The impact of substance use on brain structure in people at elevated genetic risk of schizophrenia

By

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8th August 2010

CONTRIBUTORS

This thesis has been composed using work undertaken as part of the Edinburgh High Risk Study (EHRS), hence a wide range of people have assisted with data collection.

Professor E.C. Johnstone, Professor D.G.C. Owens, and Dr S. Lawrie conceived and designed the EHRS and carried out the clinical assessments. Dr H.C. Whalley, J.N. Kestelman and Dr S.S. Abukmeil undertook the manual delineation of brain regions-of-interest. Dr B. Moorhead provided assistance with undertaking the automated image analysis. Dr A. McIntosh provided assistance with statistical issues

I implemented the automated methods of image analysis, performed the literature reviews, carried out the data analyses and wrote the manuscript.

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ABSTRACT

A variety of structural abnormalities are consistent findings in schizophrenia. These include enlargement of the lateral and third ventricles and reduced volume of the frontal lobes, medial temporal lobes and thalami. These abnormalities are present in first episode subjects and may be detectable before the onset of clinical disorder.

There is accruing evidence that substance misuse may contribute to an individual's risk of developing schizophrenia. Substance misuse is associated with similar brain abnormalities to those seen in schizophrenia and is often well established at the time of first presentation. This makes it difficult to ascertain if any of the structural abnormalities seen when individuals first present with psychosis are attributable to substance misuse. An understanding of the relationship between substance misuse and structural imaging abnormalities in people who are well but at high risk of schizophrenia is thus of great importance. It has the potential to yield important insights in to: (1) the role substance misuse may play in the development of structural brain abnormalities; and (2) how substance use may influence risk of developing the condition.

A prospective cohort study with nested case-controlled comparison design was employed to examine the relationship between substance misuse, brain imaging abnormalities and the subsequent development of schizophrenia. Substance misuse history, imaging data, and clinical information were collected on 147 subjects at high risk of schizophrenia and 36 controls at point of entry to the study. Regions exhibiting a significant relationship between level of use of alcohol, cannabis or tobacco and structure volume were identified, this relationship being elucidated through the use of both volumetric and voxel-based morphometric image analysis techniques.

Additionally, we established whether substance misuse up to the point of recruitment was associated with later risk of schizophrenia.

In addition to the baseline scan, the first 57 high risk subjects recruited to the study also had a follow-up scan after approximately 18 months. As substance use between scanning points was known, this enabled longitudinal comparison of brain structural changes in high risk subjects who did and did not use the aforementioned drugs of abuse. This comparison was made using both volumetric and tensor-based morphometric image analysis techniques.

In the baseline analysis, increased ventricular volume was associated with alcohol and cannabis use in a dose-dependent manner. Alcohol consumption was associated with reduced frontal lobe volume. Multiple regression analyses found both alcohol and cannabis were significant predictors of these abnormalities when simultaneously entered into the statistical model. The longitudinal analysis demonstrated that cannabis use between scanning points was associated with both bilateral thalamic and right anterior hippocampal volume loss. Alcohol and cannabis misuse by point of entry in to the study were associated with an increased subsequent risk of schizophrenia.

This study provides prospective evidence that use of cannabis or alcohol by people at high genetic risk of schizophrenia is associated with brain abnormalities and later risk of psychosis. A family history of schizophrenia may render the brain particularly sensitive to the risk-modifying effects of these substances.

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Chapter 1

Substance misuse and the human condition

1.1 The rise of substance use in the West

Evidence of psychoactive substance use dates to the beginnings of recorded history, and centres on the use of alcohol and plants with psychoactive properties. Once established, use of these substances tends to spread by a process of global diffusion, the pattern this takes reflecting contact and trade between different populations. The process by which the commonly consumed drugs of abuse became established in Western Europe, with a particular emphasis on how this influenced patterns of use in Scotland, will be discussed below. A class of drugs that will not be discussed in this section is opiates. This is not because opiates do not represent a major problem in Scotland (they do); rather, use of these substances was not expected to be prominent in the people who are the focus of this study. In contrast, cocaine will be considered in this section. Though use of this drug is also not expected to have been commonplace in the group under study, the strong association between stimulants and psychosis does necessitate consideration of the history of this substance.

1.1.1 Alcohol

Alcohol is the favoured drug of much of the world's population. It was probably discovered following experimental fermentation, and archaeological investigations suggest that beer was being habitually consumed earlier than 6000BC.[1] Similarly viticulture, the selective cultivation of grape varieties for making wine, probably originated where Armenia is now located, sometime between 6000 and

4000BC.[2] The spread of viticulture exemplifies the manner in which patterns of drug use have been heavily influenced by both land-based and ocean-going commerce. After being established in Europe the wine vine was successfully transported to the Americas, Southern Africa and Australia; this spread of viticulture shadowing the expanding empires of various European nations.

The technology of distilling, the process by which a concentrated, imperishable alcohol product is produced from fermented liquids, was known to the Greeks and Romans and preserved and advanced by the Arabs.[2] It re-entered Europe via Salerno in the eleventh century, but widespread knowledge of the technique was not achieved until printed books began appearing in the late fifteenth century. It then assumed increasing economic importance, with improved copper stills making the mass production of liquor possible.[2] By the mid-seventeenth century the industry was widespread throughout Europe, with the product being profitably exported around the world. Mass-produced spirits were a cheap source of intoxication, and this led to a huge increase in intoxication and alcoholism both in European and non-European societies. In Britain the problem was compounded by positive incentives to produce cheap gin being given to promote agriculture, and by 1736 consumption of spirits was approximately one gallon a head per annum.[3] Consequently drunkenness was commonplace and widespread, and adverse social consequences inevitable. These have been captured for posterity in William Hogarth's famous engraving 'Gin Lane', (Figure 1.1) drawn in 1751 and depicting the general debauchery associated with mass drunkenness.

Figure 1.1.
Hogarth's 1751 engraving 'Gin Lane'

Gradually, by means of licensing and taxation, alcohol consumption was successfully reduced. This was not to be a sustained trend however and in the 19th century, likely as a consequence of social changes accompanying the industrial revolution, consumption began to rise again.[4] It was in this period that the Scots acquired their particular reputation for heavy drinking, with reports of scenes such as the following being reported in the London press:

"The drunk and incapable on a New Year's Day are not stray eccentrics who take a drop too much and steal home as silently as they may, but are really almost as numerous as the wounded in a general engagement; they succumb in ranks and platoons and need a special ambulance service to get them to the rear."

Daily Telegraph, December 1877

In his consideration of the Scottish experience of alcohol, Daniel Paton concludes that this reputation was probably justified.[4] As he describes, industrial society had given rise to the combination of an increase in disposable income and the loss of constraints provided by the social controls of traditional society; this, when drinking was occurring in towns with high population densities, resulted in 'brutal drunkenness on an unprecedented scale'. Inevitably the backlash followed, with the rise of the temperance movement and the assembly of a formidable anti-drink coalition. Legislation was passed, and by the early 20th century the Scottish licensing system became the most restrictive in Britain. Consumption fell once more and continued to fall, this being encouraged by the harsh economic climate of the 1930s. Alcohol ceased to be a major social concern, which meant that when consumption began to rise again in the 1950s there was no culture of abstinence and no organised political opposition to campaign against it.

The recent history of alcohol consumption in Scotland from the 1950s onwards has been one of ever-increasing consumption. By the 1970s the vast majority of Scots were drinkers and alcohol had regained an almost universal acceptance not known in the country for a century.[4] The rise continued, and consumption levels are now more than double what they were in the 1950s.[4] Indeed, recent survey data have reported that 60% of Scottish men and women aged 16-24 'binge drink' (i.e.

consume more than 6 units (women) and 8 units (men) in a single drinking session) on a weekly basis, and 38% of Scottish men aged 45-54 exceed 21 units of alcohol a week.[5] Reflecting the particular severity of this problem in Scotland, alcohol-related death rates for males and females here are around double those for the UK as a whole.[6] It is thus the case once again that alcohol consumption is a major public health preoccupation in Scotland.

1.1.2 Tobacco

Europeans first learned of tobacco in 1492, when two members of Columbus's party observed Tainos Indians smoking leaves rolled in large cigars.[2] Widespread cultivation began in the late 16th century, and by the mid 17th century the cost of the product had fallen to the extent that widespread consumption was possible. The habit was rapidly acquired, and was quickly spread through Europe by sailors and soldiers. Consumption was further boosted by the development of the cigarette in the 19th century, and by the early 20th century this was the dominant method of nicotine administration. By the 1950s world tobacco production of tobacco was over 8.4 billion pounds annually, and the plant was a cash crop on all continents save Antarctica. With growing awareness of the health concerns associated with use of the product consumption levelled off in Western nations during the 1960s and 1970s, but use continued to expand in developing nations; indeed, by the mid-1990s a third of the world's population were believed to be smokers.

The prevalence of smoking in Scotland is approximately 27% in those aged 16 years or over.[7] This exceeds the UK adult prevalence of 21%.[8] Twenty four percent of

all deaths in Scotland in 2004 were attributable to smoking.[8] It is clearly a major public health concern, and a number of initiatives (such as the smoking ban, operating in Scotland since 2006) have been introduced to try and address it.

1.1.3 Cannabis

Cannabis originated in central Asia and was first extensively cultivated in China 6,000 years ago. It was a valuable, multipurpose crop, yielding a potent drug as well as cooking oil, edible seeds, animal fodder and hempen fibres.[2] Cannabis also has a long history in India, early Indian texts dating from 2000-1400 BC referring to its psychoactive properties. Indian cannabis use apparently peaked during the Mogul era (1526-1856), when cultivation and preparation of various cannabis drugs flourished in all parts of the subcontinent. By this time cannabis had spread through the Islamic world, down the East coast of Africa and in to Europe.

Both Indian and Chinese cultures promoted the medicinal use of cannabis, and there was periodic interest in applying cannabis products for this purpose in Britain from at least the 18th century.[9] Before the 1950s however the general population had little knowledge of the intoxicating properties of cannabis and it was little used as a recreational drug. Nevertheless, it had been illegal in Britain since 1928, when the 1925 Dangerous Drugs Act came in to force.[10] The profile of cannabis in the UK began to be raised in the late 1950s, when a drug subculture seemed to emerge in the West End of London linked to bohemian and jazz cultures.[1] This gained momentum in the 1960s, particularly in the context of the 'hippie' movement, and cannabis is now well established as the most commonly used illicit drug in the UK.

The high prevalence of cannabis use in Britain is supported by findings from surveys of drug use. The British Crime Survey for example reported that in 2008-2009 18.7% of people aged 16-18 had used cannabis in the last year, and 10.4% in the last month.[11] These figures do represent a decline from earlier years of the millennium, but clearly cannabis use remains very widespread among young people. Given such widespread use, any health risks related to use of the drug would clearly be of great concern. The data supporting a relationship between cannabis and psychosis will be discussed in subsequent chapters.

