evidence of prominent white matter loss in alcoholics, which may exceed grey matter loss.[77, 82] The frontal lobes and cerebellum are particularly vulnerable.[48, 84,75] Furthermore, white matter loss in the temporal lobes has been linked to alcohol withdrawal seizures.[85] Neuroimaging studies have replicated these findings.[54, 205, 244] The corpus callosum is known to be affected in alcohol-related nutritional deficiency conditions such as Marchiafava-Bignami,[245] but thinning in alcoholics has also been described independent of nutrition.[89, 90] It is additionally implicated in foetal alcohol syndrome and prenatal alcohol exposure.[246, 247] Abnormalities in white matter microstructure have also been reported in alcoholics,[248-250] and there is some suggestion that white matter hyperintensities may be more prevalent in such individuals.[251] Interestingly, some of these white matter abnormalities may improve after an extended period of abstinence.[252]

Whether more moderate alcohol consumption can influence white matter is unclear. Some studies have described reduced white matter volume,[132] including in selected regions such as superior temporal and parahippocampal gyrus,[128] and the anterior corpus callosum.[253] In contrast, one small study found lifetime alcohol consumption positively correlated with white matter density in men.[131] Others have found no association.[227] Conflicting results regarding the relationship with white matter hyperintensities have also been reported. Mukamal et al. found reduced visually rated

WMHs in those drinking 1- <7 units (8-<56g) weekly compared to those who were abstinent (OR 0.68).[127] Similarly Fukuda et al. found moderate drinkers (>7 units (<56g) weekly) had 0.13% lower WMLs (%ICV) than non-drinkers.[133] However others have found no such relation.[124, 126, 132, 254] We are not aware of any studies to date examining the relationship between moderate alcohol consumption and white matter microstructure using diffusion tensor imaging.

5.2 Objectives and hypotheses

Our aims were two-fold:

1. To establish whether there is a relationship between non-dependent alcohol consumption and macroscopic white matter changes, namely:

    a. Total white matter volume
    b. White matter hyperintensities

2. To establish whether there is a relationship between non-dependent alcohol consumption and microscopic white matter changes, as inferred from diffusion tensor indices.

Hypotheses:

1. Mean alcohol consumption across the study is negatively correlated with total white matter volume.

2. The extent of white matter hyperintensities is inversely correlated with alcohol intake.

3. White matter integrity, inferred by diffusion tensor indices, is negatively associated with alcohol consumption.

5.3 Methods

Detailed methods of subject selection, data collection and initial MRI analysis are in Chapter 4.

5.3.1 Diffusion tensor imaging

Diffusion tensor imaging (DTI) measures the directional preference of water diffusion in neural tissue and allows inferences about the structural integrity of white matter tracts. In healthy myelinated fibres diffusion is restricted perpendicular to the longitudinal axis of the fibre, i.e. it is anisotropic. Voxelwise statistical analysis of fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD) and mean diffusivity (MD) data was carried out using Tract-Based Spatial Statistics (TBSS).[255] This involves non-linear registration followed by projection onto an alignment-invariant tract representation (the "mean FA skeleton"). This avoids alignment problems for multiple subjects and avoids arbitrariness of spatial smoothing extent, improving the sensitivity, objectivity and interpretability of analysis of multi-subject diffusion imaging studies.[256] Multiple diffusion indices were analysed to allow a

richer investigation of localised connectivity related changes. AD describes diffusion parallel to, and RD perpendicular to the to the principal fibre direction. MD is the apparent diffusion coefficient averaged over all directions. FA reflects AD in relation to RD and is widely used as a marker of tract integrity.[257] [258] Diffusion images were corrected for head movement and eddy currents (eddy_correct) and brain masks generated using BET. Fractional anisotropy, mean diffusivity, axial diffusivity and radial diffusivity maps were generated using DTIFit (http://fsl.fmrib.ox.ac.uk/fsl/fdt) that fits a diffusion tensor model at each voxel. Tract-based spatial statistics (TBSS) were used in a 4-stage process. Pre-processing prepared images for registration to standard space. Mean fractional anisotropy (FA), diffusivity (MD), radial diffusivity (RD), axial diffusivity (AD) and skeletonized FA, MD, RD and AD images were created, and thresholded. Lastly each FA, MD, RD and AD image was projected onto the relevant skeleton and 'randomise' used for statistical analyses. Masks of specific white matter tracts were created using ICBM-DTI-81 white-matter labels atlas[259] and used to extract mean white matter integrity indices.

5.3.2 Visual rating of white matter hyperintensities

White matter changes on FLAIR images were graded independently, by three researchers using the Fazekas scale depending on the presence and size of deep white matter changes (DWMC; 0-3), and the presence or extent of periventricular white matter changes (PVWMC; 0-3).[260] This scale provides two different scores each, rated on a 4-point scale.

5.3.3 Automatic segmentation of white matter hyperintensities

WMHs were automatically segmented on FLAIR images with a newly developed tool, BIANCA (Brain Intensity AbNormality Classification Algorithm), a fully automated, supervised method for WMH detection, based on the k-nearest neighbour algorithm.[261] Briefly, BIANCA classifies the image's voxels based on their intensity and spatial features, where the intensity features were extracted from FLAIR, T1 and FA images (additional options used: local average intensity within a 3D kernel of size = 3 voxels; MNI coordinates as spatial features, with a weighting factor of 2; 24 manually segmented images as training dataset). The output image represents the probability per voxel of being WMH. The total WMH volume was calculated from the voxels exceeding a probability of 0.9 of being WMH and located within a white matter mask, adjusted for the total intracranial volume.

Mean FA values were extracted for WMHs for each subject. This was done in FA space, by first transforming each BIANCA WMH mask (in FLAIR space) into DWI space. Secondly, the mask was binarised with a threshold of 0.5 (determined by visually checking a sample of alternative thresholds) to ensure only voxels from within lesions were sampled without contamination from marginal voxels. Finally, mean FA values were extracted from the masked lesion areas.

5.3.4 Statistical analyses

Mean alcohol consumption (units per week) across all study phases was included as an independent variable in TBSS analyses (FA/MD/RD/AD as dependent variable).

Voxelwise, a Generalised Linear Model (GLM) was applied using permutation-based non-parametric testing (randomise),[217] correcting for multiple comparisons across space (threshold-free cluster enhancement, tfce). Results were judged significant, if adjusted $p<0.05$.

Multiple linear regression analyses were performed, with a white matter outcome (total WM volume % ICV/WMH volume/mean FA of lesions) as the dependent variable. Average alcohol consumption over the study was an independent variable. In order to test whether low levels of alcohol consumption protects against regards white matter hyperintensities, weekly alcohol units were categorized as follows: <1 (<8g) (reference), 1- <7 (8-<56g), 7-<14 (56-<112g), 14-<21 (112-<168g), and >21 (>168g). Fazekas visual rating scores were binarised into mild (0/1) and severe (2/3). Odds ratios for severe versus mild periventricular and deep white matter hyperintensities were calculated using binary logistic regression.

In analyses the following potential confounding variables (identified from knowledge of the literature) were included as independent variables: age, sex, premorbid IQ, education, social class, Framingham Risk Score (a composite measure including smoking, cardiovascular disease or diabetes, cardiovascular medication), current psychotropic medications (number), lifetime history of Major Depressive Disorder (SCID, binarised yes (2)/no (1)), exercise frequency, club attendance, voluntary work, visits with friends and family.

5.4 Results

5.4.1 White matter measures

511 subjects were included in the TBSS analysis, after the exclusion of 16 with absent or poor quality DTI. A further 52 subjects were excluded for the automatic extraction of white matter hyperintensities due to missing FLAIR images or inadequate image quality (Figure 5.1).

Periventricular white matter lesions, as assessed by the Fazekas scale, were ubiquitous (Figure 5.2). Deep white matter lesions, at least of mild severity, were present in 94.8% of subjects. Agreement between visually rated lesions and the automatically extracted hyperintensity volumes (using BIANCA) was high (Pearson's correlation coefficient 0.6, $p<0.0001$) (Figure 5.3).

5.4.2 White matter volume

Average alcohol consumption across the study did not predict total white matter volume (adjusted for intracranial volume) (Table 5.1).

5.4.3 White matter lesions

Average alcohol consumption did not predict white matter lesion volume, as calculated using automatic segmentation with BIANCA. Neither did it predict periventricular nor deep white matter lesions visually rated using the Fazekas scale (Table 5.1). Furthermore, there was no protective benefit of low levels of alcohol

consumption on the odds of having severe versus mild white matter hyperintensities (Table 5.2).

5.4.4 White matter microstructure

Participants, who drank on average more alcohol across the study, had greater abnormalities in white matter microstructure (Figure 5.4). This was reflected by lower corpus callosum fractional anisotropy, and higher radial, axial and mean diffusivity. These associations were focused on the anterior corpus callosum (genu and anterior body), and were independent of vascular risk and depressive illness. The opposite contrasts (i.e. positive correlation with fractional anisotropy and negative correlations with radial, axial and mean diffusivity were not statistically significant.

5.5 Discussion

Addressing our original aims, higher alcohol consumption over the course of the study was associated with differences in white matter microstructure, as assessed by DTI, but not with either measure of macrostructural damage, total white matter volume or white matter hyperintensities, as discovered with FLAIR. The association between alcohol consumption and white matter microstructure found in non-dependent drinkers is novel and appeared to be driven by greater radial relative to axial diffusivity.

We observed a high occurrence of white matter hyperintensities amongst subjects (Figure 5.2). Direct comparison with other published studies is difficult, given the differing imaging protocols, rating scales and rater expertise. Nonetheless, the Rotterdam scan study, for example, reported a slightly lower prevalence of white matter lesions compared with our findings (92% versus 94.8% DWMC, 80% versus 100% PVWMC).[262] This could reflect a true increased burden of pathological changes or increased detection by our higher resolution MRI protocol (all the above studies used a field strength of 1.5T in contrast to 3T in this study).

The finding that moderate alcohol was not associated with white matter lesions is consistent with the existing literature. A number of previous studies have also failed to find associations,[124, 126, 132] although there are exceptions.[127, 133] However, this study has three main methodological advantages over previous work in the field. First, white matter hyperintensities are defined using both a visual rating scale and additionally a fully automated method, rather than simply the former as in previous studies. Results using both independent methods were consistent. Visual rating scales are time-consuming, require user expertise, and suffer from intra- and inter-operator variability. They provide discrete measurements only. In contrast, the automated extraction method we use provides volume measurements on a continuous scale and can be applied rapidly to a large group of subjects. It does, however, require manual labelling of a small training set before application, and hence these manual masks may compromise its accuracy. As a consequence, it works best in those with a higher lesion load.[261] Despite these potential limitations, it compares favourably with both visual ratings and other existing methods.[261] Second, alcohol consumption is recorded

prospectively over 30 years, which is likely to be more relevant than current intake when considering structural brain changes. Third, we also examined the relationship between non-dependent alcohol consumption and microstructural white matter, using DTI, which has not been attempted before to our knowledge.

In animals, radial diffusivity reflects differences in myelination.[263, 264] Prior studies have highlighted the corpus callosum as an area affected in foetal alcohol syndrome,[265] and in chronic alcoholism in Marchiafava-Bignami disease,[86, 266] and volume changes have been described in moderate drinkers.[253] One study reported increased mean diffusivity in the amygdala in a post-hoc analysis of female non-dependent drinkers.[134] We are not aware of any studies investigating white matter microstructural changes in moderate drinkers using a data driven skeletonised tract approach to diffusion tensor images, such as TBSS. Alternative voxel-wise methods may compromise optimal analysis of multiple subjects, as there are alignment problems causing potential difficulties with interpretation of voxel-wise statistics.[256] The associations we found were more frontally focused. A frontal predominance to white matter damage in alcoholics is well described.[48] The relationship between moderate alcohol and white matter microstructure was not mediated by vascular risk, which is known to affect white matter quality.[254] Postulated mechanisms for white matter damage in alcoholics include thiamine deficiency, liver dysfunction, and head injury. These do not easily translate into moderate alcohol consumption.

In summary, we have found novel evidence for an association between non-dependent alcohol consumption and microstructural white matter indices in a cohort of

community-dwelling older subjects. There was no evidence for a protective effect of moderate alcohol intake over abstinence on white matter.

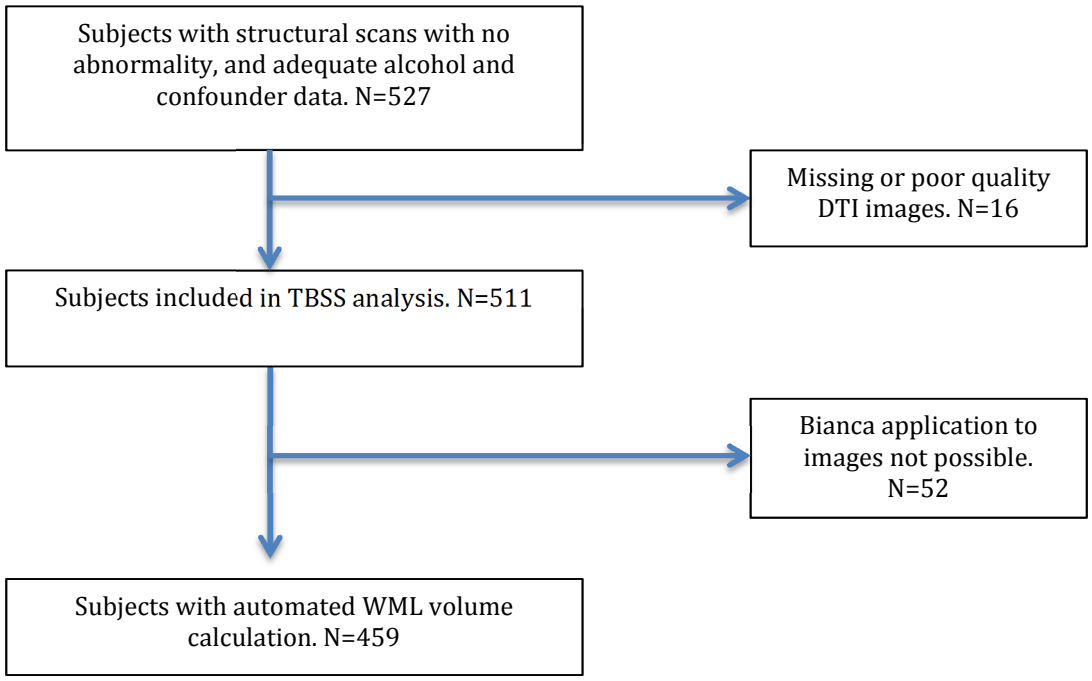


Figure 5.1 Flow chart of participants included in analysis.

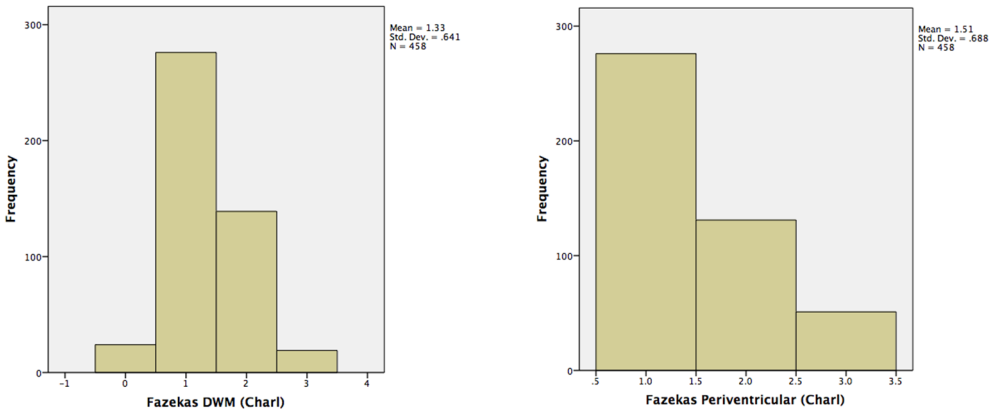


Figure 5.2 Frequency histograms of Fazekas visual rating scores of deep (DWM) and periventricular white matter hyperintensities.

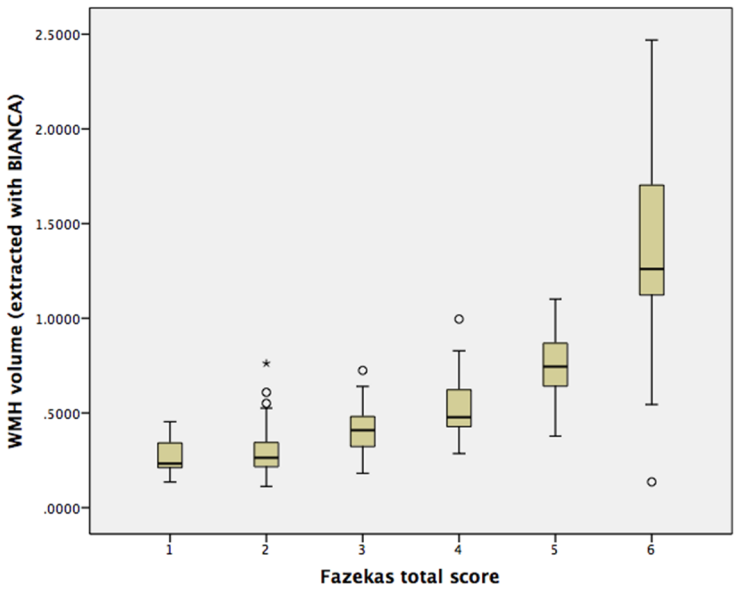


Figure 5.3 Relationship between Fazekas visual rating scores and automatically extracted white matter hyperintensity volume (using BIANCA).