1.1.4 Cocaine

Evidence of the chewing of coca leaves, the raw material from which cocaine is derived, date back to 3000BC.[2] It was however not until 1860 that Albert Niemann, a graduate student at Gottingen, described the isolation of cocaine in his dissertation.[12] He did not profit from this advance. By contrast however, a number of entrepreneurs who marketed foodstuffs utilising coca extract did, Coca-Cola being among the glut of coca-based products to achieve international success from 1863 onwards. In the 1880s, promoted by figures such as Sigmund Freud, interest in the drug escalated further, the resulting shortages of coca supply from Peru precipitating global cultivation. The Dutch established Java as a rival producer, transporting the product to Europe for the extraction of cocaine. Increased supply resulted in lowered prices, and this prompted a global epidemic of cocaine use that lasted from the 1890s to the 1920s.

The manner in which different countries experienced the 'cocaine epidemic' varied substantially, but the British experience was of some interest. It is described by Mark Kohn in Paul Gottenberg's global history of cocaine.[12] It was essentially restricted to the West End of London, and regarded as associated with the women who worked there as actresses, nightclub dancers, and prostitutes. The West End was a fertile habitat for an underground drug scene at the time, being small, crowded, and a centre of hedonism. With the outbreak of the First World War efforts were made to control the consumption of alcohol, and restrictions placed on pub opening hours. This represented a boom for underground clubs in the West End, potentially bringing more people in contact with cocaine-using groups. In this context all it took was for a number of stories to appear in the press of servicemen being sold cocaine for a moral panic to ensue. Newspapers talked of cocaine use 'spreading like wildfire' and women 'preying on soldiers'. As is so often the case with drugs policy, this period of moral panic on a background of grumbling concerns resulted in immediate legislation, and cocaine possession was made a criminal offence.

In the late 1920s cocaine use subsided, and world exports began a sustained decline. Indeed, there was little interest in cocaine for almost half a century, and little further research undertaken with the drug. This, of course, was all to change in the 1970s, when cocaine returned to mass consumption in North America.[2] This continued in to the 1980s, when the drug also made its way back to the UK, being the favoured drug of high-earning professionals in the City. Subsequently prices have fallen, and crack (a pure and smokeable form of the drug with a more intense high) has also arrived. Now cocaine is widely available to all socioeconomic classes, and has firmly re-established itself in the UK.

Cocaine exposure has progressively risen in the UK over the last decade. Whereas 3.8% of people aged 16 to 59 had ever used cocaine in 1998, this figure was 9.4% in 2008/2009.[11] Comparable changes have occurred in those aged 16-24; 7.1% had ever used the drug in 1998, rising to 12.4% in 2008/2009. Use in the last month, predominantly representing regular users, has risen from 0.6% to 3.7% in the same age group over the same period.

1.1.5 Other illicit drugs

The other drugs expected to have a reasonable prevalence of use in the group under study are amphetamines, LSD and ecstasy. These three substances are synthetic products, and each was initially developed in the hope they may have medical utility. In each case initial promise gave way to disappointment, and as the problems associated with the substance became apparent use shifted from medical to popular consumption. The history of amphetamine is archetypal of this process, recognition of its propensity to cause dependence and induce psychotic symptoms resulting in restrictions being placed on its availability.[2] As the history of amphetamine use is intertwined with early conceptualisations of the dopamine hypothesis of schizophrenia, the history of this drug will be considered in greater detail in the relevant subsequent section; the history of the other two substances will be discussed below.

The Swiss chemist Albert Hoffman first synthesized LSD in 1938 while studying derivatives of the fungus ergot for use as potential medicines. It was set aside for five years, but in 1943 he decided to re-examine it. When he accidentally absorbed

some LSD during a laboratory session there followed an intense experience of perceptual and emotional effects.[13] By the late 1940s psychiatrists were beginning to experiment with LSD; this work included exploration of the possibility that psychedelics might be used as 'psychotomimetics', to mimic the mental states of patients with schizophrenia, and that it could be used as an adjunct to psychotherapy.[14] It was in this context that Timothy Leary, then a research psychologist at Harvard University, first had exposure to the drug.[2] He promoted widespread use of the substance, coining the catchphrase 'tune in, turn on, drop out' and advocating youth to utilise the drug for self-discovery. With such promotion LSD leaked from the scientific community to a wider audience, and by 1966 LSD use had become regarded as a problem in the USA and its use made illegal.[14] Nonetheless, use of the drug continued to rise, intimately associated with the hippy movement. A similar escalation was seen in Britain, this being associated particularly with the 1967 summer of love.[15] Inevitably media hysteria followed, this predicting mass violence and chromosomal abnormalities in children.[16, 17]

Despite the decline in popularity of the 'hippy' culture, use of LSD remained fairly prevalent in the UK through the 1980s and 1990s. The most recent British Crime Survey reports that 5.5% of people aged 16-59 have ever used the drug, a figure which has changed little since the mid-1990s. Since 1996 however, use of LSD in those aged 16 to 24 has actually fallen dramatically; whereas 13.1% had ever used the drug in 1996, this had fallen to 2.5% by 2008/2009.[11]

MDMA (or ecstasy) was patented in 1913 by the German company Merck. They did not market the drug however, and no uses are mentioned in the patent. The drug re-emerged in the 1970s. The American biochemist Alexander Shulgin synthesised the drug, and being impressed by the effects it had began to promote it as

a tool in psychotherapy.[18] By 1984 the drug was still legal in the USA, but had seeped in to popular use, predominantly among college students. The escalation in use combined with concerns about its safety led to pressure for the drug to be made illegal, this occurring in 1985.[19]

Interestingly, MDMA had actually been illegal in the UK since 1977, under legislation banning a number of psychedelic amphetamines.[20] Nonetheless, use of the drug became rapidly established in Britain in the late 1980s; it quickly became the drug of choice at the all-night dancing parties (raves), which came to dominate the youth scene of the time. The sub-culture associated with raves, the affordability of the drug and its highly desirable behavioural effects (with apparently few side effects) all contributed to an explosion of recreational ecstasy use.[20] In recent years, although the popularity of raves has declined, the use of ecstasy has shifted to nightclubs and discos, where it remains a highly popular recreational drug. Ecstasy is thought to be the second most commonly used controlled drug (after cannabis) in Europe,[21] and it is estimated that 12-24 million people worldwide have taken ecstasy.[22]

In the UK it is estimated that 8.6% of the population of 16 to 59 year olds has ever used ecstasy, with 0.6% having used the drug in the last month.[11] A total of 9.9% of 16 to 24 year olds have ever used the drug, a slight decline since the 1990s. Of these 1.5% have used the drug in the last month; the majority of these individuals are likely to constitute a regularly using population.

1.2 General effects of drugs on brain

Drug dependence is a phenomenon which, while deeply embedded within a social context, is increasingly appreciated to be underpinned by neuropathological abnormalities. As the current study will focus on the brain structural abnormalities existing at the interface between drug use/dependence and risk for schizophrenia, it is important that the neuropathology underpinning addiction is appreciated. In this section I will thus briefly discuss the most important pathways and neurotransmitters believed to be central to the development of dependence. It is important to note at the outset that, despite the diversity of pharamacological effects exhibited by drugs of abuse, there is believed to be a 'core' addiction syndrome. This is illustrated by the fact that the diagnostic criteria for the dependence syndrome are the same regardless of the drug under discussion (see Table 1.1, outlining the Diagnostic and Statistical Manual of Mental Disorders, (DSM), criteria).[23] This review will thus focus on the circuitry underpinning this core syndrome; when warranted the specific pharmacological effects of particular drugs of abuse will be discussed in subsequent sections.

A maladaptive pattern of substance use, leading to clinically significant impairment or distress, as manifest by three (or more) of the following, occurring at any time in the same 12-month period:

(1) Tolerance as defined by either of the following:
  (a) a need for markedly increased amounts of the substance to achieve intoxication or desired effect
  (b) markedly diminished effect with continued use of the same amount of the substance
(2) Withdrawal, as manifested by either of the following:
  (a) the characteristic withdrawal syndrome for the substance
  (b) the same (or closely related) substance is taken to relieve or avoid withdrawal symptoms
(3) The substance is often taken in larger amounts or over a longer period than was intended
(4) There is a persistent desire or unsuccessful efforts to cut down or control substance use
(5) A great deal of time is spent in activities necessary to obtain the substance, use the substance, or recover from its effects
(6) Important social, occupational, or recreational activities are given up or reduced because of substance use
(7) The substance use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance

Table 1.1
The DSM-IV dependence syndrome.[23]

Addiction to drugs and alcohol is now understood to be based on pathological changes in brain function produced by repeated pharmacological insult to the brain circuitry underpinning motivation. In their normal state the function of these brain circuits is to learn about and behaviourally adapt to important environmental stimuli, whether these be how to best approach rewards such as food or sex, or to avoid dangerous situations.[24] By interacting with and changing this motivational circuitry, addictive drugs impair the development of behavioural strategies towards biological stimuli in favour of progressively greater orientation of behaviour towards drug-seeking and drug-taking strategies.[25] These changes are long-lasting and not readily reversed, resulting in an individual's life being increasingly dominated by the seeking and consumption of drugs of abuse. Most disturbingly, even if abstinence is achieved, this recurring desire to take drugs can persist for many years or even a lifetime. Consequently, an individual remains at risk of relapse even after many years of abstinence.

If an impaired ability to regulate the drive to use substances is at the core of addiction, then it is understandable that abnormalities of motivational circuitry are implicated in these conditions. The most significant structures constituting this motivational circuitry are outlined in Figure 1.2. Within these circuits the mesolimbic dopaminergic pathway is regarded as central. All drugs of abuse, albeit through different molecular mechanisms of action, (and with the exception of benzodiazepines), increasing dopamine here.[26] This release has two related consequences; it results in the experience of pleasure (reward), and it imbues the prevailing circumstances with salience (so promoting memory).[27] There is nothing innately pathological about this circuit; indeed, as discussed above, it is essential in providing the drive to undertake and continue the activities essential for life. The power of drugs of abuse derives from their ability to directly activate this circuit, doing so to a degree both of greater amplitude and duration than can be achieved through biological mechanisms. Thus, when an individual consumes a particular drug they both experience pleasure, and the memory of this experience and the circumstances surrounding it are promoted.[27] Given that networks so central to conferring salience are being directly stimulated, this memory promotion is much more effective than that associated with most other stimuli, which become of increasingly secondary importance. Conversely, with repeated exposures drug-related associations become further strengthened, and stimuli associated with substance use precipitate craving, (an intense urge to use the substance). The process of the development of dependence has begun, and with further episodes of use becomes further entrained.

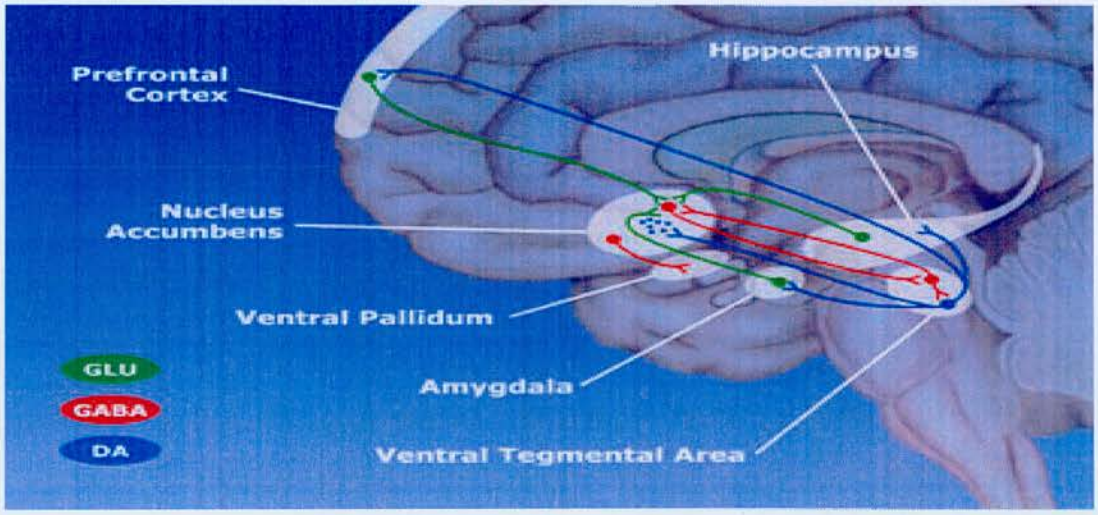

Figure 1.2
The primary constituent structures underpinning motivation and drug dependence (reproduced from Ferrer *et al.*).[28]

Though preserved in its basic form, in recent years our understanding of the processes outlined above has been further refined. This understanding is summarised by Kalivas *et al.* in two recent reviews, these attempting to integrate understanding of neurotransmission in the circuits involved in motivation and salience with advances in the understanding of neuroplasticity.[25, 27] In their model the importance of repeated drug intake resulting in repeated release of dopamine from cells in the ventral-tegmental area (VTA) into the prefrontal cortex (PFC), striatal complex (including the nucleus accumbens, NA), and amygdala remains central. As discussed above, this imbues an event with salience, creating an internal sense that this is a relatively important event requiring the development of a behavioural response.[29] As use continues, so enduring changes in neuronal physiology occur. This occurs through mechanisms such as excitatory synaptic plasticity driven by glutamate-associated long term potentiation, and results in ever-increasing associations being made between the drug and life events.[27] In time the drug comes to dominate an individual's life, the primacy given to drug use manifesting as maladaptive drug-seeking behaviours and

occurring at the expense of biologically important stimuli such as seeking food and avoidance of harm.

In characterising the processes underpinning learning and behaviour, Kalivas *et al.* further distinguish between those which are important in promoting new behaviour (i.e. establishment of a drug use problem) and those involved in maintaining an existing problem (relatively stable changes promoting ongoing vulnerability to relapse).[27] Kalivas *et al.* argue that in establishing a drug problem dopaminergic afferents to the amygdala, nucleus accumbens and prefrontal cortex are indeed central. Even at this early stage however, corticofugal glutamate projections are also involved (predominantly from the PFC and amygdala into the NA), these resulting in greater efficiency of memory retrieval in response to drug-related cues. At this stage however, drug use does still occur as a consequence of a conscious choice, the glutamatergic projections simply facillitating the retrieval and integration of drug-associated memories. As the behaviour is repeatedly executed however, this network becomes less important in favour of glutamate projecting from sensory motor cortical areas to the dorsal striatum.[30] In this way, they argue, behaviour evolves from being a declarative process involving prefrontal executive functions into a habitual behaviour utilizing working memory circuitry.[31] It is all too easy to see the implications of this for drug dependence. As this transition from prefrontal circuitry to habit motor circuitry occurs, so drug use becomes less of a conscious decision, and an individual's ability to control their intake is further diminished. Indeed, this is inkeeping with findings from neuroimaging studies, hypofrontality having been identified as a strong indicator of reduced ability to regulate drug-seeking.[27] In individuals so affected, it seems, drug-seeking is now permitted to occur free of conscious intervention.

It is thus the case, as the above summary illustrates, that a substantial body of research now points to enduring changes in mesocorticolimbic circuitry in the established dependence syndrome. These data provide a neurocircuitry template for the primary features of addiction, and yield a neurobiological explanation for why a dependent individual experiences an excessive, uncontrolled responding for drug-related cues (clinically described as craving), but conversely experiences poor or inappropriate responding for more appropriate and biologically important stimuli (clinically manifest as neglect of alternative pleasures and interests). Consequently, an individual finds it very difficult to change damaging behaviours, and their substance misuse problem is perpetuated. I will now examine if it is indeed the case that people with schizophrenia are particularly susceptible to substance misuse problems.

Chapter 2

The extent of the problem of comorbidity and how an association between substance use and schizophrenia first came to be recognised

2.1 Prevalence of substance misuse in schizophrenia

It is generally regarded as an accepted truth in psychiatry that rates of substance misuse are particularly elevated among people with schizophrenia. In the ECA study, for example, it was estimated that 47% of patients with schizophrenia also had a lifetime diagnosis of substance abuse disorder.[32] As always with such accepted truths however, it is both prudent to be cautious in accepting its veracity and if genuinely present to question why such an association should exist. It is thus important to establish (1) if other studies also support the claim that there is an increased prevalence of substance misuse in schizophrenia, (2) if this increase exists independent of any potential bias that may be inherent in sampling methods, (3) if this increased propensity to use substances is not simply a general feature of major mental illness (rather than specific to schizophrenia itself), and (4) if this increased propensity to use substances arises not as a direct consequence of schizophrenia but as a consequence of other factors associated with the condition (i.e. the association exists as a consequence of confounding). Confounders which could be very relevant to the association between substance misuse and schizophrenia are factors such as poverty and long term unemployment. Of particular importance in investigating this association will be data from disparate cultures; if substance use is indeed a central feature of schizophrenia, then it would be expected to be observed across cultures. With these considerations in mind, I will consider the prevalence data for each of main groups of substances of interest in turn. Given the high profile that cannabis use in schizophrenia has received, it will be considered first in this section.

Prior to considering the data relating to the prevalence of substance misuse in schizophrenia an important issue of classification needs to be clarified. In much of the

data relating to this area of research both the specific diagnosis of schizophrenia and the less specific term 'psychosis' are frequently used. Often, as well as encompassing schizophrenia, the latter term also includes other schizophrenia spectrum psychoses and also the affective psychoses. In this report, when research refers specifically to schizophrenia then this term will be used; when the term 'psychoses' is used this means that the work being referred to relates to the broader concept.

2.1.1 Cannabis

Systematic reviews of the prevalence of cannabis use disorder in people with schizophrenia were published in 1990 and 2001 by Muesner and Cantor-Graae respectively.[33, 34] Both reviews noted that the studies included were markedly heterogeneous, and that this hindered interpretation. Nevertheless, they both drew some conclusions. Mueser concluded that a history of cannabis abuse was related to fewer symptoms of schizophrenia and fewer hospitalizations, suggesting more socially competent schizophrenia patients were more prone to cannabis use. On the basis of six reports, in addition to their own data, this study estimated that people with schizophrenia were no more likely to use cannabis than other psychiatric patients. According to Cantor-Graae *et al.*, alcohol was generally the most frequently used substance among schizophrenia patients, with cannabis use frequent among younger patients. Seventeen studies were reported as specifically ascertaining the proportion of patients with a (generally lifetime) history of cannabis abuse or dependence, the vast majority of these studies originating from the USA. Rates varied from 13.0% in a first admission German sample to 42% in an American inpatient sample. No comparison is

made with prevalence rates in either other psychiatric populations or the general population.

The first meta-analysis of this topic was published in April 2009 by Koskinen *et al.*[35] It focused on studies published from 1996 to January 2009. Only studies that ascertained the presence of cannabis abuse or dependence (fulfilling either International Classification of Diseases (ICD)[36] or DSM criteria[23] and collectively deemed cannabis use disorders, CUD) were included, with 'schizophrenia' being regarded as one of the DSM schizophrenia-spectrum diagnoses (schizophrenia, schizophreniform disorder, schizoaffective disorder, delusional disorder). At least 80% of the subjects in the study had to have a schizophrenia spectrum disorder diagnosis for the study to be included in the meta-analysis. Thirty-five studies met the inclusion criteria; as before the majority were from America or Europe, though a study from Turkey and one from Lebanon were also identified. The total number of cases was 5540. The total median rate of CUDs in schizophrenia was 27.0% (range = 0.0–65.6, 35 studies). The median rate of lifetime CUDs was 27.1% (IQR = 12.2–38.5, 28 studies) and that of current CUDs was 16.0% (IQR = 8.6–28.6, 10 studies). CUDs were more common in younger (<30 years) than older (>30 years) patient samples. Additionally this study compared CUD rates between first-episode and long-term schizophrenia patient samples. In samples other than first-episode patients, the minimum reported average duration of illness was 9 years, and all these studies were categorized as having long-term patient samples. In studies presenting results from first-episode samples, the median CUD rate was higher for these patients than for those with chronic illness (28.6% vs 22.0% for current and 44.4% vs 12.2% for lifetime diagnoses).

Some general features of CUDs were notable. Firstly, as well as being more common in younger and first-episode patient samples, they were also more common in samples with a high proportion of males. The CUD rate was however not affected by the study location (only Europe vs. North America was considered), classification system used (DSM-III-R vs. DSM-IV vs. ICD-10), or patient type (inpatient vs. outpatient). On the basis of a small subset of studies, schizophrenia patients with CUDs were reported to have more positive symptoms and fewer negative symptoms. A weakness of this meta-analysis is that it did not consider rates of cannabis use in comparison populations. Indeed, this was not practicable as most studies did not include a comparison group of appropriately matched controls from either the general population or other psychiatric patient groups. Instead, the authors compared prevalence rates reported in schizophrenia to those reported by the United Nations in the respective countries from which the samples arose (these mainly being in the age range 16-65 years).[36] Perhaps surprisingly, in these comparisons no consistent pattern emerges; in some samples of patients with schizophrenia prevalence of CUD is greater than the general population, while in others it is less. It is the case however that the UN and patient samples differ substantially in factors such as age and gender distribution. This makes such comparisons challenging, and (as the authors state), they should be interpreted with caution.

Update of systematic review

To augment the above reviews, a further literature search was undertaken with the aim of identifying relevant papers published after the period included in the review of Koshkinen *et al.* Comparably to that meta-analysis, the search terms included were ''schizophrenia,'' ''psychosis,'' ''psychoses,'' and ''psychotic'' to

locate studies on schizophrenic psychoses, as well as ''cannabis,'' "drug abuse" ''substance abuse,'' and ''dual diagnosis''. Searches were conducted in PsychInfo, Medline and EMBASE, with both free text and expanded subject headings being used. Only studies focusing on subjects over 16 years of age in which at least 80% of subjects had diagnoses of schizophrenia spectrum disorders (i.e. schizophrenia, schizoaffective disorder or delusional disorder) were included. Specific rates of use of cannabis had to be given, rather than simply rates of generic 'substance misuse'. Given that the meta-analysis of Koshkinen *et al.* included studies published up to the beginning of January 2009, the search was limited to reports published after this date. The above methodology identified 489 studies. On examination of abstracts 13 were potentially relevant and attempts made to obtain the full text report. A number of studies had to be excluded either because the subjects included were too young, [37] or the study included substantial numbers (>20%) of subjects with conditions other than schizophrenia spectrum disorders.[38-41] Four studies yielded useable additional data, of which only two included control groups; details of these are given in Table 2.1.