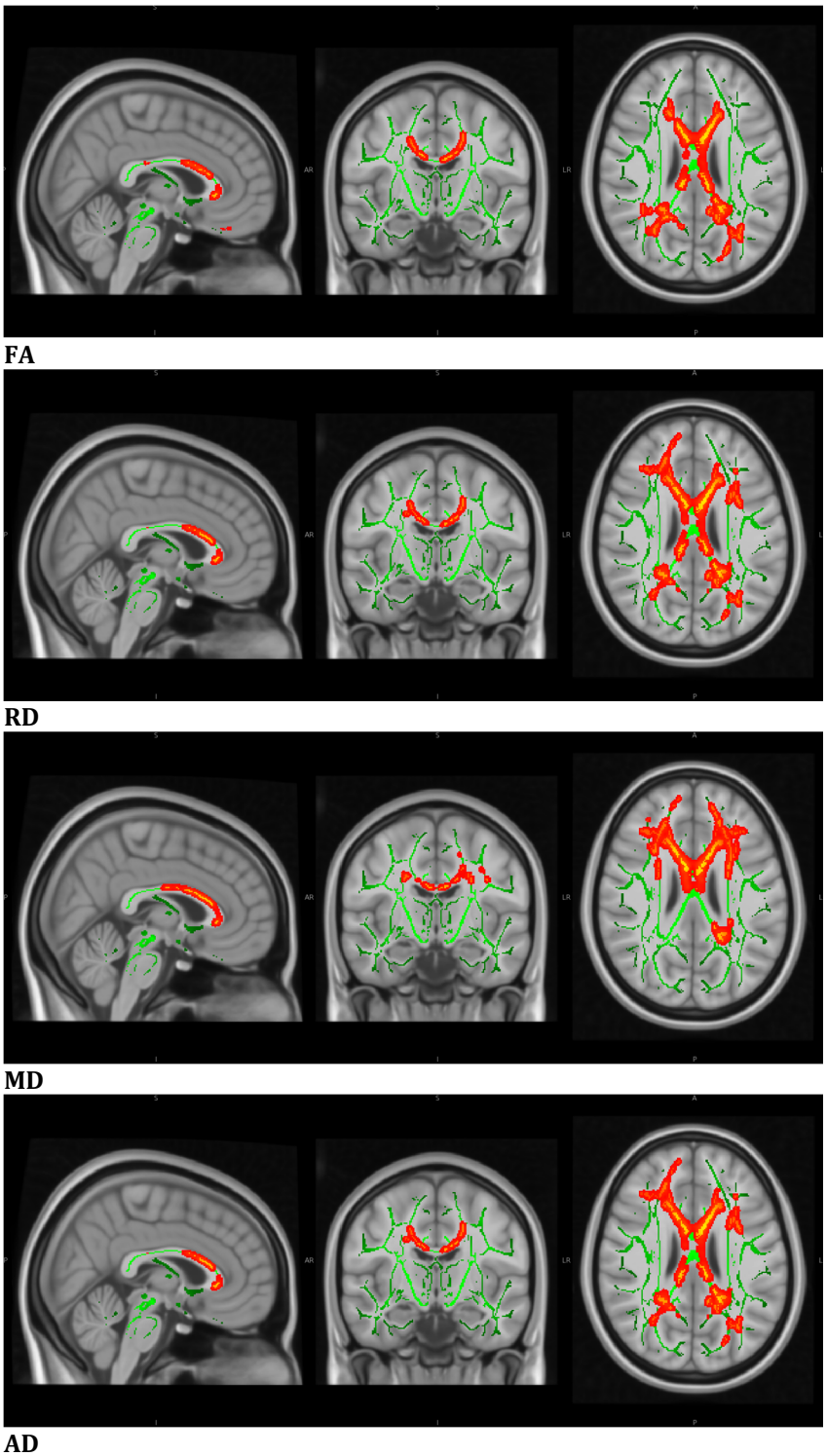
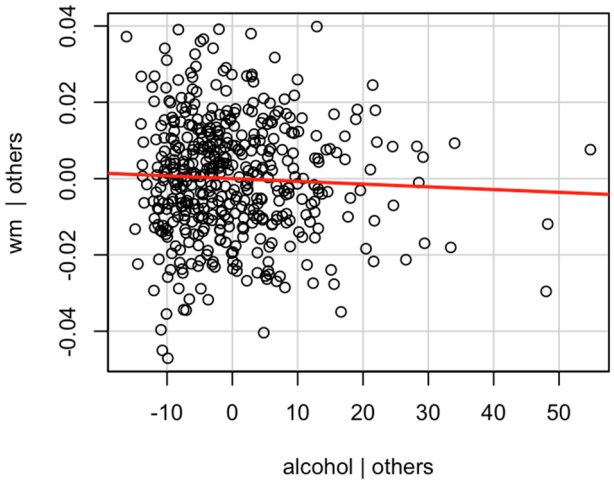
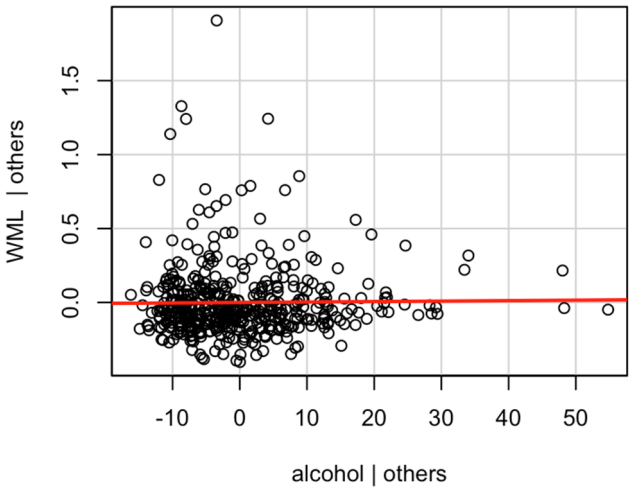


Figure 5.4 Tract-based spatial statistics results (threshold-free cluster enhancement, tfce, corrected) showing negative correlation between average alcohol across study (all phases) and fractional anisotropy (top), radial diffusivity, mean diffusivity and axial diffusivity (bottom). N=511. Significant results (p<0·05) in red/yellow Adjusted for age, sex, education, premorbid FSIQ, social class, physical exercise, club attendance, social activity, Framingham Stroke Risk Score, psychotropic medication, and history of Major Depressive Disorder.



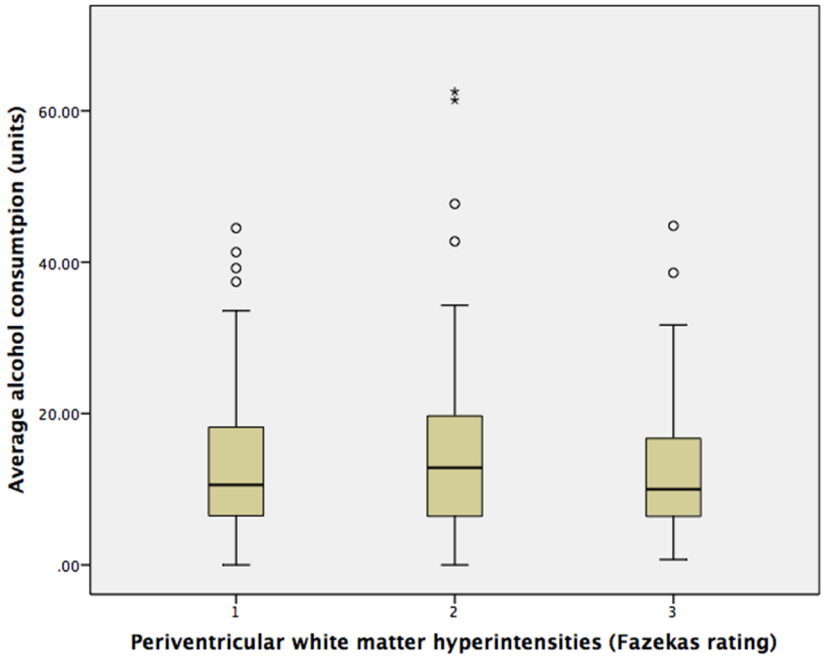
$y = -5.08^{e-17} + -8.63^{e-5} * x$

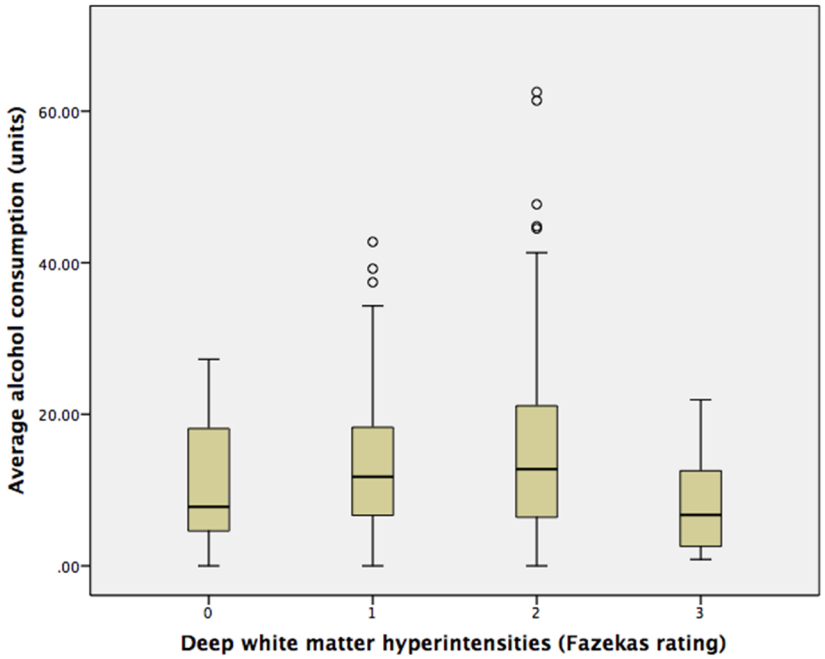
Figure 5.5 Partial regression plots for multiple linear regression analysis with total white matter volume (% of intracranial volume) as the dependent variable, and average alcohol consumption as an independent variable, adjusted for: age, sex, education, premorbid FSIQ, social class, physical exercise, club attendance, social activity, Framingham Stroke Risk Score, psychotropic medication, and history of Major Depressive Disorder.



$y = 7.18^{e-16} + 9.84^{e-4} * x$

Figure 5.6 Partial regression plot for multiple linear regression analysis with white matter hyperintensity volume (estimated using BIANCA) as the dependent variable, and average alcohol consumption as an independent variable, adjusted for: age, sex, education, premorbid FSIQ, social class, physical exercise, club attendance, social activity, Framingham Stroke Risk Score, psychotropic medication, and history of Major Depressive Disorder.





Figures 5.7 Box plots showing alcohol consumption on average across the study categorized by periventricular and deep (DWM) white matter hyperintensities rated using the Fazekas score.

Dependent variable	Standardised coefficient Beta (p value)	Unstandardised coefficient B	95% CI
Total white matter volume (%ICV)	-0.04 (0.4)	-6.9^{e-05}	-2.2^{e-01} to 7.7^{e-05}
White matter lesion volume (BIANCA extracted, %ICV)	0.001 (0.4)	0.04	-0.001 to 0.003
Mean FA BIANCA lesion	-0.05 (0.9)	2.0^{e-05}	-0.0004 to 0.0004
Fazekas rating of deep white matter lesions	0.03 (0.3)	0.003	-0.003 to 0.009
Fazekas rating of periventricular white matter lesions	0.04 (0.4)	0.003	-0.004 to 0.009

Table 5.1 Multiple linear regression results, with varying white matter markers as the dependent variable, and average alcohol consumption as the independent variable. Adjusted for: age, sex, education, premorbid FSIQ, social class, physical exercise, club attendance, social activity, Framingham Stroke Risk Score, psychotropic medication, and history of Major Depressive Disorder.

	PVH (severe vs. mild)			DWH (severe vs. mild)		
Average alcohol U (g)/week	***N (Total)***	***OR (95% CI)***	***P***	***N (Total)***	***OR (95% CI)***	***P***
0-<1 (0-<8)	(33)			(33)		
1 to <7 (8-<56)	49 (117)	1.1 (0.5-2.5)	0.8	45 (117)	1.3 (0.5-3.1)	0.6
7 to <14 (56-<112)	52 (1144)	0.9 (0.4-2.0)	0.8	44 (144)	0.9 (0.4-2.1)	0.8
14 to <21 (112-<168)	35 (88)	1.0 (0.4-2.4)	1.0	29 (88)	1.0 (0.4-2.3)	0.9
>21 (>168)	34 (77)	1.2 (0.5-3.0)	0.6	31 (77)	1.4 (0.6-3.6)	0.5

Table 5.2 ORs for severe (>1 on Fazekas visual rating scale) vs. mild (0-1 on Fazekas scale) periventricular (PVH) and deep white matter (DWH) hyperintensities, by average weekly alcohol consumption (units (g)) over course of the study. Adjusted for: current age, sex, education, FSIQ and Framingham Risk score.

Chapter 6: Alcohol and Brain Function

6.1 Introduction

Chronic heavy drinking can lead to Korsakoff's syndrome and alcoholic dementia, characterised by multiple cognitive deficits including memory impairments, aphasia, apraxia, agnosia and executive dysfunction (DSM IV criteria).[267] Several longitudinal studies have supported the association between heavy drinking and increased cognitive decline.[41, 44] In contrast, the long-term effects of non-dependent alcohol consumption on cognition are poorly understood. Some authors have suggested an inverted U-shaped relationship of alcohol use with cognitive function, similar to that with cardiovascular disease.[4] Claims of a lower dementia risk,[7, 8] a reduced incidence of myocardial infarction[5] and stroke have been made.[6] However, meta-analyses have produced conflicting results. Whilst Peters et al.[116] reported some evidence of a protective effect of moderate drinking on dementia risk, there was no such effect on cognitive decline, and they advised caution in interpreting the results due to significant heterogeneity. Similarly, Anstey et al.[117] found no significant effect of moderate alcohol on cognition.

The effect of alcohol consumption on functional connectivity of the brain is poorly characterised. This is largely due to the paucity of studies examining resting state fMRI in relation to drinking. Studies in healthy volunteers have investigated the effects of acute alcohol administration. These have shown functional connectivity changes in a number of networks including the visual network,[29] default mode

network,[268] amongst others.[269] Other studies in those with alcohol use disorders, defined variably, report connectivity changes in the anterior and posterior cingulate,[270] reward network,[271] cerebellum,[272] visual network,[30] and the default mode network.[273] There are no studies examining relationships between resting state connectivity and long term moderate alcohol consumption.

6.2 Objectives and hypotheses

With conflicting results in the existing literature or poorly investigated relationships, we explored the following two questions about alcohol and brain function:

1. What is the association between non-dependent alcohol consumption and cognitive performance?

2. Is there a relationship between alcohol consumption and resting functional connectivity of the DMN?

We hypothesised:

1. There would be a protective effect of moderate alcohol consumption on cognitive impairment.
2. Alcohol consumption would be associated with increased functional connectivity in the DMN.

6.3 Methods

6.3.1 Cognitive function

Cognitive function was assessed at Phases 3, 5, 7, 9, 11 and again at the time of the MRI using lexical (how many words beginning with a letter can be generated in one minute) and semantic (how many words in a category can be named in one minute) fluency tests. Short-term memory (recall of a 20-word list) was tested at Phases 3, 5, 7, 9 and 11. The Mini-Mental State Examination (MMSE) was performed at Phases 5, 7, 9, and 11. Further assessment at the time of the MRI included the following tests: MoCA, Trail Making Test (TMT A and B), Rey-Osterrieth Complex Figure (RCF) copying, RCF immediate recall, Hopkins Verbal Learning Test (HVLT-R) immediate recall (HVLT TR), Boston Naming Test (BNT), Digit Span (forwards - DSF, backwards - DSB, substitution - DSS) and Digit Coding (DST, from the Wechsler Adult Intelligent Scale-IV), Test of Premorbid Function (TOPF), HVLT-R delayed recall (HVLT DR), and RCF delayed recall. Full-scale intelligence quotient (IQ) was estimated using the TOPF with sex and education adjustment.

Full-scale intelligence quotient (IQ) was estimated using the Test of Premorbid Functioning - UK Version (TOPF UK, assessed at the time of the scan) with sex and education adjustment.

6.3.2 Resting state functional connectivity

Two sets of resting state networks' (RSN) spatial maps with differing numbers of dimensions (25 and 100 – see later for explanation) was generated from the Whitehall II subject's images using an independent components approach (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/MELODIC). MELODIC pre-processing includes motion correction, brain extraction, high-pass temporal filtering (cut-off 150s) and field map correction.[215, 274] To reduce noisy components, a data-cleaning approach was used. Following single-subject ICA, FMRIB's ICA-based X-noisefier (FIX) was used to automatically classify and regress out artefactual components.[275, 276] This followed FIX being "trained" on hand-classified ICA components on a matched training set (Whitehall II MB6).

Data was then affine registered to structural images using FLIRT (and optimized using boundary-based registration.[209, 210, 277] FNIRT was used to register images into standard (MNI) space.[211] MELODIC then performed a group-level ICA to identify spatial maps (RSNs).

To analyse relationships with functional connectivity within networks, spatial maps of interest based on *a priori* hypotheses e.g. the default mode and executive networks, were identified by visual inspection and used as a common template to extract time series and spatial maps from Multiband and Standard EPI sequence data using the dual regression approach (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/DualRegression).[278, 279] Correlations with average alcohol consumption were tested for using FSL's randomise permutation-testing tool, adjusted for multiple potential confounders including grey matter. Using network masks, mean connectivity values for the DMN were extracted.