Study	Country, Study period	Diagnosis	Duration of schizophrenia (mean years)	Mean age (y) (SD)	Sample size (m/f)	Rate of cannabis use disorder (%)	Comments
Galderisi *et al.*, 2009[42]	13 European countries and Israel,	DSM-IV	First-episode psychosis	25.9 (5.6)	454 (273/181)	24.7 (cannabis use rather than abuse or dependence) In controls: 4.5, SD (P = .00001)	Prevalence rates compared to matched group of healthy controls *without* substance abuse/dependence diagnosis
Mufic *et al.*, 2009[43]	Croatia, 2004-2007	ICD-10	First-episode psychosis	24.9 (6.5)	58 (41/17)	8.7 (ICD-10 substance abuse at point of admission)	Included all subjects with first-episode psychosis; no further diagnostic breakdown is made
Ongur *et al.*, 2009[44]	USA,	DSM-IV	13.9 (SZ) 16.0 (SA)	37.7 (11.5)	80	22.5 in SZ, 32.3 in SA, (lifetime use disorder) In BPAD: 23.8. NSD	Prevalence rate in SZ and SA compared to that in BPAD
Turkington *et al.*, 2009[45]	Northern Ireland, 2003-2004	ICD-10	First-episode psychosis	No data	103	26.2 (abuse or dependence in year preceding initial presentation with psychosis)	Exclusively subjects with SZ-spectrum disorder

Table 2.1
Studies reporting prevalence of cannabis use or abuse/dependence in people with schizophrenia spectrum disorders published after 2008.
SZ: schizophrenia; SA: schizo-affective disorder; BPAD: bipolar affective disorder; SD: significant difference; NSD: no significant difference

A number of findings from these studies are notable. Firstly, rates of cannabis use in the Croatian study are rather lower than in most other reports, with only 8.7% reporting active cannabis abuse at the point of admission to hospital.[43] Conversely, a Northern Irish first episode study reported rates of cannabis abuse or dependence over the year preceding their presentation as 26.2% in those people diagnosed with schizophrenia.[45] This rate, together with that reported by Ongur *et al.*,[44] is broadly comparable with rates reported in the meta-analysis of Koshkinen *et al.* As Galderisi *et al.* reported on cannabis use rather than abuse/dependence their data are not directly comparable to Koshkinen *et al.*'s meta-analysis.[42]

In reviewing the studies outlined above, it is of course important to consider the potential implications of how the patient samples were derived. In these studies, (both the review of Koshkinen *et al.* and my supplementary review), the schizophrenia populations in which prevalence of cannabis use was ascertained were all patients in contact with services. It is possible that the prevalence of cannabis use in patients in contact with services differs from those who are not, and thus these estimates are not representative of the population with schizophrenia as a whole. If this were the case, more representative data would be expected to be obtained from population studies.

To address the above question Green *et al.* reviewed data from community population studies, comparing cannabis use and misuse prevalence estimates among individuals with and without psychosis.[46] They identified 6 such studies published between 1990 and 2002, a haul I was unable to supplement, being unable to identify any relevant studies published after this date. All of these studies showed higher odds of cannabis use or misuse for people with psychosis. Rates of use in the last twelve months in the psychosis groups ranged from 6.9 - 69.4%, while in the non-psychosis comparison groups were 2.5 - 50.6%. Rates of lifetime use ranged from 17.7 – 34.5% in the former group and 9.4 – 21.9% in the latter. In the discussion accompanying this report Green *et al.* underline the importance of demonstrating a higher prevalence of cannabis use in people with schizophrenia in population studies as well as treatment cohorts. Such findings increase confidence that the reports of elevated rates of cannabis use in treatment cohorts do not simply represent a sampling artefact; i.e. they demonstrate that the increased prevalence of cannabis use observed in psychosis is not

restricted to those in contact with services and thus available for study. An obvious proviso to these epidemiological studies is of course whether or not the relationships observed are simply a consequence of sociodemographic confounders. A number of the studies have controlled for these factors however. The findings of Degenhardt *et al.*, for example, (which admittedly did focus on level of psychotic symptoms rather than diagnoses of schizophrenia), were robust to the inclusion of demographic characteristics, personality and other mental health problems in the regression analysis.[47] It does thus seem to be the case that rates of cannabis use in psychosis are genuinely elevated over those in the general population, and that this increased risk is related to the mental illness itself.

Of fundamental importance in establishing if the increased risk of substance misuse is a feature specific to schizophrenia/psychosis, are studies comparing the prevalence of substance misuse in the condition to that in other major psychiatric disorders. Unfortunately, only a handful of studies have done this. Muesser *et al.* compared patients with schizophrenia, bipolar affective disorder or depression. They reported that diagnosis was not clearly related to type of substance misuse, with demographic variables such as gender, age and race being stronger predictors.[48] Patients with schizophrenia and bipolar affective disorder had similar rates of lifetime cannabis use disorder, a rate approximately double that seen in people with depression. Similarly, in a more recent study comparing bipolar affective disorder and schizophrenia, rates of cannabis use were similar in the two conditions.[49] Lifetime rates of cannabis abuse or dependence were also reported as similar in the two conditions by Ongur *et al.*[44] Conversely, a Lebanese study reported that cannabis use was more prevalent in those with schizophrenia compared to either bipolar affective disorder or depression.[50]

Summary

As is clear from the above, a large number of studies have examined the prevalence of cannabis use in schizophrenia, generally finding it substantially elevated compared to rates in the general population. Given this, it is perhaps surprising that so few studies have formally compared rates in those with schizophrenia to a comparable population with other major psychiatric conditions. The few studies that have attempted this have generally compared rates to bipolar affective disorder; generally rates have been similar in the two conditions. This is perhaps not surprising, bipolar affective disorder itself being generally recognised as having a strong association with substance misuse.[51] Studies compared rates of cannabis use/abuse in schizophrenia with rates in psychiatric conditions other than bipolar affective disorder are even rarer, but the limited data available does suggest rates are elevated in comparison to depression.[50]

In summary, it does seem to be the case that rates of cannabis use in schizophrenia are indeed elevated compared to the general population. This association has now been demonstrated in many different nations, though most studies have been undertaken in Western Europe or North America. Though this is at times regarded as a problem specific to schizophrenia however, it is also a prominent feature of bipolar affective disorder. Surprisingly, despite the large number of studies investigating the prevalence of cannabis use in schizophrenia, studies comparing this with the prevalence in what is likely the most appropriate comparator group (i.e. individuals with another major mental illness) are actually rather rare.

2.1.2 Alcohol

Koshkinen *et al.*, the group who undertook the meta-analysis addressing the prevalence of cannabis use/abuse in schizophrenia, also applied this methodology to ascertain the prevalence of alcohol use disorders in the condition. As with the cannabis meta-analysis, this built on the two earlier systematic reviews undertaken by Muesser *et al.* and Cantor-Graae *et al.* which had focused on the use of various substances by people with schizophrenia.[33, 34] The nine relevant studies identified by Muesser *et al.* reported lifetime rates of alcohol abuse or dependence rates ranging from 8.8 to 47%. Their interpretation of these data is that people with schizophrenia were no more likely to abuse alcohol than patients with other psychiatric disorders or general population controls. The more recent review of Cantor-Graae *et al.* identifies 25 relevant studies. They report that abuse rates are greater for alcohol than any other substance (tobacco is not considered), and report lifetime abuse rates ranging from 6.7 to 50.7%. When considered together with their own data, they believe rates of alcohol abuse to be elevated in schizophrenia compared to the general population.

The methodology of the Koshkinen *et al.* meta-analysis was essentially the same as the cannabis review. The only significant alteration was that cannabis-specific terms were altered to their alcohol-specific equivalents; e.g. 'cannabis abuse' changed to 'alcohol abuse'. As with the cannabis review it included studies published from 1996-2008.

Sixty studies met inclusion criteria for the meta-analysis. On the basis of these studies the median prevalence of alcohol use disorder (AUD, essentially fulfilling ICD or DSM diagnostic criteria for alcohol harmful use/abuse or dependence) either at any point in lifetime or currently was determined. The median of lifetime AUD

prevalence was 20.6% (IQR 12.0–34.5, 47 studies) while that of current AUD prevalence 9.4% (IQR 4.6–19.0, 18 studies). As was the case with the cannabis meta-analysis, there was a paucity of studies comparing the prevalence of alcohol abuse/dependence in people with schizophrenia to matched controls derived either from the general population or people with other psychiatric conditions. Indeed this was done in only one of the studies they identified, which reported that rates of both current alcohol abuse and dependence were approximately doubled in those with schizophrenia compared to the general population.[52] In lieu of these data, AUD prevalence in schizophrenia was compared with alcohol consumption in the countries in question as reported by WHO.[36] In general, in studies presenting results from countries with high consumption of alcohol, the prevalence of AUD in patients with schizophrenia was also higher than in studies from countries with lower alcohol consumption. Direct contrasts between AUD prevalence in people with schizophrenia and either the general population or those with other psychiatric disorders were not made.

Update of systematic review

As was the case with cannabis, the above reviews were augmented by a supplementary literature search, aimed of identifying relevant papers published after the period reviewed by Koshkinen *et al.* The search undertaken was identical to that for cannabis, with the exception that 'alcohol' was substituted for 'cannabis' in the search terms.

On examination of abstracts yielded by the above search, 12 potentially relevant studies were identified and the full text papers obtained. As previously, a number of studies had to be excluded either because the subjects included were too

young,[37] or prevalence figures included substantial numbers of subjects with conditions other than schizophrenia spectrum disorders.[39-41] Three of the four studies which augmented the cannabis review yielded useable additional data; details of these are given in Table 2.1. As can be seen, rates reported are comparable to those in the meta-analysis.

Study	**Country, Study period**	**Diagnosis**	**Duration of schizophrenia (mean years)**	**Mean age (y) (SD)**	**Sample size (m/f)**	**Rate of alcohol use disorder (%)**	**Comments**
Mufic *et al.*, 2009[43]	Croatia, 2004-2007	ICD-10	First-episode psychosis	24.9 (6.5)	58 (41/17)	10.3 (current abuse)	Included all subjects presenting with FEP
Turkington *et al.*, 2009[45]	Belfast, 2003-2004	DSM-IV	First-episode psychosis		103	49.8 (abuse or dependence in year preceding presentation with psychosis)	Only subjects with schizophrenia-spectrum disorder included
Ongur *et al.*, 2009[44]	USA,	DSM-IV	13.9	37.7 (11.5)	80	Lifetime use disorder: 27.5 in SZ, 52.3 in SA, In BPAD 38.6. NSD	Prevalence rate in SZ and SA compared to that in BPAD

Table 2.1
Studies reporting prevalence of alcohol use or abuse/dependence in people with schizophrenia spectrum disorders published after 2008.
SZ: schizophrenia; SA: schizo-affective disorder; BPAD: bipolar affective disorder

Overall studies identified with this supplementary search report rates of AUDs in schizophrenia which exceed those reported by Koshkinen *et al.*. This is particularly so in the Northern Irish study.[45]

Interpreting the data ascertaining rates of alcohol abuse/dependence use in people with schizophrenia

In their discussion Koshkinen *et al.* compared the rates of AUDs in schizophrenia in more recent studies to those observed in the earlier reviews. Overall their impression was that though AUDs in patients with schizophrenia remained

common, there may be a descending trend. The findings of my supplementary search do not however seem to support this.

Interestingly, when Koshkinen *et al.* compared findings from the cannabis and alcohol meta-analyses, rates of cannabis use disorders appear to be higher than alcohol use disorders. Specifically, median current and lifetime rates of the former are 16.0% and 27.1%, while that of the latter are 9.4% and 20.6%.[35] On considering this finding however, it does seem likely that the relative prevalence of the two conditions will be substantially affected by geographical location. In countries such as Scotland, for example, where alcohol problems are so common in the general population, it seems likely this will be reflected in particularly high rates of use of this drug by people with schizophrenia. Evaluation of specific studies does in fact provide support for this supposition. In countries with generally high rates of alcohol consumption (such as Northern Ireland or Scotland), the rates of alcohol abuse/dependence exceed that of cannabis use disorders.[45, 52] In populations in which alcohol use is generally less prevalent however the converse is true.[50, 53] These observations clearly underline the fact that substance misuse by people with schizophrenia does not occur in isolation from the prevailing patterns of substance misuse in a particular population.

As was the case with cannabis use, studies comparing rates of alcohol abuse/dependence in schizophrenia to that in other psychiatric conditions are particularly rare. Mueser reported similar rates of lifetime diagnoses of alcohol use disorder in schizophrenia, bipolar affective disorder and major depression.[33] A small number of studies have directly compared rates of alcohol abuse/dependence in schizophrenia and bipolar affective disorder; though rates are reported as greater in the latter condition, any difference is non-significant.[44, 49] Again, as was the case with

cannabis, this underlines that though rates of alcohol problems may be elevated in people with schizophrenia, this problem is not necessarily specific to this diagnosis.

2.1.3 Tobacco

Clinicians have long observed that people with schizophrenia are markedly prone to smoke tobacco, but prevalence estimates have varied widely. To facilitate accurate estimation of the prevalence of cigarette smoking in schizophrenia, de Leon *et al.* undertook a meta-analysis in 2005.[54] A primary aim of this meta-analysis was to enable comparison of prevalence rates across countries and cultures; thus the methodology aimed to include all possible studies from all over the world, with inclusion not being restricted to studies published in English.

A major strength of this review is that in each included study smoking rates in schizophrenia were incorporated into the meta-analysis as an odds ratio; the odds of smoking in schizophrenia relative to that in the general population (and, where data were available, relative to the odds in other major psychiatric conditions). As some articles provided smoking data from schizophrenia patients but did not provide comparable data from the general population, in these cases comparable data were obtained from independent (usually government) surveys. This means that the output from the meta-analysis is an odds ratio of worldwide smoking rates in schizophrenia relative to that in population controls (or, alternatively, non-schizophrenic people with a major mental illness). This odds ratio is more meaningful than a simple estimation of prevalence.

The review process identified 42 samples from 20 different nations, investigating a total of 7593 schizophrenia patients (62% of whom were current smokers). The weighted average of the ORs comparing prevalences for schizophrenia patients versus the general population was 5.3 (CI, 4.9–5.7). This suggests that the odds that patients with schizophrenia are current smokers are 5.3 times higher than people from the worldwide general population. The association between schizophrenia and current smoking remained after using severe mentally ill controls (18 studies across 9 countries, weighted average OR was 1.9, CI 1.7–2.1). The severely ill control group was heterogeneous and varied between studies, but patients with affective disorders generally made up the largest single component of it.

I updated the study discussed above by searching for studies investigating the prevalence of smoking in schizophrenia published after 2004 (and thus not included in the above meta-analysis). The methodology employed was comparable to the approach taken for the searches supplementing the cannabis and alcohol meta-analyses, with the substitution of the search terms 'cigarette', 'tobacco smoking' OR 'smoking' for those substances.

The above approach yielded 924 potentially relevant studies, of which on examination of abstracts 34 were potentially relevant, and attempts were made to retrieve full text articles. One of these was unobtainable,[55] the main reason for exclusion of the others was that the recruitment methods meant that the sample may be unrepresentative of the population with schizophrenia as a whole e.g.[56, 57] Nine studies provided additional usable information which could potentially augment the de Leon *et al.* meta-analysis. These are detailed in Table 2.2 below. Data from these studies will be considered together with findings from the aforementioned meta-analysis.

Study	Country, period	Diagnosis	Duration of schizophrenia in mean years (SD)	Mean age (y) (SD)	Sample size (m/f, if given)	Rate of tobacco smoking in SZ and (if included) comparator population (%)	Comments
Bernardo *et al.*, 2009[58]	Spain, 2004-2005	DSM-IV	12.7	37.8 (11.3)	526/ 200	69.8	Patients were consecutive psychiatric admissions
Birkenaes *et al.*, 2007[59]	Norway, 2002-2005	DSM-IV	7.1 (8.8)	33.6 (10.3)	94/69	54.9 General population: 27.7, (SD, $P < .001$) BPAD: 50.0, NSD	Patients identified on basis of being in contact with mental health services. Compared to controls from same geographical area.
Campo-Arias *et al.*, 2006[60]	Colombia,	DSM-IV	No data	42.2 (10.8)	40/33	26.0 Matched controls: 10.0, (OR=3.1, 95% CI, 1.4–6.8) Mood disorder: 7.0, (OR=4.3, 95% CI, 1.6–11.9)	Patients recruited from outpatient clinics. Matched population control group and unmatched mood disorder comparator group
Galderisi *et al.*, 2009[42]	13 European countries and Israel,	DSM-IV	First-episode psychosis	25.9 (5.6)	273/181	54.4 Matched healthy controls: 32.7, (SD, $P < .000001$)	Prevalence rates compared to matched healthy control group without substance abuse/dependence diagnosis (excluding tobacco)
Gurpegui *et al.*, 2005[61]	Spain	DSM-IV	14.2	36.1 (9.5)	195/55	69.0 General population:35.0, (OR=4.3; CI, 3.0-6.1)	Patients recruited from outpatient clinics. General population controls, without mental illness
Harrison *et al.*, 2007[62]	London	DSM-IV	First episode	25.3	110/42	60.0	Most recruited at point of first admission to hospital. No control group
Kavanagh *et al.*, 2004[63]	Australia, 1997-1998	DSM-III-R	First episode	Not given	430	74.4 (current or prior tobacco use)	Epidemiological sample of people in contact with psychiatric services
Laqueille *et al.*, 2008[64]	Tunisia	DSM-IV	13.9	38.4 (8.3)	74	79.7 General population (male): 48.0	Random selection from sequential attendees at outpatient clinic
Ucok *et al.*, 2004[65]	Turkey	DSM-IV	No data	30.9 (7.9)	39/27	57.5 Healthy controls: 47.3, NSD Bipolar disorder: 55.1, NSD	Consecutive presentations to outpatient and inpatient services. General population and bipolar controls

Table 2.2
Studies investigating the prevalence of smoking in schizophrenia published after the de Leon *et al.* review.
Unless the control group is specifically described studies were uncontrolled.
Period during which data were collected is included if this is reported in study

Interpreting the data ascertaining rates of tobacco use in people with schizophrenia

An important outcome of the meta-analysis of de Leon *et al.* was to yield a global odds ratio, quantifying the strength of the association between smoking and schizophrenia when these individuals are compared to general population controls. This was convincingly achieved, people with schizophrenia being demonstrated to be at a hugely elevated risk of smoking. This strong association between schizophrenia and smoking is further illustrated by the additional studies detailed in Table 2.2; on

considering these supplementary data, only in Turkey did the smoking rate in people with schizophrenia not exceed that in the general population.[65]

It is the case however that a specific objective of the meta-analysis, (and my subsequent review), was also to compare data from across cultures. As discussed by de Leon *et al.*, by this means the hypothesis that the association between schizophrenia and tobacco smoking is relatively independent of sociocultural factors could be further explored. This being the case, even in the context of generally consistent findings overall, any notably anomalous studies do need to be examined a little more carefully. While these may of course simply represent chance findings, they may potentially offer important insights in to the relationship between smoking and schizophrenia in particular social contexts.

Firstly the potential explanations for the anomalous negative Turkish result, which demonstrated no increase in the prevalence of smoking in people with schizophrenia when compared to the general population, must be considered. It may indeed represent a chance finding, or be consequent to a ceiling effect arising from the very high rate of smoking in the population in general in Turkey, or reflect anomalous features of the control group (drawn as they were from the relatives of neurology outpatients).

A number of other studies included in de Leon *et al.*'s meta-analysis also reported negative findings however, and these must also be considered. Specifically, three Columbian (in contrast to the Columbian study of Campo-Arias *et al.*, above, in which the difference was smaller in magnitude than in other studies but still significant[60]) and one Indian study included in the meta-analysis also reported that rates of smoking were not increased in those with schizophrenia compared to the general population. In contrast to the Turkish study, these non-significant findings are

in the context of populations which have a very low prevalence of tobacco use. Clearly the explanation of a ceiling effect does not apply here, and nor did the comparator groups have any obviously unusual characteristic. The explanation for these apparent anomalous results seems different, and likely stems from the fact that the degree of drug accessibility will have a major influence on smoking prevalence. Thus, even if the association between schizophrenia and smoking were *entirely* biologically (rather than socio-culturally) determined, restriction in access to tobacco would still impact on the manifestation of this association in some countries and/or societies. As an example, if a society is very poor then very few people will be able to afford to buy cigarettes, and consequently very few people will ever be exposed to tobacco. If they never try tobacco they can never become a smokers, and thus the association between schizophrenia and tobacco use cannot become manifest in that society. Similarly, if it is not socially acceptable for women to smoke then they will be unlikely to ever be exposed to the drug, and thus the association between schizophrenia and smoking will not be observed in that subset of the population. The lack of smoking by people with schizophrenia in these circumstances does not refute the association between schizophrenia and tobacco smoking; it only proves economical and social factors may be a major deterrent to nicotine use initiation and consequent addiction. Thus, on considering these issues, the Columbian and Indian reports are in fact not inconsistent with there being a strong association between schizophrenia and tobacco smoking.