Time courses were also fed into FSLNets (v0.6) to perform network modelling.[280] For this analysis group maps from a higher dimensionality ICA were used, thought to be more meaningful for between component network analysis.[281] Nodes were classified as 'good' (n=58) or 'bad' (n=42) (representing white matter, physiological noise, MRI or movement artefacts) according to a recent paper by our group.[282] The netmat was created, which is a Node x Node correlation matrix showing the correlation strength (connectivity/edge) between pairs of nodes. Full and partial correlations were calculated; the latter thought to better estimate direct connections. Matlab was used to reorder the nodes after a hierarchical clustering of the group-average correlation netmat. A subject x edges matrix was created by unwrapping each subject's correlation netmat into a single row and combining across subjects. Average and current (time of scan) alcohol consumption over the study were correlated with this matrix, using randomise, controlling for multiple comparisons (family-wise error), age, sex, education and FSIQ.

6.3.3 Statistical analyses

Cognitive test scores were summarised using means (standard deviations) or medians (interquartile ranges) depending on their distribution. Regression models were fitted to check whether average alcohol consumption over the study (independent variable) predicted cross-sectional performance on a range of memory tests (dependent variable) performed at the study end point. Age, sex, education and FSIQ were included as covariates. Where the test score represented a continuous variable, multiple linear regression was used. For count data (e.g. digit coding), initially Poisson regression was fitted and checked for over dispersion. If this was found, a negative binomial model was used. For the remainder of the tests, where the upper

score is bounded, regression models were initially fitted using binomial distributions. If over-dispersion was in evidence then a folded transformation was performed and checked for approximate normality using Q-Q plots of residuals. The same models were re-fitted with and without alcohol consumption, and a hypothesis test (likelihood ratio) was performed. Calculated p values were used to test whether alcohol made a significant difference to the model.

In order to model longitudinal cognitive data, mixed effects models were used with test score as the dependent variable. For count data (word recall from list of 20 – 'memory') a binomial regression was used, and for lexical and semantic fluency (performed within a time constraint) Poisson regression. The following fixed effects were included: time from study baseline, average alcohol consumption across the study (categorised as: abstinent (reference group, <1 unit (<8g) weekly), 1- <7 (8-<56g), 7- <14 (56-<112g), 14- <21 (112-<168g), >21 (>168g)), age, sex, education, social class, FSIQ and FRS. Contrasts between other drinking categories were also checked to test for significance differences in cognitive decline e.g. those drinking 1-<7 (8-<56g) vs. >21 units (>168g). Learning effects have been well demonstrated when the same cognitive test is presented more than once to a subject. In an attempt to control for learning effects when the same cognitive test is presented more than once to a subject (i.e. the second and subsequent times the performance may be better),, which may obscure true cognitive decline, we added a dummy variable to code for first time the test was taken (First – yes(1)/no(0)). We also dummy coded for the test being performed at Oxford (Oxford – yes(1)/no(0)), as there was an atypically short time interval between Phase 11 and the last measurement point, which we hypothesised may result in an increased learning effect. Interaction terms were

included for alcohol*time, to check for different declines in cognition over time according to alcohol consumption; and FSIQ*First and FSIQ*Oxford, to check if learning effects differ with FSIQ. Subject ID was included as a random effect. Usual diagnostic checks were performed on the models. The resulting coefficients from binomial regression equate to log(odds) and from Poisson regression to log(Poisson mean count). Regression coefficients were converted into interpretable differences in lexical decline per year compared to abstainers by: 100*(1-(exp (estimates)). Models identical to those described above, except excluding alcohol, were also computed and compared using likelihood tests and p values to the models including alcohol, to estimate the difference in prediction adding alcohol. Models were visually presented using graphs to predict trends in cognitive test scores over the study for a male participant of approximately mean age (70 years), education (15 years), social class I and IQ (118), FRS (10%).

In resting state (dual regression) analyses, a Generalised Linear Model (GLM) was applied voxelwise using permutation-based non-parametric testing. This was corrected for multiple comparisons across space (threshold-free cluster enhancement, tfce), for age, sex, premorbid FSIQ, and grey matter (voxelwise covariates of no interest using maps prepared using FSL feat_gm_prepare script) to ensure that differences in functional connectivity were not driven by structural differences). The threshold used to determine significance in all analyses was two-tailed $p < 0.05$.

Structural equation modelling (SEM) (Amos 24 for Windows) was used post-hoc for hypothesis testing and to generate fit statistics for models of relationships between

alcohol use, brain measures and cognitive decline. SEM allows simultaneous analysis of multiple variables in one model, and time series with auto-correlated errors. The hypothesised underlying structure of the model was constructed following the VBM, TBSS and mixed effects analyses, with average alcohol consumption as an exogenous variable, hippocampal volume, corpus callosum MD (generally the most sensitive measure of loss of white matter integrity), and decline in lexical fluency (slopes from mixed effects model) included as endogenous variables (using latent variables to account for measurement error). Covariance of alcohol with sex and IQ, and between brain measures was modelled. The model was improved by iteratively eliminating paths with $p>0.1$, and monitoring of the successive improvement of the model fits statistics (chi square, Comparative Fit Index, Root Mean Square Error of Approximation and the Tucker-Lewis Index) until the most parsimonious model was identified. Bootstrapping was performed to derive 95% confidence intervals for estimates.

In all analyses results were judged significant, if adjusted $p<0.05$.

6.4 Results

6.4.1 Cognitive data for subjects

Summary data for the subjects' cognitive tests is presented in Table 6.1. The median MoCA score for the group was 28.0 (3.0). 25.2% of subjects had scores below the widely used screening cut off of <26/30.

6.4.2 Alcohol and cognitive function

No significant relationships were found between alcohol consumption and cross-sectional performance on cognitive tests performed at the time of scanning (Table 6.1).

Higher alcohol consumption over the study predicted faster decline on lexical fluency but not semantic fluency or word recall (Figure 6.1 and Table 6.2). Quadratic and cubic terms for time were tested but the models did not fit significantly better. Those drinking 7 - <14 (56-<112g), 14- <21 (112-<168g) and >21 units (>168g) weekly declined faster in terms of lexical scores than abstainers. This effect was independent of age, sex, premorbid FSIQ, education, social class and FRS. The size of the difference can be interpreted as follows: subjects drinking 7 - <14 units (56-<112g) experienced a 0.5% greater reduction from their baseline in lexical fluency per year (over 30 years 14%), those drinking 14- <21 units (112-<168g) 0.8% greater per year (17% over 30 years) and those drinking >21 units (>168g) 0.6% per year (16% over

30 years) than abstainers (Table 6.2). Although the three higher consumption categories (7-<14 (56-<112g), 14- <21 (112-<168g) and >21 units (>168g) weekly) demonstrated significantly greater decline than abstainers, the only significant difference in trends between these three groups was between those drinking 14-<21 units (112-<168g) and those drinking 7-<14 units (56-<112g) (14-<21 units (112-<168g) experience 0.3% faster decline per year, p=0.02). There was no evidence for light drinkers being relatively protected from cognitive decline compared to abstainers.

We found evidence of learning effects on lexical and categorical fluency tests ($p \leq 0.01$), such that the second time a subject was presented with a test they performed better. A greater learning effect was predicted by higher premorbid FSIQ (p=0.002-5).

6.4.3 Modelling alcohol consumption and brain structure and function

In order to see how alcohol consumption and the associated brain regions interacted with cognitive decline, structural equation modelling was used. Hippocampal volume and corpus callosum MD were included as exogenous variables. Age, sex and premorbid FSIQ were also incorporated.

Removing regression arrows from age, sex, FSIQ and hippocampal volume to lexical fluency decline improved the model fit. Alcohol consumption independently predicted decline in lexical fluency. The final parsimonious model explained 21% of

corpus callosum MD, 14% of right hippocampal volume, and 2% of lexical fluency decline variance (see Figure 6.2 & Table 6.4), with good model fit (Chi square=5.6, degrees of freedom=5, p=0.23, Root Mean Square Error of Approximation=0.03, Comparative Fit Index=0.99, Tucker-Lewis Index=0.97). Alcohol consumption (in addition to age) predicted smaller hippocampal volume, and greater corpus callosum MD. Through its relationship with corpus callosum MD, and through a direct path, increased alcohol consumption predicted faster decline of lexical fluency.

6.4.4 Resting state functional connectivity

6.4.4.1 Within the DMN

A current history of higher alcohol consumption was associated with increased functional connectivity in the anterior default mode network (anterior paracingulate gyrus) (Figure 6.3), after adjustment for multiple potential confounders (age, sex, IQ, grey matter mask). In contrast, no association was found between average alcohol consumption throughout the study and functional connectivity in the anterior or posterior default mode network after adjustment for multiple potential confounders (age, sex, IQ, grey matter mask).

Connectivity in the anterior DMN was inversely associated with delayed recall on the HVLT, even after adjustment for age, sex, education, FSIQ, hippocampal volume and corpus callosum FA (B= -0.13 95% CI= -0.16 to -0.03), but no other cognitive test.

6.4.4.2 Between network nodes

A matrix of full and partial correlations between network nodes was created (Figure 6.4). Increased alcohol consumption across the study was associated with anticorrelation between nodes 16 and 40, on the full correlation analysis only (p=0.0004) (Figures 6.5, 6.6, 6.7). This was after correction for potential confounders and multiple comparisons (family-wise error). However, this finding was not significant in the partial correlation analysis (Figure 6.8), suggesting it may represent an indirect rather than direct connection. Similarly, greater weekly alcohol intake at the time of the scan (Oxford phase) was associated with anticorrelation between nodes 16 and 40 on full (p=0.001, Figure 6.9) but no partial (Figure 6.10) correlation.

6.5 Discussion

Although we found no cross-sectional association between alcohol consumption over the study and cognitive function, the longitudinal analysis did show a significant decrease in lexical fluency performance over time in those drinking 7 units (56g) or more compared to abstainers. There was no relationship between average alcohol and functional connectivity within or between resting state networks.

Compared with previous studies, the proportion of participants with global cognitive changes (defined as MoCA <26/30) was high (25%).[283] Potential lack of health concerns may have induced some participants to avoid participation in the study, so the potential for selection bias cannot be dismissed, as those concerned about memory problems may have been more likely to attend. No dementia cases were identified,

which is unsurprising given the study inclusion criteria (community resident and ability to travel to Oxford).

Unlike the original MoCA validation study[284] this sample was not a healthy control group, but a community sample, which included those with a history of major depression (16% of sample). Deficits in executive function and attention are known to persist in euthymic patients with a history of unipolar depression,[285] although analyses were controlled for this and CES-D scores.

Higher alcohol consumption was associated with a significantly faster decline of lexical fluency over the study. Lexical fluency involves selecting and retrieving information based on spelling (orthography), and has characteristically been associated with frontal executive function,[286, 287] in contrast to semantic fluency which may depend more on temporal lobe integrity.[286, 288] However, the distinction may not be as clear cut, as functional networks overlap.[288] The inverse relationship between alcohol consumption and lexical performance was perhaps unsurprising, given the frontal predominance of the negative associations with white matter integrity. We suggest two possibilities for the lack of more widespread associations with cognition, particularly with semantic fluency and short-term memory decline, given the structural brain findings (hippocampal atrophy). First, there are clear practice effects over the study, i.e. at least some subjects improve their performance after repeated testing, and this is positively associated with FSIQ. These may be greater for the semantic compared to lexical fluency tests. Variables predicting the ability to learn may be different from those protecting against cognitive impairment due to a neurodegenerative process. Although we have attempted to control for both FSIQ and learning effects, this may be insufficient to remove the confounding effect if a third

variable, for example diet, mediates the relationship between IQ and learning but is not in the model. Second, the brain changes may reflect an intermediate phenotype and cognitive change is not yet evident. It is now well documented that hippocampal atrophy precedes symptoms in Alzheimer's dementia by several years,[289] so a similar phenomenon in alcohol-related changes is plausible.

Perhaps surprisingly, we found no protective effect of light drinking on cognitive decline over abstinence, in contrast to some others. Stampfer et al.[118] reported reduced 10 year cognitive decline in women drinking approximately 15g daily (HR 0.85, 0.74-0.98). Similarly, Ganguli et al.[119] found less decline over a 7-year period in moderate drinkers. Previous studies of the larger WHII cohort have also been done. Whilst Britton[290] found those drinking >1 g/week were less likely to have poor cognitive function, there were minimal associations with cognitive decline after adjustment for social class (based on occupation) except with Mill Hill vocabulary – essentially a verbal intelligence test. Sabia[41] found worse cognitive decline in those drinking >34g/day only. There are two major methodological limitations of many studies citing a protective effect of moderate alcohol. First, many include elderly subjects at baseline, which increases the possibility of reverse causation, i.e. that subjects with cognitive impairment change their pattern of drinking as a result of noticing worsening performance or limited social activity, rather than drinking directly affecting cognition. For instance in Stampfer at el.[118] the mean baseline age was 74 years. Second, potential sources of confounding, including learning effects, are often poorly controlled. Stampfer el al.[114] and Ganguli et al.[119] adjust for education but not premorbid intelligence or social class. In Britton et al.,[290] most of the associations with cognitive decline became insignificant after social class was adjusted for.

This is the first study, to our knowledge, to find a relationship between alcohol consumption at the time of scanning and resting state functional connectivity (default mode network) in moderate drinkers. In contrast, there was no association with average alcohol intake over the study. Resting state connectivity reflects current brain activity, which may well be associated with contemporary alcohol consumption in a direct way, e.g. rebound from previous day, rather than chronic use. We also found that alcohol consumption (both on average over the study and current) was associated with anticorrelation between nodes of the Default Mode Network. This appears to be an indirect connection, as it was only significant on the full rather than partial correlation analyses. Participants were unlikely to have been under the influence of alcohol during the resting scan, although we did not measure alcohol levels. Higher weekly consumption while sober during the scan could theoretically be associated with intermittent mild or subclinical withdrawal symptoms or some more persistent "silent" change in brain metabolism that is reflected in changed activity patterns. Of the few studies that have examined resting state networks in alcoholic individuals, several have reported reduced functional connectivity in a range of networks, including visual, executive [291], salience [273, 292] and default mode networks [50] [293]. Functional connectivity in the anterior DMN was inversely associated with delayed recall on the HVLT, independently of age, sex, FSIQ, hippocampal volume and corpus callosum FA. The resting state associations we found with increased current alcohol intake therefore appear to reflect a negative consequence of heavier drinking rather than a compensatory strategy.

Alcohol is vasoactive,[294] therefore it can disrupt coupling between neuronal activity and cerebral blood flow[295] used to make inferences in fMRI. Resting state fMRI may

be less sensitive to effects of vasodilation, but we cannot exclude such a phenomenon.[296]

In conclusion, these results provide evidence for faster cognitive decline in non-dependent drinkers consuming great than over 7 units (56g) weekly compared to abstinence. There is no support for a protective effect of light drinking on cognition. We also found evidence for an association between non-dependent alcohol consumption and resting brain activity, in particular the functional connectivity of the Default Mode Network.

Cognitive test	Range	Mean (S.D.)[1]/ median (IQR)[2]	P value for likelihood test[3]
MoCA (adjusted for education)	17-30	28.0 (3.0)[2]	0.4
TMT A (sec)	13-160	31.3 (13.4)[1]	0.7
TMT B (sec)	0-321	68.7 (36.9)[1]	0.3
Rey copy	6-36	32.0 (5.0)[2]	0.6
Rey immediate	0-32	16.0 (9.8)[2]	0.9
Rey delay	0-30	15.5 (8.5)[2]	0.8
HVLT total recall	10-36	28.0 (6.0)[2]	0.4
HVLT delayed recall	0-12	10.0 (3.0)[2]	0.9
BNT	15-60	59.0 (3.0)[2]	0.01*
Digit span total	13-46	30.0 (8.0)[2]	0.8
Digit substitution test	13-112	63.0 (17.0)[2]	0.3

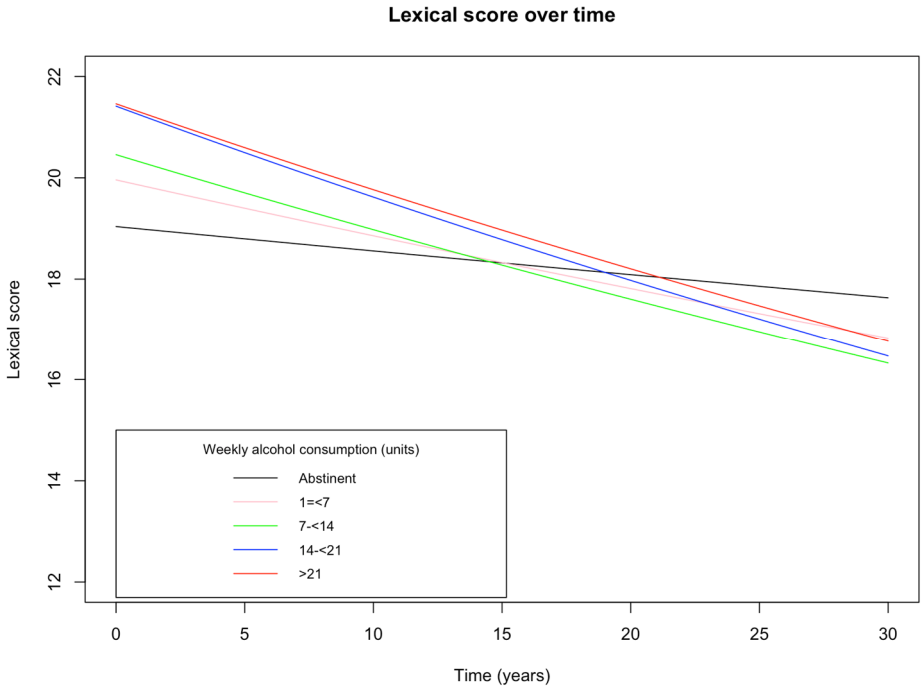
[1] **Mean (S.D.) for normally distributed continuous variables.**

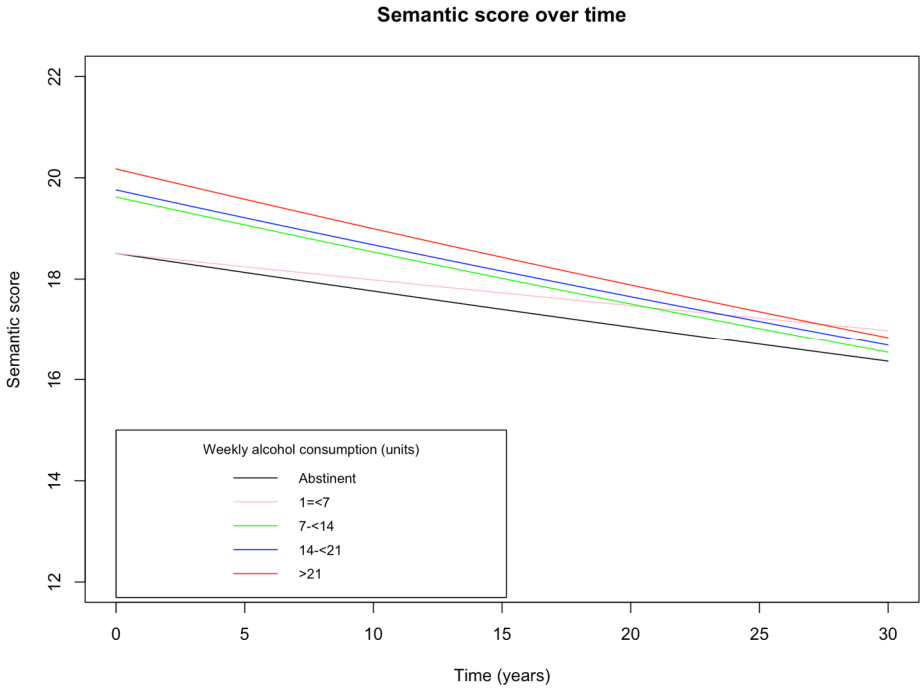
[2] **Median (IQR) for discrete variables.**

[3] **Hypothesis test for significant difference between models with and without alcohol included.**

*** Not significant after Bonferroni correction (0.05/9 = 0.006 significance level).**

Table 6.1: Summary of cross-sectional cognitive test data and its relationship with average alcohol consumption across the study. Mean and standard deviations are given for normally distributed data and median and interquartile range for non-normally distributed data. MoCA=Montreal Cognitive Assessment, TMT=Trail making test, Rey=complex figure task, HVLT=Hopkins Verbal Learning Test, BNT=Boston naming test (associated with IQ, hence positive association). P values result from hypothesis tests (likelihood tests) comparing regression models with cognitive test as the dependent variable, adjusted for age, sex, education and FSIQ, with and without alcohol consumption in the model.


Lexical score over time
Lexical score
Time (years)
Weekly alcohol consumption (units)
Abstinent
1=<7
7-<14
14-<21
>21


Semantic score over time
Semantic score
Time (years)
Weekly alcohol consumption (units)
Abstinent
1=<7
7-<14
14-<21
>21

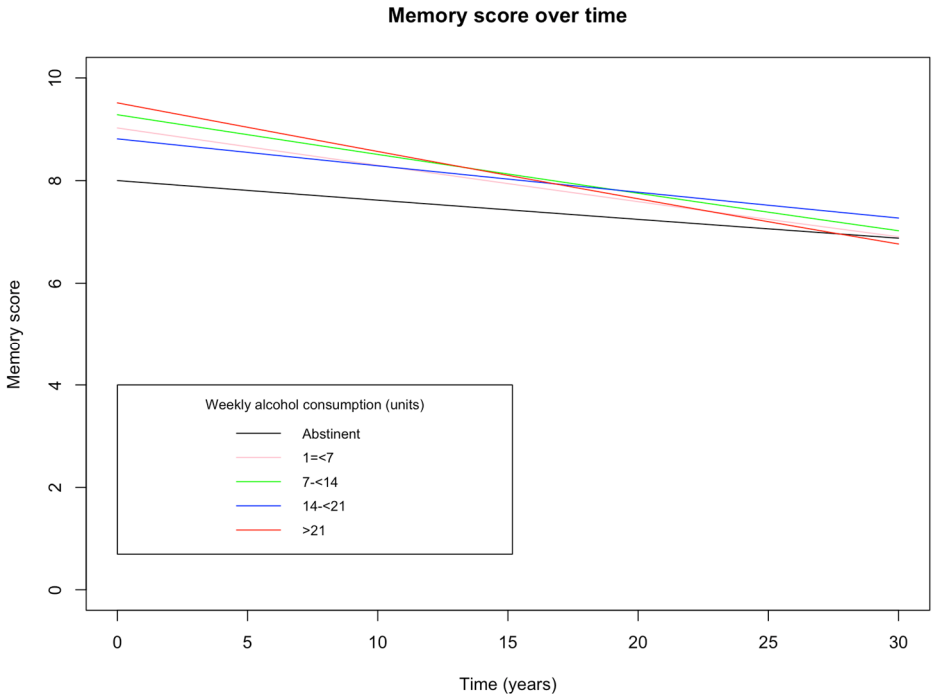


Figure 6.1: Predicted longitudinal change in cognitive test scores (lexical and semantic fluency, word recall 'memory') for a male subject of mean age (70 years) and FSIQ (118), median education (15 years), social class I and FRS (10%) according to average alcohol consumption (weekly units). Predictions are made on the basis of mixed effects models with cognitive testing performed at phases 3, 5, 7, 9, 11 and time of the scan.

	Estimates[7]	S.E.	95% CI	P value
LEXICAL FLUENCY				
Age[1]	-0.82	0.18	-0. 12 to -0.47	<0.001***
Time[1]	-0.26	0.19	-0.63 to 0.12	0.18
Sex[2]	0.06	0.02	0.02 to 0.11	0.005
First	0.35	0.14	0.07 to 0.63	0.01**
Social class[3]	-0.06	0.02	-0.11 to -0.02	0.006**
FSIQ[4]	0.90	0.12	0.66 to 1.0	<0.001***
Education[5]	-0.05	0.04	-0.12 to 0.02	0.14
FRS[6]	-0.18	0.12	-0.40 to 0.05	0.12
1 - <7	0.05	0.05	-0.06 to 0.15	0.38
7 - <14	0.07	0.05	-0.03 to 0.18	0.17
14 - <21	0.12	0.06	0.01 to 0.23	0.03*
>21	0.12	0.06	0.01 to 0.23	0.03*
Time[1]x 1 - <7	-0.31	0.21	-0.72 to 0.09	0.13
Time[1]x 7 - <14	-0.50	0.20	-0.90 to -0.10	0.015*
Time[1]x 14 - <21	-0.62	0.21	-1.00 to .-0.20	0.004**
Time[1]x >21	-0.57	0.22	-0.99 to -0.14	0.009**
FSIQ[4]x First	-0.34	0.12	-0.57 to -0.10	0.005**
SEMANTIC FLUENCY				
Age[1]	-0.11	0.16	-1.39 to -0. 74	<0.001***
Time[1]	-0.41	0.21	-0.82 to 0. 00	0.05
Sex[2]	0.06	0.02	0.02 to 0.10	0.002**
First	0.46	0.16	0.15 to 0.78	0.004**
Oxford	0.74	0.10	0.56 to 0.93	<0.001***
Social class[3]	-0.08	0.02	-0.12 to -0.04	<0.001***
FSIQ[4]	1.20	0. 11	0. 99 to 1.4	<0.001***
Education[5]	-0.10	0.03	-0.16 -0.03	0.03*
FRS[6]	-0.15	0.11	-0.36 to 0.05	0.15
1 - <7	0.00	0.05	-0.11 to 0.11	1.0
7 - <14	0.06	0.05	-0.05 to 0.16	0.28
14 - <21	0.07	0.06	-0.05 to 0.18	0.25
>21	0.09	0.06	-0.03 to 0.20	0.14
Time[1] x 1 - <7	0.12	0.22	-0.31 to 0.56	0.58
Time[1] x 7 - <14	-0.16	0.22	-0.59 to 0.27	0.47
Time[1] x 14 - <21	-0.15	0.23	-0.60 to 0.30	0.50
Time[1] x >21	-0.19	0.23	-0.65 to 0.27	0.41
FSIQ[4] x First	-0.42	0.13	-0.69 -0.16	0.002**
FSIQ[4] x Oxford	-0.33	0.08	-0.49 -0.17	<0.001***
WORD RECALL				
Age[1]	-2.20	0.34	-2.86 to -1.53	<0.001***
Time[1]	-0.80	0.52	-1.83 to 0.22	0.13
Sex[2]	0.20	0.04	0.12 to 0.28	<0.001***
First	0.40	0.36	-0.30 to 1.11	0.26
Social class[3]	-0.08	0.04	-0.16 to 0.01	0.08
FSIQ[4]	1.60	0.23	1.16 to 2.05	<0.001***
Education[5]	-0.22	0.66	-0.94 to -0.35	0.008**
FRS[6]	-0.40	0.22	-0.83 to 0.02	0.06
1 - <7	0.21	0.12	-0.03 to 0.45	0.09
7 - <14	0.26	0.12	0.03 to 0.50	0.03*
14 - <21	0.17	0.13	-0.08 to 0.41	0.19
>21	0.31	0.13	0.05 to 0.56	0.02*
Time[1] x 1- < 7	-0.68	0.57	-1.18 to 0.44	0.24
Time[1] x 7- < 14	-0.77	0.56	-1.85 to 0.34	0.18
Time[1] x 14 - <21	-0.27	0.59	-1.43 to 0.89	0.65
Time[1] x >21	-1.11	0.60	-2.30 to 0.07	0.07
FSIQ[4] x First	-0.68	0.30	-0.13 to -0.09	0.02*

[1]Expressed as a fraction of one hundred years.
[2] Females vs. reference group males.
[3]Classes 2,3 and 4 vs. reference group class 1.
[4]Expressed as a fraction of 100 points.
[5]Expressed in ten year multiples.
[6]Expressed as a fraction of 100%.
[7]Estimates represent log(odds) for memory recall (binomial regression) and log(mean count) for lexical and semantic fluency (Poisson regression).

*** P value <0.05**
**** P value <0.01**
***** P value <0.001**

Table 6.2: Results from mixed effects models fitting longitudinal cognitive test scores over time according to average alcohol consumption (1-<7 units (8-<56g), 7-<14 units (56-<112g), 14-<21 units (112-<168g), >21 units (>168g), reference group abstainers) across the study. For lexical and semantic fluency Poisson regression is applied, and for memory recall binomial regression. Adjusted for: age, sex, education, social class, FSIQ, Framingham Risk Score, learning effects of the test and time from study baseline.

Test	Change in lexical fluency slope* for every 10 unit increase in weekly alcohol consumption	P value	95% CI
Average alcohol[1]	-0.28	0.03	-0.53 to -0.02
Average alcohol[2]	-0.23	0.08	-0.46 to 0.57

[1] Adjusted age, sex, education, FSIQ
[2] As in [1] and additionally corpus callosum MD
*As a multiple of 100

Table 6.3: Results from multiple linear regression analysis, with decline in lexical fluency (slopes, Phase 3 to time of scan) as the dependent variable, and average alcohol consumption (weekly units) as an independent variable.

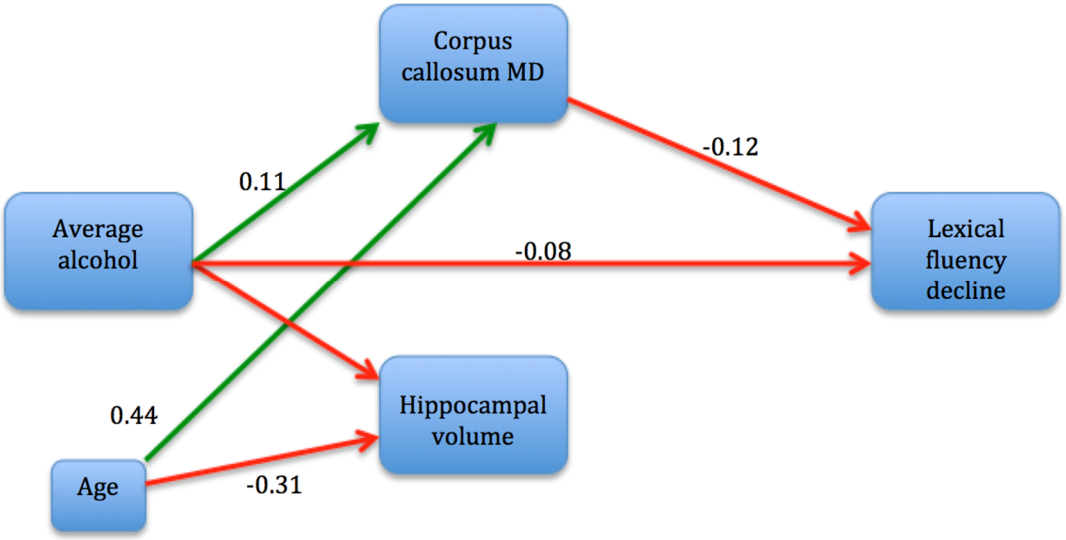


Figure 6.2 Final parsimonious structural equation model illustrating relationships among alcohol consumption (average across study phases, as a fraction of 100 units weekly), hippocampal volume (average, %ICV), corpus callosum mean diffusivity (MD, as a multiplicative of 1000), decline in lexical fluency (slopes), and age. N=511. Values on arrows represent standardised coefficients. Red arrows indicate inverse relationships and green positive relationships. Model explained 21% corpus callosum MD, 14% of hippocampal variance and 2% of lexical fluency decline variance (R^2). Model fit: Chi-square=5.6, degrees of freedom=4, p=0.23, Root Mean Square Error of Approximation=0.03, Comparative Fit Index=0.99, Tucker-Lewis Index=0.97.

Path		Change in y (in S.D.) for each 1 S.D. increase in x	95% CI (P value)
From (x)	**To (y)**		
Average alcohol[1]	Hippocampal volume	-0.20	-0.28 to -0.12 (0.008)
Average alcohol	Corpus callosum MD[2]	0.11	0.05 to 0.18 (0.008)
Average alcohol[1]	Lexical fluency decline	-0.08	-0.18 to 0.01 (0.07)
Corpus callosum MD[2]	Lexical fluency decline	-0.12	-0.25 to -0.03 (0.004)
Age	Hippocampal volume	-0.31	-0.40 to -0.24),0.006
Age	Corpus callosum MD[2]	0.44	0.34 to 0.49 (0.04)

[1]As a fraction of 100 units weekly
[2]As a multiplicative of 1000

Table 6.4: Standardised regression coefficients for paths in the final structural equation model (Figure 6.2) with their bias-corrected 95% confidence intervals and p values. N=511.

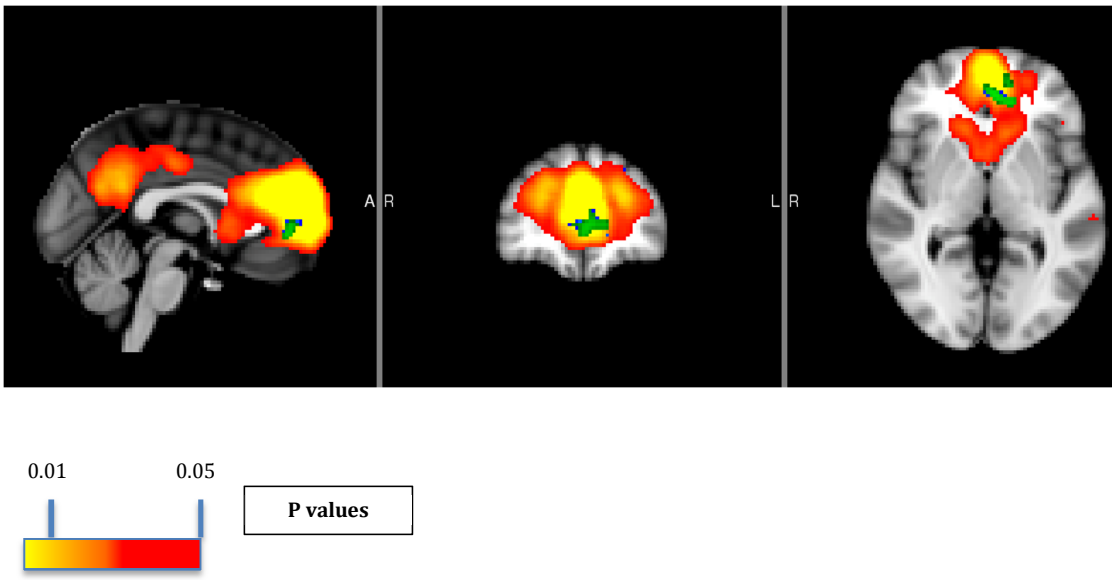


Figure 6.3: The resting state Default Mode Network (DMN) resulting from a group independent component analysis (MELODIC) is shown in yellow-red. A significant positive correlation within the DMN with current alcohol consumption is shown in the voxels overlaid in green. Adjusted for: age, sex, IQ and GM mask and tfce.