A further observation made in the meta-analysis is that, (though the increase is substantially less than when compared to the general population), smoking rates in schizophrenia are elevated relative to that seen in people with other severe mental

disorders. Specifically, 14 of the 18 studies included in the meta-analysis that make this comparison report an elevated rate of smoking in the former group.[54] People with other major mental illness share many of the potential confounding factors exhibited by people with schizophrenia (e.g. institutionalisation, high levels of unemployment), so these data are particularly important in making the case that an elevated vulnerability to smoking is a characteristic directly attributable to this condition. It has also been argued however that the association between schizophrenia and smoking may be a specific consequence of antipsychotic treatment, an exposure which would be expected to be greater in people with schizophrenia even than in those with other major mental illnesses. It is the case however that in the small number of studies controlling for this (and other potential confounders such as race, use of other substances etc), the association between schizophrenia and smoking did remain (3 studies, adjusted ORs ranged 2–3).[66-68]

Some of the studies in which differences in prevalence of tobacco use between schizophrenia and controls are least apparent are those in which the latter group is made up of people with bipolar affective disorder.[59, 65, 69] Thus, though it is clear that smoking rates in schizophrenia are elevated above those in the general population, and likely also in the generality of people with major psychiatric disorders, any elevation above that in bipolar affective disorder is likely considerably more modest, if indeed it is present at all. Clearly exploration of the reasons why bipolar affective disorder may have a strong association with tobacco use is an important topic, but beyond the scope of this review. I will however further explore the potential reasons for an association between schizophrenia and tobacco use in Section 3.2.1.3.

2.1.4 Other drugs

Process by which stimulants were recognised as having a relationship with psychosis

The other class of drugs which have been long associated with psychosis are stimulants. Though this group is now loosely regarded as containing amphetamine, cocaine and MDMA, it was with amphetamine that this association was initially observed. This drug was first synthesised by Lazar Edeleanu in Berlin in 1887.[70] By the 1930s it was being marketed as a treatment for narcolepsy and a decongestant, but as well as addressing these conditions was also noted to result in general psychological stimulation, increased confidence and a degree of euphoria.[71] A potential for abuse was also noted, as was a tendency for it to worsen the somatic features of anxiety. Nevertheless, it was widely promoted as an antidepressant in the USA in the 1930s and 40s.[72] Additionally, it and methamphetamine, (a related drug synthesised in Japan in 1918), were widely used by British, German and Japanese troops during the Second World War to combat fatigue. It was during this period of military application that reports of amphetamine/methamphetamine induced delusions began to emerge.[73] Indeed, such reports together with increasing concerns about addictive potential prompted the Germans to limit availability of the drug. Further cases of stimulant associated psychosis subsequently arose, culminating in Philip Connell's 1958 monograph in which he reviewed cases of stimulant psychosis, noting that they tended to resolve rapidly on discontinuation of the drug.[74] He also observed that the psychopathology of this psychosis closely resembled paranoid schizophrenia, an observation soon confirmed by other researchers.[75, 76]

Given the apparent psychotomimetic effect of this drug class, their use by people with schizophrenia would be expected to have detrimental consequences.

Clearly this makes ascertainment of the extent of use of these drugs by people with the condition important to ascertain. Thus, though other issues such as *how* stimulant use can give rise to psychosis, and the *consequences* of stimulant use for prognosis of schizophrenia are clearly important, I will first try to clarify the extent of their use by people with schizophrenia.

Prevalence of use of amphetamines and other substances in schizophrenia

In 1995, LeDuc and Mittelman undertook a systematic review of the prevalence of stimulant use in schizophrenia. It covered articles published between 1975 and 1994, and focused on amphetamine and cocaine (including crack cocaine).[77] They included studies which ascertained rate of use of either amphetamine or cocaine, or the combined rate of use of either drug. Only studies that classified patients' psychosis according to diagnostic criteria in the DSM-III, DSM-III-R, or RDC, including only patients diagnosed within the schizophrenia spectrum (i.e., schizophrenia, schizoaffective disorder, schizophreniform disorder, or schizotypal personality disorder) were included. As was the case in the reviews of Koshkinen *et al.*, studies of subjects selected on the basis of being substance misusers were excluded. The definition of substance misuse itself varied between studies. While in a substantial proportion it was a requirement to fulfil DSM criteria for abuse or dependence, in others criteria were more variable; in some instances it was regarded as, for example, two consecutive weeks of use or positive urinalysis.

The review of LeDuc and Mittelman identified 20 studies fulfilling their inclusion criteria, yielding a total of 1512 patients. They all originated from North America, with 19 of the 20 studies arising from the USA. Thirteen of these studies focussed on 'stimulants' in general, six on cocaine, and one on amphetamines. The

rate of psychostimulant abuse in these identified studies averaged 26.5 %; as described above, the term 'abuse' had a rather broad interpretation. It is acknowledged that age is a significant factor influencing drug abuse,[78] and the average age of the subjects with schizophrenia included in this review was 33. Rates of psychostimulant abuse in schizophrenics were thus compared to rates reported in American subjects over 26 years of age in The National Household Survey on Drug Abuse (NIDA 1991);[79] at the time of the study this had last been conducted in 1990. This indicated that the incidence of psychostimulant abuse in schizophrenics was 2-5 times higher than that of the American general public. The researchers also compared rates of stimulant use in people with schizophrenia spectrum disorders to those with affective disorders. Rates of recent stimulant use in patients with schizophrenia spectrum disorders were significantly elevated when compared to rates in the 160 identified patients with a primary diagnosis of affective disorders (in whom the rate of recent use was 20%, $P < .01$).

The researchers also examined trends in levels of stimulant use with increasing age. They noted that unlike the decline in use that was being seen with increasing age in adults in the American general population, high rates of abuse appear to be maintained in older schizophrenics.

Update of systematic review

Though in recent years North America has experienced an epidemic of amphetamine use (in the particularly potent form of methamphetamine),[80] this review has not been updated since initial publication. Given the close relationship between amphetamine and psychosis, such an update is clearly important. Not least, it is essential in providing a context from which to consider the potential significance of

any brain structural abnormalities that may be associated with use of amphetamine in the study population. It is also the case however, that since the review of LeDuc and Mittelman cocaine use has also become more widespread in Europe, and use of a new drug with stimulant properties, MDMA (or ecstasy), has also become commonplace.[81] Thus, in addition to amphetamine and cocaine, the following review will also identify studies which consider the prevalence of use of ecstasy by people with schizophrenia.

In order to maximise identification of articles reporting the prevalence of amphetamine and/or cocaine use (or the use disorders of abuse or dependence) in patients with schizophrenia published in 1994–2010, we conducted a search using three electronic databases (PsycINFO, Medline and EMBASE). The keywords used were schizophrenia OR psychosis to find studies on schizophrenic psychoses, combined with amphetamine, stimulants OR cocaine. At the same time a similar search was performed substituting the latter search terms for MDMA or ecstasy.

The searches outlined above were augmented by a further search employing more general terms (substance use OR dual diagnosis combined with schizophrenia OR psychosis), this methodology being comparable to that employed in the searches undertaken for alcohol and cannabis.

The inclusion criteria for studies were comparable to those employed by Koshkinen *et al.* in their recent meta-analyses of alcohol and cannabis use in schizophrenia. These were that: the study reported information on the prevalence of substance of interest (i.e. amphetamine, cocaine, or MDMA) use or abuse/ dependence; the sample consisted (at least 80%) of individuals with schizophrenia spectrum diagnosis (schizophrenia, schizophreniform disorder, schizoaffective disorder and delusional disorder) or articles reported the prevalence rates by diagnostic group (in samples that were less than 80% schizophrenia spectrum); the

subjects were older than 16 years of age; and that the study sample was larger than 15. Only articles reporting schizophrenia spectrum disorders and specifying that they were *either* diagnosing substance abuse or dependence according to DSM or ICD diagnostic system criteria *or* simply reporting that they determined rates of exposure were included. We excluded studies with samples that might have biased the presented prevalence of substance use in the study, e.g. samples recruited from prisons, forensic psychiatry units or shelters for the homeless, or studies including only subjects on depot preparations (as substance misuse is so strongly associated with non-compliance). Trials and intervention studies were also excluded. Where the same sample of subjects appears to have been included in more than one publication, only data from the publication with the largest sample size was included. Only articles written in English were included.

On applying the above methodology the first search (aiming to identify studies investigating the prevalence of amphetamine and/or cocaine use) yielded 2924 articles, of which 99 were evaluated in detail after analysing the abstracts. The second, (aimed at identifying studies ascertaining the prevalence of ecstasy use), yielded 251 articles, of which 23 were retrieved in full text form. The more general search, designed to augment the previous two searches, yielded 2150 articles, of which 68 were retrieved in full text form.

Data from studies identified that fulfilled these inclusion criteria are presented in the tables below. In contrast to the review of LeDuc and Mittelman (in which rates of amphetamine or cocaine use were generally combined), these data are presented separately for each substance of interest. As determined by the inclusion criteria employed, studies which ascertain combined levels of use of both amphetamine and cocaine have not been included. Unless specified, prevalence of substance use has

been ascertained by self-report. For each substance a distinction has been made between studies in which a diagnosis of substance abuse or the dependence syndrome is made and studies simply reporting a history of use. Studies ascertaining either of the former are displayed separate from those simply reporting a history of use.

Four studies were identified which were published during the period of interest and reported rates of amphetamine abuse or dependence in schizophrenia (Tables 2.3). Five studies reported rates of cocaine abuse or dependence (Table 2.5), while none reported rates of ecstasy abuse or dependence. Generally studies more commonly reported any level of use rather than abuse or the dependence syndrome, this being ascertained either for any point previously or in a defined period preceding the study. Eight studies reported any use of amphetamines (Table 2.4), 10 any use of cocaine (Table 2.6) and four any use of ecstasy (Table 2.7).