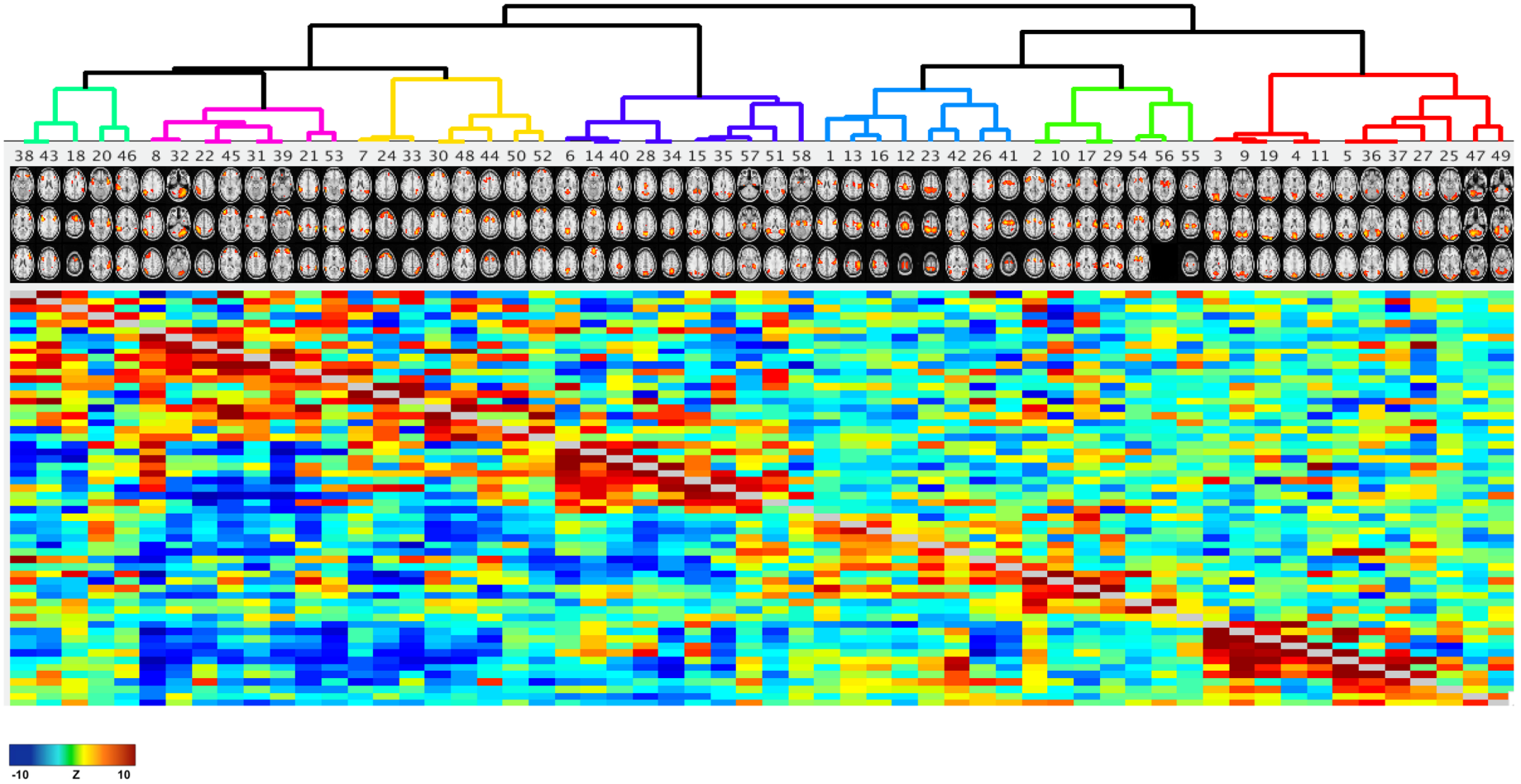


Figure 6.4 Functional connectome consisting of hierarchical ordering of the 58 functionally distinct brain regions as network nodes, and the connectivities between these as network edges. The strengths of the estimated network edges are elements in an N_{nodes} x N_{nodes} network matrix. Boxes above the diagonal represent full correlations (thought to represent direct and indirect connections) and those below the diagonal represent partial correlations (used to infer only direct connections between nodes).

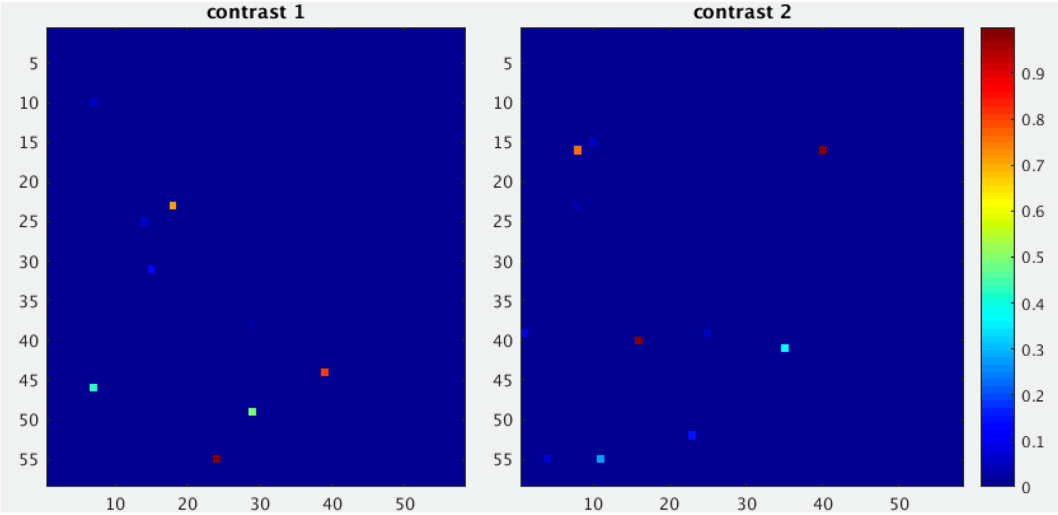


Figure 6.5 Matrices showing *full correlation* (contrast 1=positive, contrast 2=negative) associations between nodes (x and y axis numbers corresponding to Figure 6.4) and average alcohol consumption across the study, adjusted for: multiple comparisons (family-wise error), age, sex, education and FSIQ. 1-p values indicated by colour of squares corresponding to right-hand colour bar. Significant (p=0.0004) negative correlation between nodes 16 (lateral temporal lobes, default mode network, see Figure 6.6) and 40 (posterior cingulate cortex, default mode network, see Figure 6.7).

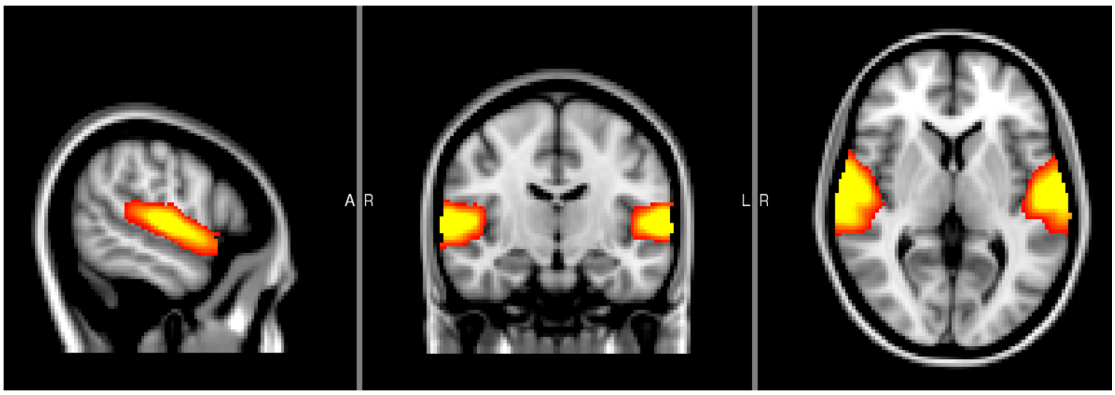


Figure 6.6 The resting state network of node 16 is shown in yellow-red, identified from a group independent components analysis (MELODIC) of 500 subjects, thresholded at Z=3. This is focused on the lateral temporal nodes of the default mode network. R and L define respectively the right and left hemisphere.

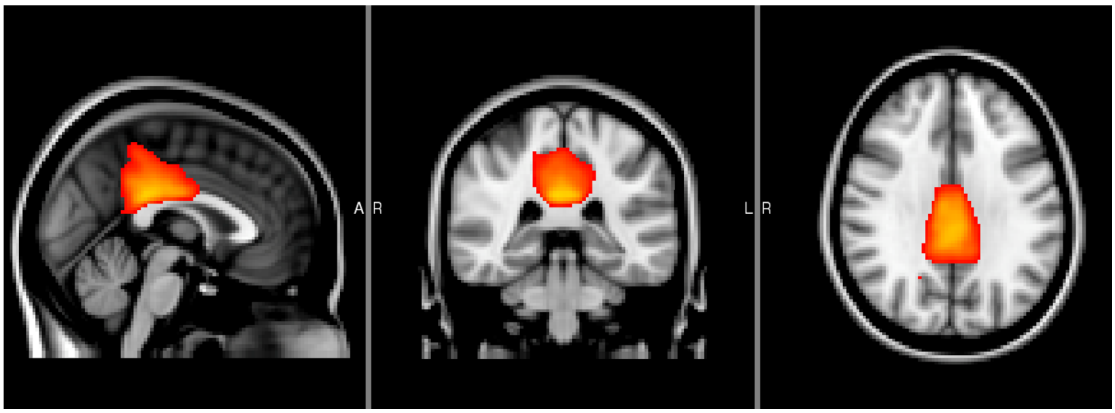


Figure 6.7 The resting state network of node 16 is shown in yellow-red, identified from a group independent components analysis (MELODIC) of 500 subjects, thresholded at Z=3. This is focused on the posterior cingulate node of the default mode network. R and L define respectively the right and left hemisphere.

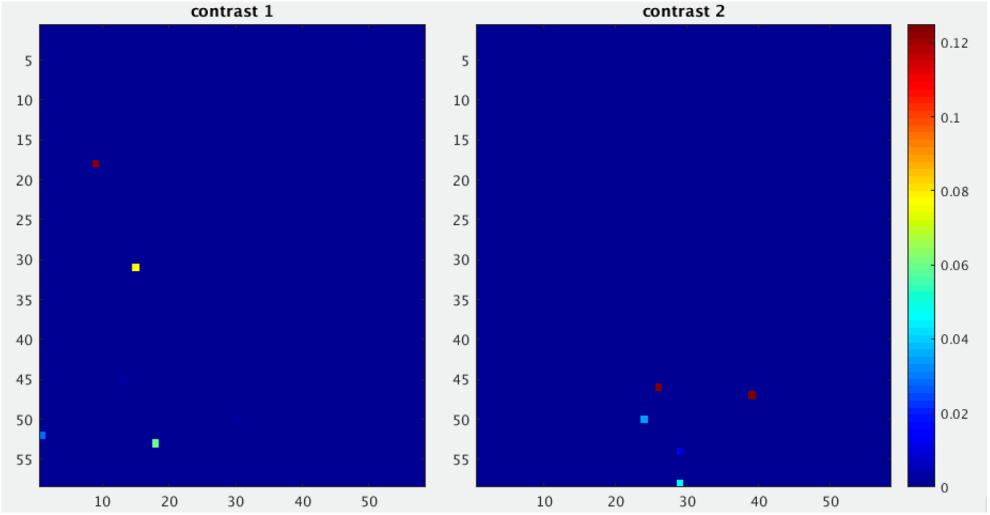


Figure 6.8 Matrices showing *partial correlation* (contrast 1=positive, contrast 2=negative) associations between nodes (x and y axis numbers corresponding to Figure 6.6 & 6.7) and average alcohol consumption across the study, adjusted for: multiple comparisons (family-wise error), age, sex, education and FSIQ. 1-p values indicated by colour of squares corresponding to right-hand colour bar. No significant (p<0.05) correlations between nodes.

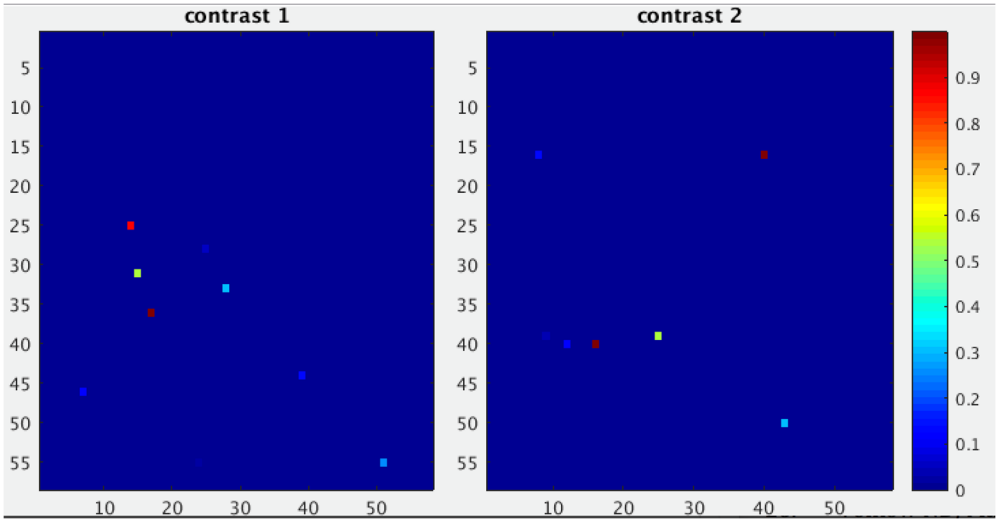


Figure 6.9 Matrices showing *full correlation* (contrast 1=positive, contrast 2=negative) associations between nodes (x and y axis numbers corresponding to Figure 6.4) and current (time of scan) alcohol consumption across the study, adjusted for: multiple comparisons (family-wise error), age, sex, education and FSIQ. 1-p values indicated by colour of squares corresponding to right-hand colour bar. Significant (p=0.001) negative correlation between nodes 16 (lateral temporal lobes, default mode network, see Figure 6.6) and 40 (posterior cingulate cortex, default mode network, see Figure 6.7).

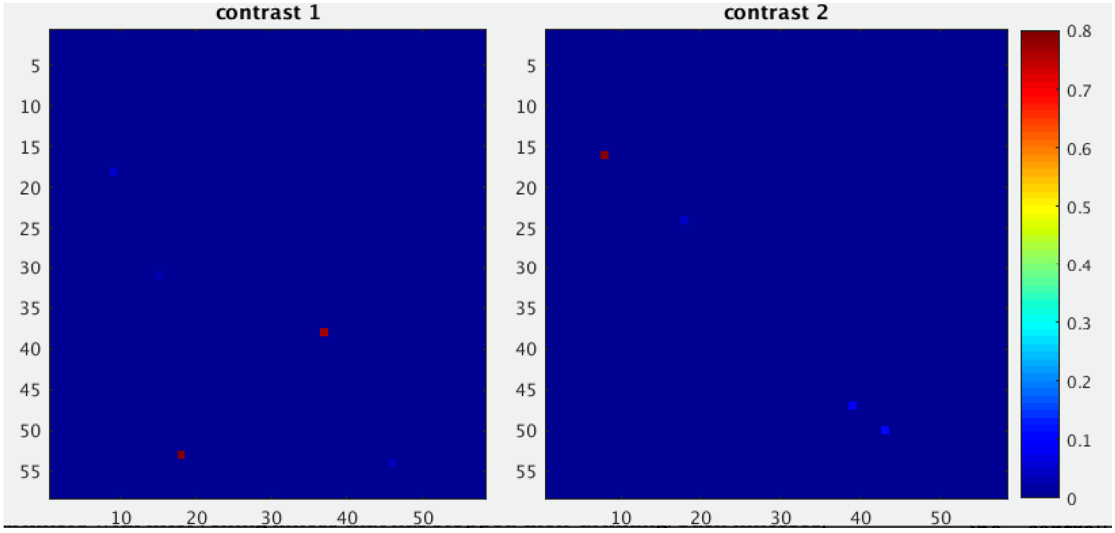


Figure 6.10 Matrices showing *partial correlation* (contrast 1=positive, contrast 2=negative) associations between nodes (x and y axis numbers corresponding to Figure 6.6&6.7) and current alcohol consumption across the study, adjusted for: multiple comparisons (family-wise error), age, sex, education and FSIQ. 1-p values indicated by colour of squares corresponding to right-hand colour bar. No significant (p<0.05) correlations between nodes.

Chapter 7: Resilience

7.1 Introduction

In the preceding chapters I have shown that non-dependent alcohol consumption in this sample was associated with hippocampal atrophy, microstructural indices in white matter, and differences in the functional connectivity of the Default Mode Network. Despite evidence for links between these structural brain changes and lexical fluency decline,[297] including within our dataset,[179] we found minimal direct associations between alcohol consumption and cross-sectional cognitive performance or decline on semantic fluency or word recall.

An example from neuropsychiatry

To give a comparable example from neuropsychiatry, the natural history of early changes in of Alzheimer's disease is now fairly well characterized.[298] Biomarkers become abnormal in sequence, beginning two decades prior to the development of clinical symptoms, with amyloidosis, and progressing to tau-mediated neuronal injury and brain atrophy closer to the time of first symptoms and diagnosis.[289] However, there is substantial heterogeneity in the length of this prodrome, with some resilient individuals appearing to tolerate pathology without displaying detectable cognitive impairment. Previous studies have found approximately one fifth of those positive for Alzheimer biomarkers to be normal on cognitive testing, regardless of whether these markers are neuropathological findings on autopsy,[299] amyloid on PET imaging,[289] or abnormal levels of cerebrospinal fluid tau and amyloid.[300]

The mechanism underlying such resilience is unclear. Proposed hypotheses include greater network efficiency or capacity, or additional compensatory networks in the face of brain damage.[301, 302] All require highly functioning white matter connections.

Compensatory effects occurring with age

Elders who perform as well cognitively as their younger peers appear to shift their brain activation from a posterior to anterior (PASA)[302] and less lateralised (HAROLD) pattern.[301] Increased recruitment of frontal brain areas has been demonstrated in functional fMRI studies also in those with mild Alzheimer's disease, to a degree correlated with performance.[303] Reduced coherence of anterior with posterior components of Default Mode Networks (DMNs) is associated with poor executive function, processing speed, and memory.[304] This "inefficient processing" may be somewhat compensated for by a frontal shift of brain activity in resilient individuals.[301] However, once the burden of brain lesions becomes too large, compensatory mechanisms are exhausted and clinical decline ensues.[305]

To quantify the effect of resilience in our study, we will identify factors that might moderate the relationship between hippocampal atrophy (one of the most striking findings associated with alcohol use in this cohort) and cognition, i.e. those factors that predict good neuropsychological performance in the presence of atrophy. Such resilience may also explain the difficulty in finding behavioural consequences associated with alcohol use in our sample.

7.2 Objectives

My specific aims were to identify features, whether socio-demographic or brain-related, that appeared to protect participants from the adverse effects of smaller hippocampi on cognitive performance. Hippocampal volumes are used as an in vivo proxy for neuropathological burden and verbal memory as a measure of cognitive current performance. Hippocampal volume correlated highly with visual ratings of hippocampal atrophy in our sample and in others.[179, 222] Hippocampal atrophy (HA) has been identified as one of the five most robust biomarkers for AD and is a supportive diagnostic sign in the revised NINCDS-ADRDA criteria.[306] It effectively differentiates between Alzheimer's disease patients and controls,[307] and is correlated with neuropathological progression including amyloidosis.[222] Verbal memory is frequently first affected in Mild Cognitive Impairment and most tightly coupled with hippocampal atrophy and volume.[297] Any variable that adds resilience would be positively associated with memory after correction for hippocampal size. Additionally, the correlation between verbal memory and resilience variable may be stronger in participants with atrophied hippocampi than in those with normal hippocampi, i.e. show an interaction effect, which could imply a ceiling for memory performance measures in subjects with normal hippocampi.

7.3 Methods

7.3.1 Participants and study design

As in Chapter 4.

7.3.2 MRI analysis

As in Chapter 5.

7.3.3 Statistical analysis

Analyses were performed using R[216] and SPSS.[308] Significant differences ($p<0.05$) between subjects included and excluded from the analysis were explored using t tests of means (continuous variables) and chi-squared tests (categorical variables). All cognitive tests performed at the time of the scan were included in a factor analysis using an oblique rotation (allowing for correlation between factors – 'direct oblimin' in SPSS (SPSS 24 for Windows (IBM Corporation, Armonk, N.Y.). The only test not included in the factor analysis was the MoCA, as it is a screening test. A reduced number of cognitive factors (with eigenvalues >1) were generated (see Figure 7.2 and Table 7.2 for scree plot and factor loadings). Verbal memory factor scores (largest factor, eigenvalue>6) were used in this analysis, as these were the best predictor of 'MCI' (defined using MoCA <26) (see Table 7.3).

Sociodemographic and neural predictors of resilience were identified using hierarchical multiple linear regression in a six-stage process, with verbal memory as the dependent variable. First, hippocampal volume (adjusted for intracranial volume, ICV) was entered in the first block of independent variables. Second, the following putative protective or confounding factors, identified from the literature, where entered together in the second block of independent variables: age, sex, education, premorbid IQ (estimated from TOPF, education and gender), social class, living with spouse or partner, frequency of moderate exercise, Framingham Risk Score, systolic and diastolic blood pressure, alcohol, history of Major Depression (SCID), CESD

score, psychotropic or cardiovascular medication, social activity, voluntary work, bilingualism. Third, predictors with high multicollinearity (Variance Inflation Factors >2.5 was deemed concerning) were removed in an order determined by their scale characteristics, starting with the highest p value and progressing until p=0.1. Fourth, variables with p>0.1 were removed sequentially. Fifth, white matter variables (Fazekas total score and corpus callosum FA (CC-FA; commissural fibres are essential in some models of resilience)[301] were added in the third block of independent variables. Sixth, white matter variables with p>0.1 were removed, leaving the final parsimonious model. Interaction terms between significant predictor variables in this final model (age, sex, FSIQ, social class, bilingualism, exercise, social visits, psychotropic medication, CC-FA) *and* hippocampal volume were then generated using demeaned values and entered into a GLM along with their demeaned main effects, with verbal memory again as dependent variable, to check for inhomogeneity of regression slopes between subjects with and without hippocampal atrophy.

Post-hoc, WMH volume, as estimated using BIANCA, was added as an additional independent variable to the final parsimonious resilience model. Separate multiple linear regressions were also performed, post-hoc, where significant interactions were found in those with and without hippocampal atrophy (defined per Scheltens scores, using a cut off >0 for atrophy).

For all regression analyses unstandardised regression coefficients (B), their 95% confidence intervals, standardised coefficients (beta) and p values are reported. All tests were two-tailed and the significance level was defined as <0.05.

Finally, to investigate the tract distribution, over which white matter integrity was associated with higher memory performance independently of hippocampal size, a TBSS analysis was performed with FA/RD/AD/MD as the dependent variable. Verbal memory factor scores were entered into a generalised linear model as independent variable of interest, adjusted for other variables (excluding FA) conferring resilience from the hierarchical multiple linear regression analysis above. Each variable is orthogonalized with respect to the others, so only white matter measure variance uniquely attributable to verbal memory after accounting for hippocampal volume (and other confounders) remained. GLM was applied using permutation-based non-parametric testing (FSL 'randomise'), correcting for multiple comparisons across space (threshold-free cluster enhancement, tfce).

7.4 Results

7.4.1 Participant characteristics

Four hundred and eighty-six subjects were included in the analyses (Figure 7.1). The only difference between the excluded and included groups was a higher prevalence of major depressive disorder history on the SCID in those excluded (32.1% vs. 19.2%; χ^2=11.4; p=0.001). Subjects were median age 42.0 years old (IQR=7.7) at the beginning of the study in 1985, predominantly male (81.2%), and of high average premorbid IQ (118.0, S.D.=10.1) and education level (14.0 years, IQR=5.0). Their median MoCA score was 28.0 (IQR=3.0). Four cognitive factors were identified (Figure 7.2 and Table 7.1). HVLT loaded high on "Verbal Memory", digit span tests on "Working Memory", TMT on "Executive Function", RCF on "Visuospatial Function", and TOPF and BNT on "General Intelligence". Factors were highly correlated. "Verbal memory" was the factor accounting for the largest proportion of overall variance, with an eigenvalue >6, and scores followed a skewed distribution in the sample (skewness -0.89 S.E.=0.11) (Figure 7.3). The difference between the means for high (>26) versus low (<26) MoCA groups was largest for the verbal memory factor (Table 7.2).

Over two thirds of participants had hippocampal visual atrophy ratings of 1 and higher on at least one side of the brain (69%). Scheltens scores correlated with intracranial volume-adjusted hippocampal sizes in our sample (r= -0.4 p<0.0001), as it did in other studies.[179, 222] All subjects had at least some periventricular or deep white matter hyperintensities per the Fazekas visual rating score. Partial correlations corrected for hippocampal volume with verbal memory performance were observed

for age, sex, premorbid IQ, education, social class, FRS, cardiovascular medication, exercise, non-native English speaker, FA, Fazekas score and MoCA scores (Table 7.3).

7.4.2 Variables that protect against adverse effects of small hippocampal size on verbal memory

Hippocampal volume (normalized to ICV) explained 4.4% of verbal memory variance (adjusted R^2) in the hierarchical multiple linear regression (block 1). Addition of not directly brain-related independent variables (block 2) increased the explained variance to 27.2%. Years of education and systolic BP were removed from the model due to high multicollinearity (R^2=23.3%), along with the following non-significant ($p \geq 0.1$) independent variables: CES-D, history of MDD (SCID), club attendance, diastolic BP, FRS, cardiovascular medication, voluntary work and alcohol (R^2=23.8%). Fazekas scores and corpus callosum (CC) FA were added in block 3 (R^2=25%), but only the latter was retained in the final model (R^2=25.1%) (Table 7.4). The only significant interaction term with hippocampal size able to predict verbal memory in the GLM analysis was CC-FA x hippocampal size (p=0.01) (Figure 7.5).

In a post-hoc analysis, WMH volume (extracting using BIANCA) was added as a further independent variable to the model. This did not mediate the CC-FA effect. Multiple linear regression with verbal memory factor scores as dependent variable and corpus callosum FA as an independent variable (adjusted for age, sex, premorbid IQ and social class) found that corpus callosum FA significantly predicted verbal

memory in those with visually rated hippocampal atrophy (n=323; B=7.2, 95% CI: 3.2 to 11.2; β =0.2; p<0.0001), but not for those with normal (Scheltens=0) hippocampi (n=145; B=2.0, 95% CI: -5.7 to 9.6; β =0.004; p=0.6; Figure 7.4), however, a formal test of the interaction hippocampal atrophy*FA was not significant (p=0.3) in predicting verbal memory.

7.4.3 White matter microstructure distribution

TBSS was used to explore further whether white matter tract integrity other than in corpus callosum was important to determine resilience. Verbal performance as the variable of interest adjusted for hippocampal volume (as a proxy for pathology), age, sex, premorbid IQ and bilingualism, was positively correlated with fractional anisotropy (a measure that is greater, as axial diffusion exceeds radial diffusion, i.e. as diffusion is relatively more restricted perpendicular to fibre direction compared with parallel to the fibre direction) and negatively correlated with radial and mean diffusivity (Figure 7.6). No significant correlation with axial diffusivity was found.

7.5 Discussion

Sociodemographic and neuroimaging variables explained 25% of verbal memory variance. In this sample, resilience, defined as good memory performance independent of hippocampal size, was correlated with white matter integrity across

the brain after controlling for sociodemographic variables also conferring resilience. Domain-specific cognitive tests with high performance ceiling were used to detect mild impairments in this highly educated group.[309]

Sociodemographic variables (findings and interpretation)

The finding that increased age was associated with poorer memory performance independently of hippocampal volume was not a surprise. Multiple pathways, in additional to medial temporal lobe atrophy, contribute to declining cognition with age.[305] Females were more resilient than males in this sample. This is particularly surprising considering the cognitive reserve theory that premorbid intelligence may be protective against cognitive decline,[310] given that as a group they were less educated, with lower FSIQ. One hypothesis is that their lower cardiovascular risk and alcohol intake could be contributing, although neither variable remained in the predictive model. Non-native English speakers in this sample were less resilient. This appears to be at odds to that cited with bilingualism in the literature,[311-313] although the variables are not strictly equivalent.[311] Numbers in this sample with English as a second language was small, and the finding may be an artefact of the nature of the roles immigrants held in the civil service in the 1980s. Additionally, as better mastery of English is likely to predict better verbal memory of English words, this finding may be specific to this factor rather than typical of general cognitive resilience.

The finding that higher premorbid FSIQ was a resilience factor, is supportive of cognitive reserve theory, which posits that individuals with high premorbid intelligence or education are relatively protected against cognitive decline.[309] It is unclear whether the IQ effect is due to the baseline correlation due to shared variance

('G') with general cognitive test performance, or whether there is an additional compensatory effect. In the absence of detailed baseline cognition data we cannot elucidate which is more likely. Whilst age, sex, bilingualism and higher premorbid intelligence independently predicted memory performance, there were no interaction effects between these factors and hippocampal size.

Sociodemographic variables (limitations)

Whilst participants were community dwelling, they may not be representative of the general UK population. Participants were mostly male, educated and middle-class. Furthermore, few women and those of ethnic minority origin were in the higher grades of employment at the study inception.[170] No manual workers were included. The sample was similar to the larger Whitehall II cohort in terms of age, but had a higher percentage of men and lower CES-D scores (Table 7.1). Our imaging measure may be a diagnostic marker for Alzheimer's pathology, but it is not specific in the absence of measures of tau or amyloid. Reduced hippocampal volumes have also been reported in depression, as a result of neurotoxic injuries e.g. diabetes, or from other causes of dementia such as hippocampal sclerosis, Lewy body-related damage or frontotemporal dementia.[314] Hippocampal changes have been described in mesial temporal sclerosis in association with voltage-gated potassium channel antibodies.[315-317] On the other hand, hippocampal sparing has been described in atypical Alzheimer's disease.[318] Whilst we examined for confounding by depression and diabetes, we cannot exclude the possibility that in some subjects' other pathology may be responsible for smaller hippocampal size or, on the contrary, that some

subjects with Alzheimer's pathology had large hippocampi. Visual cognitive tasks (dot counting and letter naming) were added to the cognitive battery, after the first 190 subjects in the study, as a screening for posterior cortical atrophy. This is a subtype of Alzheimer's disease where the hippocampi may be initially spared.[319] All subjects in this analysis scored full marks on letter naming, and only 13 dropped one or two points on dot counting. Of these, only three had no hippocampal atrophy on visual rating. This increases confidence that this analysis did not include a large number of individuals with Alzheimer's disease but large hippocampi. Some have argued that such potential confounders reduce the diagnostic utility of medial temporal lobe atrophy, and favour CSF or PET measures.[320] However, the cost and invasive nature mean these may clinically not be as widely available as structural MRI. Small hippocampal volumes (as % of intracerebral volumes) do not necessarily equate to atrophy. However, small hippocampi equate to abnormal clinical medial temporal lobe ratings in this and other studies.[321] If not indicative of imminent Alzheimer pathology,[307] they may at least predict risk of earlier deterioration, if additional Alzheimer type changes supervene.

Whilst endeavours have been made to control for multiple prospectively gathered factors, it is difficult to exclude the possibility of residual confounding. Resilience was quantified cross-sectionally and white matter microstructure may causally relate to memory rather than underlie a compensatory strategy. However, FA was only predictive of better verbal memory in those with evidence of pathology (visual hippocampal atrophy), supporting a compensation argument. Lastly, there may be a selection bias, as those most cognitively impaired would have been unlikely to be included in the study that required travel to Oxford, and neuropsychological

assessment may have encouraged or discouraged those with subjective memory complaints to attend.

There were no associations with other putative protective factors cited in the literature, such as physical activity[322-324] and social interactions.[325-327] The current sample may be underpowered to detect small protective effects.

Brain variables (structural and functional connectivity)

White matter integrity appears to partially mitigate the effect of hippocampal atrophy on verbal memory in this sample. Cardiovascular risk has been linked to Alzheimer's disease,[328] and hypertension is a known correlate of poor white matter quality,[329] although in this analysis Framingham Risk Score did not predict verbal memory independently of age and other sociodemographic and clinical factors. It is not clear, whether resilient subjects have avoided age-related decline in white matter (reduced "brain battering"),[330] or whether differences are due to plasticity.[331] Better inter-hemispheric connections via the corpus callosum, may facilitate compensatory mechanisms in the presence of pathology,[302, 332] as stipulated by 'hemispheric asymmetry reduction in older adults' (HAROLD).[301] This theory is based on functional neuroimaging data suggesting that successful cognitive performance is associated with less lateralised brain activity. There was supportive evidence for the Posterior to Anterior Shift in Aging (PASA) theory,[302] in better caudal-rostral white matter tract integrity in those with higher resilience. These findings were independent of hyperintense lesions as assessed by Fazekas ratings, and BIANCA-extracted WMH

volumes (post-hoc). Whether FA abnormalities are predecessors of hyperintensities or whether they represent two separate pathologies has yet to be fully elucidated.

In preceding chapters, we found alcohol consumption was associated with multiple adverse neuroimaging markers, including hippocampal atrophy, poorer white matter integrity, and greater functional connectivity of the Default Mode Network. Despite this, alcohol intake was only minimally related to cognition. The findings in this chapter may offer an insight into this mismatch in alcohol associations between brain pathology and cognition. **First**, higher premorbid IQ was associated with higher cognitive performance independently of hippocampal size. Higher premorbid IQ also correlated with higher alcohol consumption (Chapter 4). The latter confounding relationship may be obscuring the association between alcohol and cognition. **Second**, better white matter integrity may compensate for hippocampal atrophy, but higher alcohol consumption was associated with poorer white matter integrity. Hence alcohol may deliver a 'double whammy' affecting brain structure i.e. hippocampal atrophy and a potential compensatory mechanism via white matter tracts. However, we found minimal associations with cognition. One explanation is that the confounding by premorbid IQ is more powerful and hence masking any negative association.

7.6 Conclusions

These findings have clear clinical applicability for patient diagnosis and prognosis. **First**, subjects with certain sociodemographic characteristics such as higher premorbid intelligence and social class perform better for any given hippocampal

size. **Second**, white matter microstructural changes may mediate the relationship between hippocampal volume and cognitive function.