Study	Country, Study period (or publication date)	Diagnosis	Duration of schizophrenia (mean years)	Mean age (y) (SD)	Sample size (m/f)	Rate of amphetamine use disorder (%)	Control group? If so, rate in it	Comments
Addington and Addington[82]	Canada, Calgary (2007)	DSM-IV	First episode psychosis	25.0 (8.4)	203 (approx 142/61)	0.5 (current abuse or dependence)	No	Rate of use at point of entry to EPS in people who were followed up at one year
DeQuardo *et al.* [83]	USA, Michigan 1987-1990	DSM-IIIR	ND	28.7 (8.6)	42/25	3.0 (current abuse)	No	Admissions to inpatient unit
Fowler *et al.* [84]	Australia, (1998)	DSM-III-R	ND	36.3	141/53	2.0 (current abuse or dependence)	No	Outpatients with established schizophrenia
Margolese *et al.* [85]	Canada, Montreal, (2004)	DSM-IV	ND	38.8	120/87	0.5 (current abuse or dependence)	No	Outpatients with established schizophrenia

Table 2.3
Studies reporting current prevalence of amphetamine abuse or dependence syndromes in people with schizophrenia
ND: no data; EPS: Early Psychosis Service

Study	Country, Study period	Diagnosis	Duration of schizophrenia (mean years)	Mean age (y) (SD)	Sample size (m/f)	History of amphetamine use (%)	Control group? If so, rate in it	Comments
Archie *et al.*,[86]	Canada, Ontario, 2001-2003	DSM-IV	First episode psychosis	Approx 24.0	Ratio approx 3:1	6.0 (any lifetime use)	8.0 (any lifetime use) NSD	Comparison is age adjusted general population prevalence rate from Canadian Addiction Survey
Cantor-Grae *et al.*,[87]	Sweden, Malmo, 1998	DSM-IV	21.0 (11.6)	48.0 (13.2)	54/33	6.9 (lifetime abuse or dependence)	No	Successive psychiatric presentations with a diagnosis of schizophrenia
Condren *et al.*,[88]	Ireland, Dublin (2001)	ICD-10	ND	45.2	60/39	13.0 (use in last 30 days)	From GP practices in same catchment 9.0; NSD	Successive presentations with established schizophrenia
Fowler *et al.*,[84]	Australia, (1998)	DSM-III-R	ND	36.3	141/53	20.6 (any lifetime use)	No	Outpatients with established schizophrenia
Hambrecht *et al.*,[89]	Germany, 1987-1989	ICD-9	First episode schizo-phrenia	No data	No data	4.0 (used more than once a week for at least a month)	No	Admissions to hospital
Laqueille *et al.*[90]	Tunisia, Tunis, (2008)	DSM-IV	13.9	38.4 (8.3)	370	0.0 (lifetime use)	No	Consecutive outpatients with established schizophrenia
Margolese *et al.*,[85]	Canada, Montreal, (2004)	DSM-IV	ND	38.8	120/87	0.5 (any use in last month)	No	Outpatients with established schizophrenia
Mueser *et al.*,[91]	USA, New Hampshire, (2000)	DSM-II-R	ND	ND	173	3.5 (lifetime diagnosis of abuse or dependence)	7 (bipolar patients) 2 (major depression) NSD	Both schizophrenia subjects and comparators were hospitalised patients

Table 2.4
Studies reporting any history of use of amphetamines in patients with schizophrenia
ND: no data; NSD: no significant difference

Study	Country, Study period	Diagnosis	Duration of schizophrenia (mean years)	Mean age (y) (SD)	Sample size (m/f)	Rate of cocaine use disorder (%)	Control group? If so, rate in it	Comments
Addington and Addington[82]	Canada, Calgary, (2007)	DSM-IV	First episode psychosis	25.0 (8.4)	203 (approx 142/61)	0 (current abuse or dependence)	No	Rate of use at point of entry to EPS in people who were followed up at one year
DeQuardo *et al.*,[83]	USA, 1987-1990	DSM-IIIR	ND	28.7 (8.6)	42/25	4.5 (current abuse)	No	Admissions to inpatient unit
Fowler *et al.*,[84]	Australia, (1998)	DSM-III-R	ND	36.3	141/53	0 (current abuse or dependence)	No	Outpatients with established schizophrenia
Margolese *et al.*,[85]	Canada, Montreal, (2004)	DSM-IV	ND	38.8	120/87	2.9 (current abuse or dependence)	No	Outpatients with established schizophrenia
Pencer *et al.*[92]	Canada,	DSM-IV	First episode	24.2 (7.9)	175/91	2.0 (current abuse or dependence)	No	Enrolled on to EPS

Table 2.5
Studies reporting prevalence of current cocaine abuse or dependence syndromes in people with schizophrenia
ND: no data; EPS: Early Psychosis Service

Study	Country, Study period	Diagnosis	Duration of schizophrenia (mean years)	Mean age (y) (SD)	Sample size (m/f)	Rate of cocaine use (%)	Control group? If so, rate in it	Comments
Archie *et al.* [86]	Canada, Ontario 2001-2003	DSM-IV	First episode psychosis	Approx 24.0	Ratio approx 3:1	19.7 (any lifetime use)	14.0 (any lifetime use) SD P=.0001	Comparison is age adjusted general population prevalence rate from Canadian Addiction Survey
Cantor-Grae *et al.* [34]	Sweden, Malmo, 1998	DSM-IV	21.0 (11.6)	48.0 (13.2)	54/33	0.0 (lifetime abuse or dependence)	No	Successive psychiatric presentations with a diagnosis of schizophrenia
Condren *et al.*, [88]	Ireland, Dublin (2001)	ICD-10	ND	45.2	60/39	11.0 (use in last 30 days)	From GP practices in same catchment 15.0; NSD	Successive presentations with established schizophrenia
Fowler *et al.*, [84]	Australia, (1998)	DSM-III-R	ND	36.3	141/53	13.9	No	Outpatients with established schizophrenia
Hambrecht *et al.*, [89]	Germany, 1987-1989	ICD-9	First episode schizo-phrenia	No data	No data	5.0 (used more than once a week for at least a month)	No	First admissions with schizophrenia
Kamali *et al.* [93]	Ireland, Dublin, (2000)	DSM-IV	15.1 (12.0)	38.4 (12.2)	68/34	1.0 (lifetime diagnosis of DSM-IV abuse or dependence)	No	Consecutive re-admissions with psychosis
Laqueille *et al.* [90]	Tunisia, Tunis, (2008)	DSM-IV	13.9	38.4 (8.3)	370	0.0 (lifetime use)	No	Consecutive outpatients
Margolese *et al.*, [85]	Canada, Montreal, (2004)	DSM-IV	ND	38.8	120/87	3.9 (any use in last month)	No	Outpatients with established schizophrenia
McPhilips *et al.*, [94]	UK, London, 1997	ICD-9	ND	Approx 33	36	8 (use in last month)	No	Outpatients. Substance misuse detected by hair analysis
Mueser *et al.*, [91]	USA, New Hampshire, (2000)	DSM-II-R	ND	ND	173	9.8 (lifetime diagnosis of abuse or dependence)	8 (bipolar) 12 (major dep.) NSD	Both schizophrenia subjects and comparators were hospitalised patients

Table 2.6
Studies reporting prevalence of any history of cocaine use in people with schizophrenia
ND: no data; NSD: no significant difference

Study	Country, Study period	Diagnosis	Duration of schizophrenia (mean years)	Mean age (y) (SD)	Sample size (m/f)	Rate of MDMA use (%)	Control group? If so, rate in it (%)	Comments
Barnes *et al.*, [95]	UK, London, (2006)	DSM-IV	First episode	No data	110/42	32.9 (lifetime use)	No	Presentations to FEP service
Condren *et al.*, [88]	Ireland, Dublin (2001)	ICD-10	ND	45.2	60/39	4.0 (use in last 30 days)	Controls, same GP catchment: 20.0 SD <.001	Successive presentations with established schizophrenia
Duke *et al.*, [96]	UK, London, 1990	ICD-9	ND	50.3	181/156	0.8 (lifetime use)	No	All patients in catchment with schizophrenia
Harrison *et al.*	UK, London, 2008	DSM-IV	First episode	25.3	110/42	37.0 (lifetime use)	No	Recruited at point of first admission to hospital
Kamali *et al.* [93]	Ireland, Dublin, 2000	DSM-IV	15.1 (12.0)	38.4 (12.2)	68/34	2.0 (lifetime DSM-IV abuse or dependence)	No	Consecutive asmissions with psychosis

Table 2.7
Studies reporting the prevalence of any history of ecstasy use in people with schizophrenia
ND: no data; SD: significant difference

In addition to the studies reported above, Barnes *et al.* and Duke *et al.* reported on combined lifetime use of either amphetamine and/or cocaine in two London-based studies.[95, 97] Duke *et al.* reported in 2001 a lifetime history of use of either (or both) of 8.7%, which rose to 23.9% in those aged under 36. In a 2006 first episode study Barnes *et al.* reported a history of use in 37.7%. These rates, focusing as they do on general stimulant use, are similar to the rates reported in comparable age groups in the review of LeDuc *et al.* It must be noted however that the criteria for abuse in the latter study was more stringent than the 'any exposure' criteria applied in these reports.

Interpreting the data ascertaining rates of use of other substances by people with schizophrenia

To summarise the findings relating to use of either amphetamines or cocaine, this updated review reports a prevalence of current abuse of or dependence on amphetamine of between 0.5% and 3.0% on the basis of four studies, and current abuse of or dependence on cocaine of between 0% and 4.6% on the basis of five studies. As would be expected, studies reporting any history of use of the above drugs report consistently higher rates. Levels of exposure are however greatly influenced by geographical location; this is illustrated by the fact that reported rates of lifetime exposure to amphetamines ranged from 0% in Tunisia to 20.6% in an Australian study,[64, 98] while cocaine exposure ranged from 0% in both a Swedish and Tunisian study to 19.7% in a Canadian one.[34, 64, 99] It is notable that even on combining the rates of both amphetamine and cocaine abuse/dependence in these more recent studies, the rates observed in these studies fall far short of the rates of stimulant abuse reported by Le Duc *et al.* It is only when rates of lifetime exposure are considered, a level of use of substantially lesser magnitude than even the broadest interpretation of abuse

permitted in the studies included in the report of Le Duc *et al.*, that the quoted rates approach those reported in the earlier study. While this could be interpreted as implying that the prevalence of stimulant use had fallen in people with schizophrenia, I believe there is insufficient evidence for such a conclusion. Rather, this finding may well once again illustrate the importance of geographical location in influencing the findings of a study. Thus, the fact that only two of the studies identified in the period following the Le Duc *et al.* review are from the USA (and that one originates from a rural area) may provide the explanation for the lesser rates observed in these studies. Indeed, such a theory is given some weight by the findings of recent studies undertaken in Canada and Australia.[98, 99] Similarly to the USA, stimulant use is relatively common in these geographical locations; in-keeping with this it is in these studies that the prevalence of stimulant use most closely approaches that reported by Le Duc *et al.*.

Only three of the studies identified in this systematic review compared the rates of stimulant use in people with schizophrenia to that in control populations.[91, 99, 100]Each of these studies were concerned with stimulant use (rather than abuse or dependence), and in each study separate comparisons were made for both amphetamines and cocaine. In a Canadian study the rate of lifetime exposure to cocaine was higher in people with schizophrenia than the age adjusted general population prevalence.[99] This however was the only significant difference observed. When an Irish study compared cocaine use in the last 30 days by people with schizophrenia to that in a control group obtained from the same general practices, for example, no significant difference was observed (rates of use actually being non-significantly elevated in the latter group).[100] In the rural American study rates of cocaine abuse were similar in those with schizophrenia and those with both bipolar

affective disorder and major depression.[91] No significant difference in rates of amphetamine exposure was observed between schizophrenic and control groups in the Irish or Canadian study,[99, 100] and levels of amphetamine use were actually (non-significantly) higher in those with a bipolar illness than in schizophrenia in the rural American study.[91] It may thus be the case that the elevated rates of stimulant use in people with schizophrenia (when compared to the general population) seen in the urban American environment is less apparent in other contexts. As was seen with other substances, the inflated level of stimulant use in schizophrenia is even less marked when the comparator group also has a major mental illness.