As we believe this approach combining sociodemographic and brain anatomy variables to examine resilience is novel, replication will be important. Whether cross-sectionally determined resilience can predict future cognitive decline needs proof. Such prognostic information would have important utility for patients, their families and clinicians. Furthermore, this approach may be applicable to other neurodegenerative conditions or traumatic brain injury.[333] Current therapeutic research is focusing on agents to reduce the primary pathology, with limited success.[334] Exploiting and supporting neural correlates of resilience may offer a fresh approach. Possible strategies that could be investigated include tight control of cardiovascular risk factors and even physical training programs or brain stimulation to enhance connectivity. Even small increases in tolerance to pathological changes may delay or avoid onset of clinical symptoms.

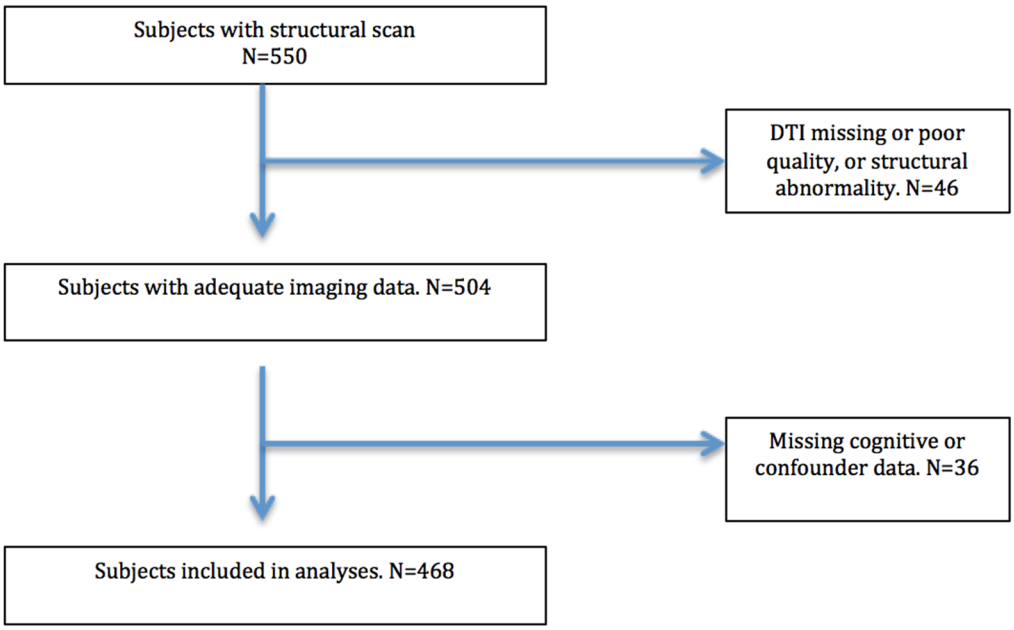


Figure 7.1 Flowchart of participants included in analysis.

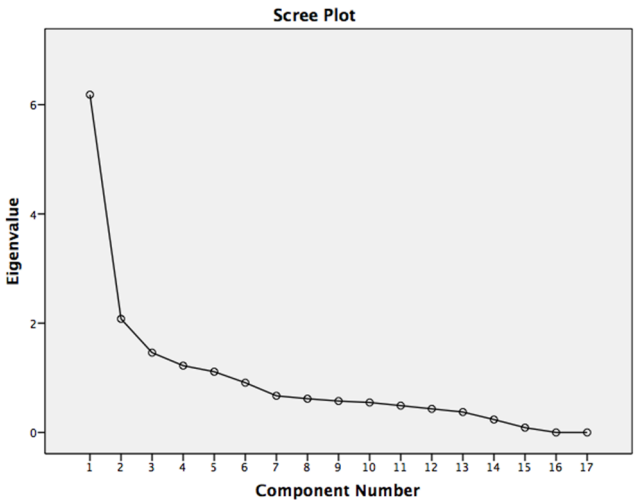


Figure 7.2 Scree plot from cognitive tests factor analysis.

	1 "Verbal memory"	2 "Working memory"	3 "Executive function"	4 "Visuospatial memory"
TOPF	-.007	.089	.033	.008
BNT	.064	-.122	-.089	-.140
Digit code	.226	.062	-.589	-.027
DSB	-.006	**.851**	.003	.011
DSF	-.152	**.860**	.060	-.101
DSS	.271	.577	-.191	.045
DST	.050	**.956**	-.055	-.017
HVLT delayed	**.842**	-.019	.007	-.087
HVLT immediate	**.834**	.056	.016	-.101
TMT_A	-.209	-.078	.623	.011
TMT_B	.090	.007	**.990**	.021
TMT_BA	.180	.036	**.950**	.021
RCF copy	-.105	.095	-.104	-.594
RCF immediate	.117	.015	.016	**-.926**
RCF delayed	.111	.018	-.014	**-.920**
Category fluency	.495	-.023	-.135	-.057
Lexical fluency	.228	.230	-.032	.172

Table 7.1 Components of four largest cognitive factors from a factor analysis with oblique rotation used in subsequent analyses (highest loadings in bold). Abbreviations: TOPF=test of premorbid function; BNT=Boston naming test.

Cognitive factor	'MCI' (MoCA <26) Mean (S.D.)	'No MCI' (MoCA >26) Mean (S.D.)	Mean difference
Verbal memory	-0.83 (1.04)	0.38 (0.71)	-1.21
Visuospatial memory	-0.33 (1.05)	0.16 (0.93)	-0.49
Executive function	0.65 (1.09)	-0.29 (0.81)	0.95
Working memory	-0.18 (0.97)	0.10 (0.99)	-0.28

Table 7.2 Mean cognitive factor scores (and standard deviations) and mean differences (all significant p<0.05) between groups for low (<26) versus high (>26) MoCA scores.

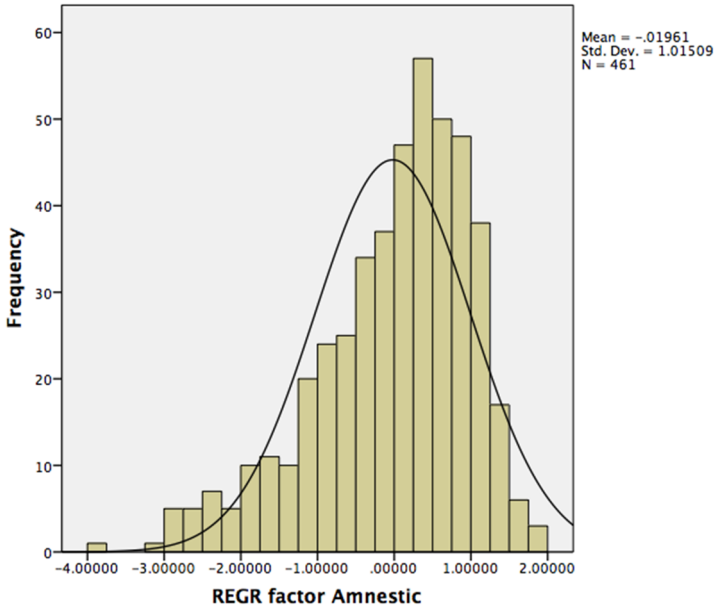


Figure 7.3 Distribution of verbal memory factor scores across the sample.

Variable	Mean (S.D.)/median (IQR)[5]/ n(%)	Partial correlations with verbal memory (after correcting for hippocampal size
Age at start (years)	42.0 (7.7)[5]	-0.3*
Sex (% male)	81.2	0.1*
Marital status (% married)	74.4	0.06
Full-time education (years)	14.0 (5.0)[5]	0.09*
Full Scale Intelligence Quotient (estimated from TOPF)*[1]	118.0 (10.1)	0.2*
Social class 1, 2, 3 & 4 (%)[2]	15.7 (1), 75.3 (2), 11.8 (3), 0.6 (4)	-0.2*
Smoker (%)	0.0	
Systolic blood pressure	120.5 (13.7)	-0.02
Diastolic blood pressure	75.4 (9.6)	-0.003
Framingham Stroke Risk total score (percentage stroke-risk in future 10 years)*	10.0 (9.0)[5]	-0.2*
CES-D[2] total (out of 60)*	3.0 (6.0)[5]	-0.02
History of Major Depressive disorder (%)*[3]	16.5	0.07
Cardiovascular medication (%)*	57.3	-0.1*
Psychotropic medication (%)*	12.8	-0.08
MoCA[3] total (out of 30)*	28.0 (3.0)[5]	
Number of moderate exercise activities (per week)	4.0 (6.0)[5]	-0.1*
Frequency of family and friends visits weekly	4.3 (3.1)	0.06
Frequency of club attendance weekly	1.7 (2.7)	0.06
Frequency of voluntary work weekly	1.6 (2.4)	-0.09*
Average alcohol over study (units per week)	12.4 (10.0)	0.1*
Non-native English speaker (%)	4.3	0.2*
Hippocampal volume (as percentage of ICV)	2.4 (0.3)	
Scheltens Scores (0, 1, 2, 3) - right	44.4(0), 41.2(1), 12.6(2), 1.7(3)	
Scheltens Scores (0, 1, 2, 3) – left	34.2(0), 42.3(1), 22.2(2), 1.3(3)	
Corpus Callosum Fractional Anisotropy	0.7 (0.03)	0.2*
Fazekas Scores (total) (0, 1, 2, 3, 4, 5, 6)	5.2(1), 44.8(2), 24.9(3), 13.7(4), 7.7(5), 3.6(6)	-0.1*

Footnotes:
[*] Assessed at time of the MRI scan.
***Significant partial correlation (p<0.05)**
[1] Test of Premorbid Function.
[2] Based on occupation at Phase 3: 1=professional, 2=managerial, 3=skilled non-manual, 4=skilled manual.
[3] Diagnosed using Structured Clinical Interview for DSM IV disorders (SCID).
[4] Assessed at Phase 7.
[5]Median (IQR) for non-normally distributed variables.

Table 7.3 Summary characteristics for participants (N=468), and partial correlations with verbal memory (after correcting for hippocampal size) for those variables included in the hierarchical multiple regression.

Dependent Variable: Verbal memory factor scores				
Block	**Variable**	**Unstandardised regression Coefficient (B) (95% CI)**	**Standardised regression coefficient (beta)**	**P-value**
1	**Hippocampal volume***	**0.4 (0.08 to 0.7)**	**0.1**	**0.01**
2	**Age**	**-0.05 (-0.07 to -0.03)**	**-0.3**	**<0.0001**
	Sex (male=1, female=2)	**0.5 (0.3 to 0.7)**	**0.2**	**<0.0001**
	Social class[1] (1=highest)	-0.2 (-0.4 to -0.01)	-0.09	0.06
	Premorbid IQ*	**0.02 (0.005 to 0.03)**	**0.2**	**0.003**
	Non-native English speaker* (yes=1, no=0)	**0.9 (0.0 to 0.5)**	**0.2**	**<0.0001**
	Exercise* (yes=1, no=0)	-0.02 (-0.03 to 0.0)	-0.08	0.05
	Psychotropic medication* (yes=1, no=0)	-0.1 (-0.3 to 0.02)	-0.07	0.08
	Hours visiting family/friends*	0.02 (-0.002 to 0.05)	0.07	0.08
3	**Corpus callosum FA***	**5.0 (1.7 to 8.3)**	**0.1**	**0.003**

*** Assessed at the time of the scan.**
[1] Based on occupation at Phase 3: 1=professional, 2=managerial, 3=skilled non-manual, 4=skilled manual.

Table 7.4 Final parsimonious model for the hierarchical multiple linear regression analysis with verbal memory factor score as dependent variable. Adjusted R-square = 25.1% (model 3). Significant (p<0.05) predictors in bold.

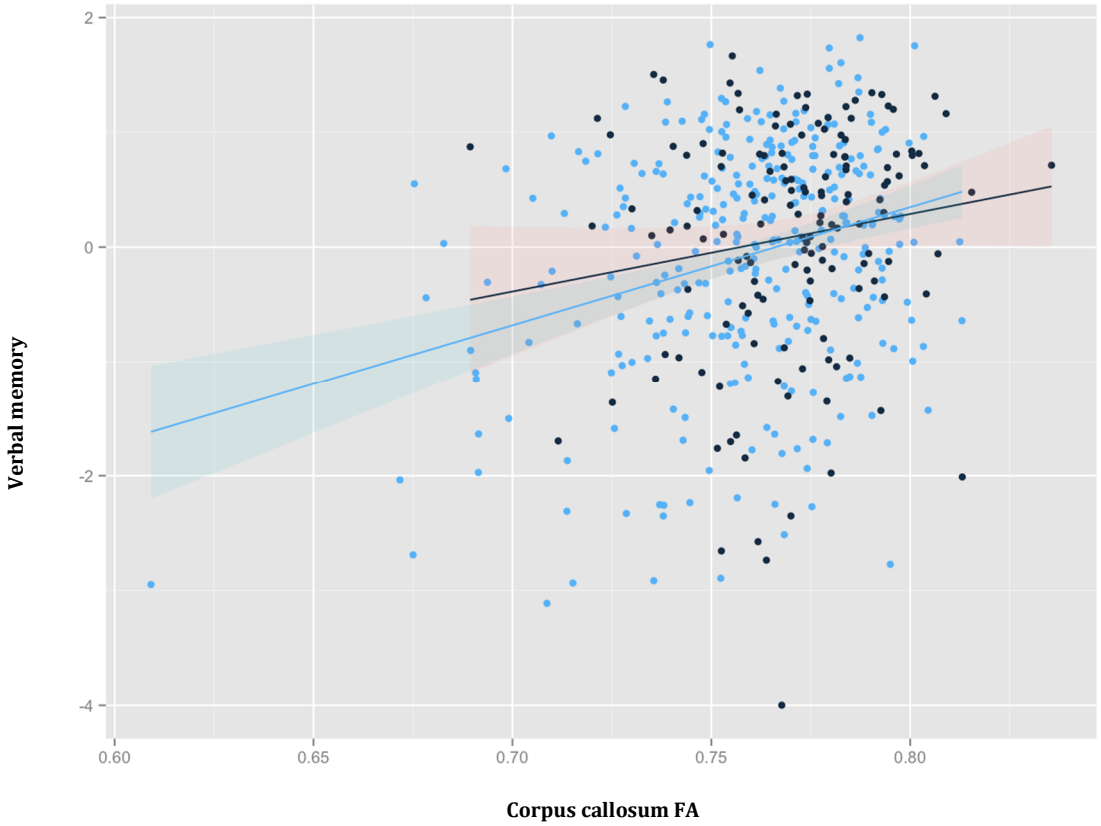


Figure 7.4 Verbal memory factor score (y-axis) against corpus callosum fractional anisotropy (FA, x-axis), plotted separately for participants with hippocampal atrophy (light-blue) and no hippocampal atrophy (dark-blue / pink) defined by Scheltens score (0 compared with 1, 2, 3).

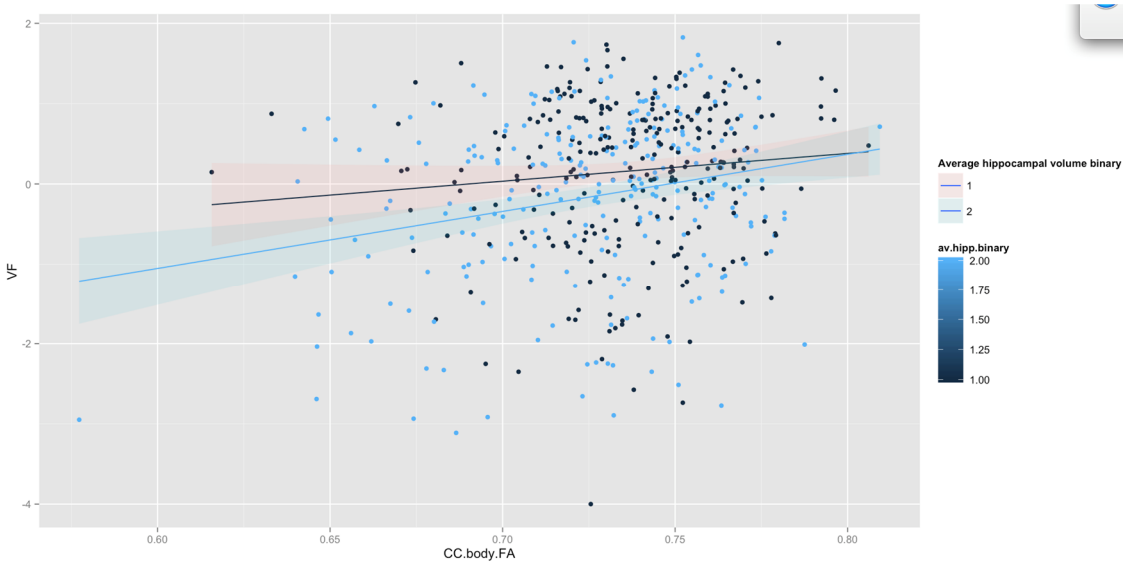


Figure 7.5: The relationship between corpus callosum fractional anisotropy (FA) and verbal memory factor (VF), according to binarised hippocampal volume (% ICV, 1=largest (pink), 2=smallest (blue)).

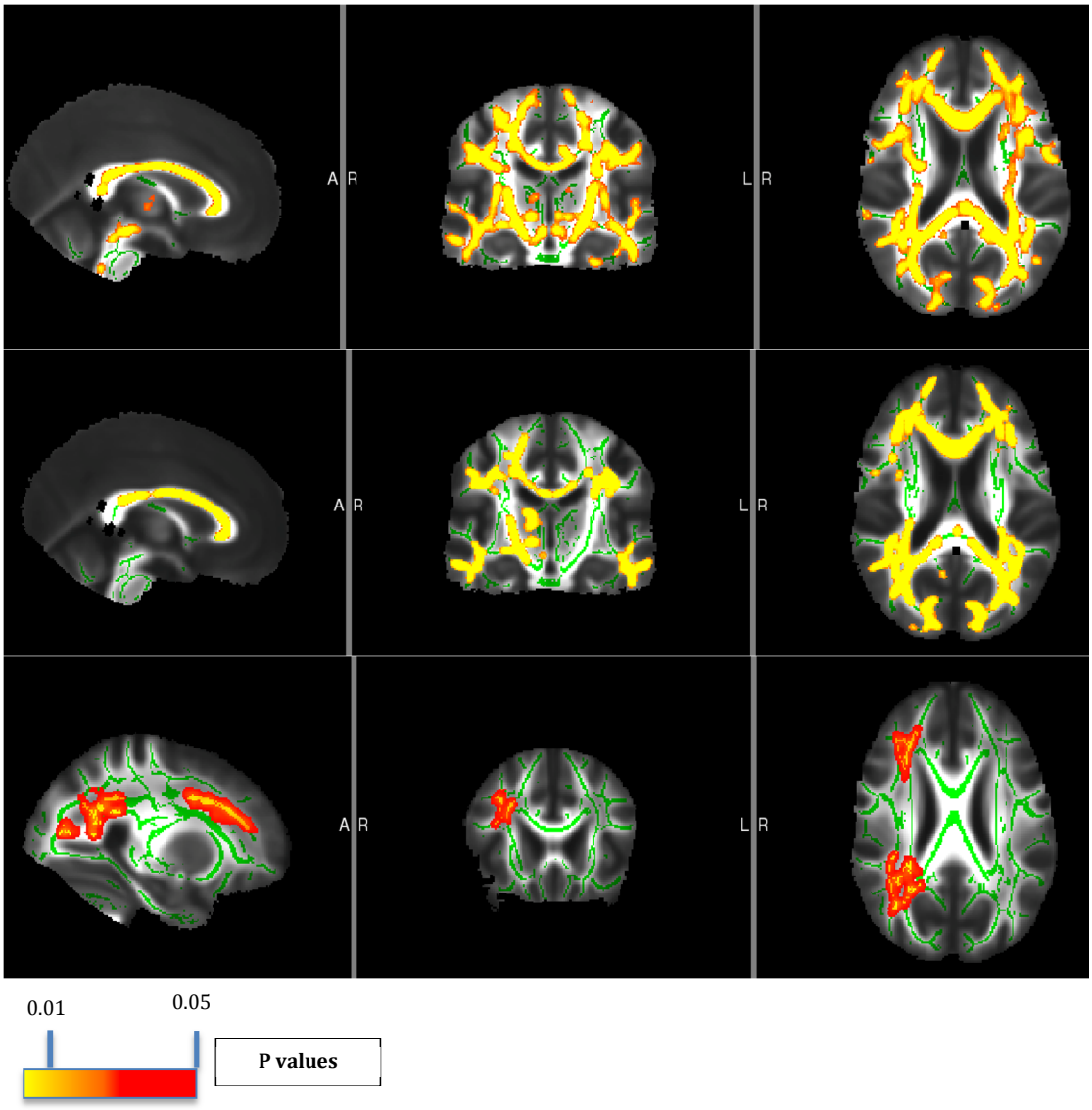


Figure 7.6 TBSS results: significant (p<0.05, tfce corrected) association of verbal memory adjusted for hippocampal volume, age, sex, premorbid FSIQ and bilingualism, superimposed on the mean FA skeleton (green) with: A) fractional anisotropy (FA, positive correlation – top panel), B) radial diffusivity (RD, negative correlation – middle panel) and C) mean diffusivity (MD, negative correlation – bottom panel).

Chapter 8 – Discussion and conclusions

8.1 Main findings

This thesis sets out to elucidate the relationship between non-dependent alcohol consumption and brain structure and function. Detailed neuropsychological testing and multi-modal MRI was performed on a sample from the Whitehall II study cohort. These individuals have been well characterised longitudinally, including their alcohol intake.

This thesis began by reviewing what can be surmised about the effects of alcohol on the brain from the current research field. Whilst long-term negative effects of heavy consumption are now well characterised, the effects of more moderate alcohol use on brain structure and function were unclear. This was in part due to contradictory findings, and also to methodological constraints of studies.

Three overall objectives of this thesis were identified in Chapter 1. These were as follows:

1. Is there a relationship between non-dependent alcohol consumption and grey matter in the brain?
2. Does alcohol intake correlate with macro- or micro-structural white matter indices?

3. What is the relationship between alcohol consumption and brain function, determined by resting state functional connectivity and cognitive performance?

I will discuss the relevant findings to each in turn.

1. Is there a relationship between non-dependent alcohol consumption and grey matter in the brain?

In the first study, associations between average weekly alcohol consumption across the study and grey matter were examined. A novel dose-dependent association between moderate consumption and hippocampal atrophy was found. This finding was replicated using three independent methods of determining hippocampal size: voxel-based morphometry, automated extracted hippocampal volumes corrected for intracranial size, and visually rated atrophy. Increased risk of right-sided atrophy was shown even in those drinking 14-<21 units (112-<168g) weekly.

2. Does alcohol intake correlate with macro- or micro-structural white matter indices?

The second study, focused on white matter associations of moderate alcohol consumption. No relationship was found with macrostructural white matter changes, namely total white matter volume (corrected for intracranial volume), or white matter hyperintensities, assessed by visual rating scale and automatically extracted volume. However, alcohol intake was negatively correlated with microstructural integrity of

white matter, as measured by diffusion tensor indices. This correlation was focused on the anterior corpus callosum, and driven by increased radial compared to axial diffusion.

3. What is the relationship between alcohol consumption and brain function, determined by resting state functional connectivity and cognitive performance?

Associations with brain function, assessed using resting state functional connectivity and cognitive function, were examined in the third study. Higher alcohol intake at the time of scanning was associated with increased functional connectivity within the anterior Default Mode Network. The relationship with average alcohol consumption was not significant. Alcohol was also positively correlated with connectivity between nodes of the DMN in the full (but not partial) correlation analyses, suggesting an indirect connection. Higher alcohol consumption across the study compared to abstinence over the study was associated with a greater decline in lexical fluency in the longitudinal analyses. There were no significant findings with declines in other cognitive domains tested or with cross-sectional performance at the study end point. This was somewhat surprising given the associations found in the preceding two chapters with structural brain changes.

The final study attempted to explore why there were minimal associations between alcohol and cognitive function, despite marked associations with brain structure. One hypothesis is that some individuals are resilient, meaning they can maintain high cognitive function in the presence of brain pathology. A comparable example is in

Alzheimer's disease, where some individuals have evidence of brain pathology with no measurable clinical symptoms. Hippocampal volume was used as a marker for pathology, given the striking associations found with alcohol consumption in Chapter 4. Verbal memory was chosen as the cognitive measure, as this explained most of the variance of cognition in our study participants. Lower age, female sex, higher premorbid IQ and white matter integrity (FA) predicted better verbal memory performance, independently of hippocampal volume. Furthermore, there was an interaction effect between white matter integrity and hippocampal volume in predicting verbal memory, such that higher FA was more protective against verbal memory involvement in those with hippocampal atrophy. Confounding by higher premorbid IQ in heavier drinkers, may explain the mismatch between brain structure and function associations with alcohol.

8.2 Overall findings

In summary, we found evidence for an association between non-dependent alcohol consumption and multiple adverse brain outcomes, including hippocampal atrophy, lower white matter integrity, and greater functional connectivity of the anterior Default Mode Network. Additionally, there was support for faster decline in lexical fluency in those reporting higher alcohol intake across the study as compared to abstainers. There was no evidence for a protective effect on brain structure or function of light drinking over abstinence.

8.3 Methodological considerations

Study-specific limitations have been discussed in detail in the relevant chapters. Here I present an overview of the general methodological considerations for the thesis.

8.3.1 Study design

Subjects included in this work were drawn from the Whitehall II cohort. This 30-year study is unique in the duration and depth of its subject health and lifestyle characterisation. However, the sample may not be representative of the general population. This was an occupational cohort, and hence the whole group was well educated and of higher than average intelligence. Due to the composition of the civil service in the 1980s at study baseline, few women were included, especially at higher occupational grades. The same is true for ethnic minorities. There may be selection bias for those remaining in the cohort, such as the health conscious or the ailing being more motivated to persist. Subjective memory problems might have induced some participants to attend Oxford for neuropsychological testing and brain scan, although they were expressly informed that no feedback on findings would be available. Additionally, there might have been a Rosenthal effect i.e. subjects pursuing more healthy lifestyles, knowing they would be reporting to a researcher with high health expectations at regular intervals.

Much of the data used in this thesis, including alcohol consumption, exercise habits and social activity, were self-reported. Participants might under- or over-report such

behaviours depending on their perception of what is "healthy". There are studies suggesting self-report of alcohol consumption may not be accurate.[147, 151, 152] The context and perceived acceptability, as well as individual factors, may determine whether individuals under- or over-estimate their intake. For example, in a health survey, subjects may perceive it socially desirable to drink less, whereas in a study of those entering addiction treatment, or amongst male university students where drinking is seen as positive, consumption levels may be inflated.[153] There is no "perfect" method to determine intake, as other measures such as blood markers lack sensitivity and/or specificity.[335] Given these limitations, many agree that self-report has adequate reliability and validity if measures are taken to minimise bias, such as prospective recording in a study where subjects do not think alcohol is the sole focus.[152, 153] Consumption in the Whitehall II study was recorded longitudinally, which is likely to be more accurate than a retrospective design, and the study was not specifically set up to study the effects of alcohol on health.

We used the CAGE screening instrument to identify alcohol dependence, as it is easily administered and well validated.[207, 336] There were some individuals with missing CAGE data, and we cannot exclude the possibility that we have included some subjects who were alcohol dependent at points during the study period. However, all included individuals had at least three (out of a total of five) CAGE measurements, and individuals with incomplete CAGE data did not on average drink significantly more than those with complete data (on a t-test of means). Some subjects reported drinking high levels of alcohol whilst screening negative on the CAGE, indicating a possible inclusion of subjects with an alcohol use disorder in the

sample. However, increased odds of hippocampal atrophy and faster lexical decline were found even in those admitting to drinking moderate amounts.

8.3.2 Imaging

Multi-modal imaging was performed on subjects. Images were analysed using both visual rating scales, used clinically, and state-of-the-art mostly automatic analysis pipelines. Whilst visual rating scales require no specialist software and hence can be used in a clinic setting, they suffer from certain limitations. These include the subjectiveness of ratings, although our inter- and intra-rater reliability results were encouraging.[179] As there are only a small number of discrete values for each scale, they may be insufficiently sensitive to detect subtle changes, especially in a non-pathological sample. However, a surprising number of our subjects did score positively on the scales.[179] Conversely, whilst automated subcortical volumes are far more sensitive to intra-subject differences, the cross-sectional nature of the measurement makes it impossible to make direct suppositions about atrophy, even when controlling for intra-cranial volume.

Although sociodemographic, health and to a limited extent, cognitive data, were gathered longitudinally, brain imaging was only performed at the study endpoint. Analyses with brain measures were, therefore, by necessity cross-sectional, which has inherent limitations. Whilst analyses were controlled for multiple potential confounders, it is possible that an unmeasured variable could be contributing to the findings. However, for this to be the case, the variable would have to both correlate with the independent and dependent variable, and unrelated to any other factor adjusted for.

Structural brain changes probably evolve over many years. Studies with longitudinal imaging over short time periods may be poorly placed to detect these. Additionally, it becomes harder to exclude reverse causation as study duration decreases (to take the alcohol example, those with atrophy may start drinking more, rather than high drinking causing atrophy). We believe a key strength of this work is the 30-year follow up. There have been substantial technological advances in MRI science since 1985. Even if baseline scans were available, much of the analysis we present in the paper would not be possible. For example, lower magnet strength and image resolution in use in the 1980's would preclude use of automated subcortical volume extraction, and diffusion tensor imaging was not in use at the study baseline. The analysis would be limited to that of visual atrophy ratings, and even this would have serious limitations, such as making comparisons between images with very different resolutions.

8.3.3 Cognition

Whilst the neuropsychological testing performed at the time of scanning was fairly comprehensive, longitudinal cognitive data were more limited. Of relevance, there was limited testing of verbal memory at earlier study phases, which may be more sensitive to hippocampal changes. This is important because the associations we found with higher alcohol consumption included hippocampal volumes.

Some cognitive tests, particularly the MMSE, are known to have ceiling effects, and may be insensitive at detecting minor cognitive change. The finding that MMSE

scores did not change over time supports this. To obviate this problem, the analysis in this thesis centred on more challenging cognitive tests, such as verbal fluency and memory. Scores on these tests were normally distributed, reducing the likelihood of ceiling effects.

When the same cognitive tests are applied multiple times, learning effects are possible. This may mask cognitive decline, as discussed in Chapter 6. The time interval between Phase 11 of the Whitehall II study and the Oxford phase was particularly short, making learning more likely. We attempted to control for learning effects in the analyses, but we cannot exclude residual confounding.

A final possible limitation is that premorbid FSIQ was established for participants using the TOPF, at study endpoint. It is possible that cognitive impairment in some participants may have also affected performance on this test.

8.4 Clinical implications

Several important learning points can be drawn from this work that may have clinical and public health relevance. First, alcohol consumption, even at moderate levels, is associated with multiple adverse brain outcomes and measurably faster cognitive decline. The links with hippocampal atrophy were found in those drinking within safe limits, as currently defined in the US. A large sector of the population drinks over this level and may be unaware of the potential health risks. This has potential important public health implications. The world's population is aging, the burden of cognitive impairment increasing, and with no effective treatment yet identified, reduction of

preventable risk factors is essential. Second, clinicians should be aware that for cognitive performance-matched patients, those with higher premorbid IQ might have more significant brain pathology indicating more advanced disease. This may also mean that adverse effects of alcohol consumption on the brain have been underestimated due to confounding by an association with higher intelligence. Finally, the mediating effect of white matter integrity on the association between hippocampal size and memory, suggests a potential role for strict vascular risk factor control in secondary prevention of cognitive impairment. Intervention studies will be needed to test whether reducing vascular risk leads to better outcomes, such as a delayed appearance of cognitive impairment.

8.5 Suggestions for future research

Given the novelty of some of these results, particularly regarding the associations of moderate alcohol and adverse brain measures, replication will be important. The development of larger platforms and cohorts, such as the Dementia Platform UK and UK Biobank, will allow for some validation of these findings and reduce concerns about representativeness for the general population of the Whitehall II cohort. Although there are complications of longitudinal neuroimaging, these studies are of such size that they may detect even small changes in brain measures, which may occur over a shorter duration. Baseline imaging at midlife would also mitigate the possibility of reverse causation. Additionally, availability of genetic data will allow investigation of possible gene-environment interactions in predicting cognitive function or brain measures.

Future studies could attempt to substantiate self-report of alcohol consumption using other means, including sales data and even wearable devices measuring alcohol intake, which are currently developed.[337, 338] One approach that has been applied to the Guangzhou Biobank is the use of Mendelian randomization using the ALDH2 genotype.[339] Individuals with inactive alleles are prone to flushing reactions following alcohol, and hence avoid alcohol. ALDH2 genotype has been shown to strongly predict alcohol consumption.[340] Mendelian randomization avoids concerns about residual confounding in studies of alcohol consumption, if there is no association between confounders and genotype. However, ALDH2 polymorphisms are rare in Western populations, limiting the application of this approach. Higher powered studies might be able to identify subgroups particularly vulnerable to alcohol-related brain changes, such as women or those with physical illness; or whether certain drinking patterns (e.g. binging vs. regular) or beverage types (e.g. wine vs. beer) are riskier. Another interesting question is, whether there are periods in life vulnerable to the effects of alcohol on the brain, for instance adolescence. However, investigating this would require large groups of individuals consuming alcohol during those time-windows only, and abstinence for the rest of their lives. It is difficult to imagine how one could conceive of a sufficiently powered cohort to study this. A related question is whether secondary prevention is possible: does abstinence in later life reduce further damage in those who already have some brain changes or cognitive impairment? This is particularly pertinent given the increasing prevalence of drinking in older adults. An intervention study would be helpful to answer this question.

To test whether associations with cognitive decline were under-detected due to test insensitivity and learning effects, future studies could perform more comprehensive longitudinal testing. Variation of the tests, for instance using different word lists or varied computerised batteries may reduce learning effects.

Whilst higher alcohol consumption was associated with smaller hippocampal size, the mechanism underlying could not be elucidated in this study. Use of other neuroimaging modalities, or even neuropathological markers *post mortem* could investigate whether Alzheimer-like pathology underlies the association, or whether another process is at work. This thesis has identified sociodemographic and brain variables predictive of better cognitive performance in the presence of a brain pathology proxy, viz. hippocampal atrophy. This work could be expanded using other tools, such as amyloid PET imaging, to define pathology better. True resilience would involve slower future longitudinal cognitive decline. Samples which have detailed neuropsychological testing over many years after the scan will be necessary to identify such factors.

8.6 Concluding remarks

In summary, this thesis has furthered knowledge about structural neuroimaging correlates of non-dependent alcohol consumption. Moderate alcohol consumption is proposed as a risk factor for adverse brain changes and faster cognitive decline. Sociodemographic and neural predictors of better cognitive performance in the presence of pathology have been identified, and may confound relationships between

alcohol consumption and cognition, obscuring the true extent of the anatomical damage. This chapter summarises the methodological limitations of this work, potential clinical implications, and suggestions for future research.

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