This is the first systematic review of levels of MDMA (ecstasy) use by people with schizophrenia. Given the chemical similarities between this drug and amphetamine however,[101] it is possible that some earlier studies (which may have been included in the review of LeDuc *et al.*) classified exposure to this drug together with amphetamine use.

No studies were identified in which prevalence of abuse of or dependence on MDMA was ascertained. Four studies were identified however in which the prevalence of some level of lifetime exposure to MDMA was ascertained. Of particular note are two of these studies which were undertaken in London with data gathering separated by many years. The data of Duke *et al.* were obtained in 1990; while the year of data gathering is not specified in the study of Barnes *et al.*, it was likely some time later as this paper was published in 2006. The rate of MDMA exposure reported in these two studies is very different. The earlier study reports that only 0.8% of schizophrenic patients had ever been exposed to MDMA, while that of Barnes *et al.* reports a rate of exposure of 32.9%. The rise in levels of exposure

between these two studies is of course dramatic, and has occurred in the context of a rise in use in the general population. Neither of these studies included a control group. It is the case however that the British Crime Survey reported in 2004/2005 that 10.8% of 16-24 year olds reported ever having used ecstasy in their lifetime;[11] this does indicate that (in Britain at least), rates of historic ecstasy use in those with schizophrenia are elevated.

Summary

In summary, it is notable from this review that in the 15 years following the paper by LeDuc *et al.*, studies ascertaining the levels of use of stimulants by people with schizophrenia have actually been relatively few. Of particular interest, only one was identified which had been undertaken in the USA, the country from which 19 of the 20 studies included in the review of LeDuc *et al.* originated. This indicates that interest in stimulant use in schizophrenia may have waned since the 1980s/early 1990s, a fact which may be attributable to the increased interest in the relationship between cannabis use and psychosis which has occurred during this period. It is clear however that use of stimulant drugs by people with schizophrenia remains common, though the degree to which this is elevated above that by people from comparable socioeconomic groups is far from clear. Additionally, it seems to be the case that in recent years there has been a considerable rise in the number of people with schizophrenia who have used ecstasy. In the case of both stimulants and ecstasy substantial evidence has accumulated of adverse psychiatric sequelae secondary to use.[76, 102] It is thus important, as has been established in this chapter, to have a clear idea of the prevalence of such exposures in the psychiatric population. It is also important of course to consider if specific psychiatric consequences would be

expected to occur in the population who have/are vulnerable to schizophrenia on exposure to drugs of abuse, and this will be considered in the next chapter. Prior to this however, the association between psychosis and stimulants must be considered in more detail. This is necessary as, as was alluded to in the opening paragraph of this section, the observation that stimulant use could result in psychosis was a seminal event underpinning the first conceptualisations of the dopamine hypothesis of schizophrenia. The reasons for an association between more general drug use and schizophrenia will be discussed later.

2.2 The association between stimulants and psychosis

As mentioned above, it has been recognised since the 1940s that use of amphetamines could precipitate psychosis in previously unaffected people. By the 1970s the work of various groups had elucidated that amphetamines act by releasing catecholamines, and a role for amphetamine in inducing the release of dopamine in the central nervous system had been suggested, (reviewed in Sulzer *et al.*[70]).

In parallel with these observations advances were also occurring in the understanding of how neuroleptic drugs produced their antipsychotic effect. Dopamine was first implicated as important in the actions of these drugs by the seminal work of Carlsson and Lindqvit, who reported in 1963 that antipsychotics increased the metabolism of dopamine when administered to animals.[103] The centrality of dopamine to psychosis became fully apparent in the 1970s, with the finding that the clinical effectiveness of antipsychotic drugs was directly related to their affinity for dopamine receptors.[104]

It was around these two strands of data that the dopamine hypothesis of schizophrenia crystallised. In its initial formulation this essentially stated that it was an excess of transmission at dopamine receptors that was responsible for the genesis of psychotic symptoms.[105] Clearly this is in-keeping with the facts both that stimulation of dopamine release by amphetamines resulted in psychotic symptoms, and dopamine blockade by antipsychotics attenuates them.

Though the original formulation of the dopamine hypothesis remains recognisable, modifications have been made as our understanding of the brain abnormalities in schizophrenia has increased. One such reformulation was made by Davis *et al.* in 1991,[106] following the publication of PET data demonstrating reduced

cerebral blood flow in the frontal cortex in schizophrenia. This enabled him to incorporate regional specificity in to the dopamine hypothesis, emphasising the importance of 'hypofrontality'. This hypofrontality was directly correlated with low CSF dopamine metabolite levels, leading to the suggestion that it indicated low frontal dopamine levels. Thus, this modified version of the dopamine hypothesis involved a shift from a global hyperdopaminergia explaining all facets of schizophrenia to regionally specific abnormalities involving prefrontal hypodopaminergia and a subcortical hyperdopaminergia. Clearly this subcortical hyperdopaminergia could still be driven by amphetamines, meaning the production of psychotic symptoms by these drugs was still compatible with this hypothesis.

Progressive advances in neuroimaging have further informed the dopamine hypothesis. Molecular imaging studies have confirmed that presynaptic striatal dopaminergic function is elevated in patients with schizophrenia and reported that this correlates with the symptom dimension of paranoia.[107, 108] It has also been demonstrated that people with schizophrenia exhibit enhanced striatal dopamine release following amphetamine challenge, and that the magnitude of this dopamine release correlates with the worsening of psychotic symptoms experienced on exposure to the drug.[109] Additionally, neurochemical imaging studies have confirmed that at clinical doses all currently licensed antipsychotic drugs block striatal D2 receptors, and that it is this blockade of heightened transmission which leads to a resolution of symptoms for most patients (reviewed in Howes *et al.*[110]).

In recent years it has also become apparent that as well as worsening symptoms in established schizophrenia, certain substances can also increase the risk of developing the condition in the first place. As will be discussed later, cannabis is now widely accepted as an independent risk factor for schizophrenia. In addition, PET

imaging work has shown that even a few doses of a stimulant may sensitize the striatal dopamine system and can lead to enduring increases in dopamine release to amphetamine even after many months of abstinence.[111] In keeping with this, it has been reported that individuals with a history of methamphetamine psychosis can experience subsequent spontaneous recurrence of their paranoid-hallucinatory states in response to stress.[112] This can occur even in the absence of further use of the drug, suggesting that the development of stimulant-induced psychosis might itself be related to persisting brain damage or changes in brain metabolism which leave the individual at increased risk of subsequent further psychoses.[113] Thus, and in contrast to the initial reports by Connell,[74] the effects of amphetamine use may not all be transient, and may actually have long term implications for an individual's risk of ultimately developing schizophrenia. Indeed, such a possibility is given further credence by Japanese studies reporting that in approximately 15% of cases methamphetamine-induced psychosis runs a chronic course, persisting even if there is complete abstinence from further drug use.[114]

In addition to substance use, accruing data have demonstrated that numerous other environmental factors can potentially contribute to dopamine dysregulation. It has been reported, for example, that striatal dopamine release in response to stress was increased in people who reported low maternal care during their early childhood,[115] and animal experiments have repeatedly shown that postnatal rearing conditions can lead to profound and lasting changes in the responsiveness of mesocorticolimbic dopamine neurons to stress and psychostimulants.[116, 117] Obstetric complications have also been associated with an increased risk of subsequent schizophrenia,[118] a possible mechanism being suggested by animal studies which demonstrated that hippocampal dysfunction (a brain structure particularly vulnerable to hypoxia-associated obstetric

complications[119]) can lead to increased striatal dopamine release.[120] Genetic variables have also been associated with dopaminergic abnormalities. Most notably, and as already discussed, variants of the catechol-O-methyltransferase gene (involved in dopamine catabolism) have been shown to interact with early cannabis exposure to increase the subsequent risk of psychosis[121]and, in other studies, to increase stress reactivity and paranoid reactions to stress (reviewed in van Winkel *et al.*[122]).

The thrust of the data outlined above has been that a variety of different factors, including drug exposure, can contribute to the common end point of abnormal striatal dopamine release. In arriving at the most recent formulation of the dopamine hypothesis of schizophrenia however, two further areas of research in have been important. The first of these is the widely replicated finding of elevated presynaptic striatal dopamine availability in schizophrenia, this being the most consistently detected dopaminergic abnormality in the illness.[123] This observation provides an explanation for the greater amphetamine-induced dopamine release observed in people with schizophrenia,[124] and in turn explains why people with schizophrenia are more prone to the psychotomimetic effects of these drugs.[125] These data are largely compatible with the dopamine hypothesis as originally formulated. The second strand of data are more genuinely novel however, deriving from work implicating a distinct role for subcortical dopamine systems in incentive or motivational salience and reward prediction.[110]

The work demonstrating the role of dopamine in motivational salience provides a conceptual framework linking the neurochemical dysfunction of abnormal striatal dopamine release to the clinical expression of the symptoms of schizophrenia. It is fundamental to the 'third version of the dopamine hypothesis of schizophrenia', recently reviewed by Howes and Kapur.[110] As outlined in this review: "The abnormal

firing of dopamine neurons and the abnormal release of dopamine leads to an aberrant assignment of salience to innocuous stimuli. It is argued that psychotic symptoms, especially delusions and hallucinations, emerge over time as the individual's own explanation of the experience of aberrant salience. Psychosis is, therefore, aberrant salience driven by dopamine and filtered through the individual's existing cognitive and sociocultural schemas-thus allowing the same chemical (dopamine) to have different clinical manifestations in different cultures and different individuals". The strength of this model is twofold. Firstly, it is compatible with multiple routes (genetic, neurodevelopmental, environmental or social) leading to the striatal hyperdopaminergia, explaining how a wide range of factors can be associated with subsequent risk of psychosis. Additionally however it provides a convincing explanation of how the symptoms themselves can come in to being, a feature that was sorely lacking from earlier formulations of the dopamine hypothesis.

It is thus the case since its initial formulation (largely as a consequence of recognition of the association between amphetamine use and psychosis), the dopamine hypothesis has been modified considerably. These reformulations have enabled it to incorporate novel data as they have come to light. As these additional data have accrued it has become apparent that a number of drugs other than amphetamines may also be associated with psychosis, this association being believed to be particularly robust in the case of cannabis. This has again been attributed, to variable degrees, to the dopaminergic effects of these drugs.[110] Thus, despite the modifications, the idea that dopaminergic transmission remains the final common pathway for psychosis has remained remarkably robust.

2.3 Integrating prevalence of use data and the dopamine hypothesis of schizophrenia

A number of robust facts can thus be taken from the preceding review. Firstly, it is the case that people with schizophrenia have a great propensity to use a variety of substances, though this may also be a feature of bipolar affective disorder. Secondly, certain substances can undoubtedly induce (at least transient) psychotic symptoms, and this effect is more pronounced in those with a diagnosis of schizophrenia. Thirdly, the dopamine hypothesis provides a model through which the consumption of drugs that precipitate the release of dopamine and the experience of psychotic symptoms can be linked. While these three facts are generally accepted, what has been more contentious is whether the induction of psychotic symptoms can be regarded as causally related to the (generally chronic) disorder known as schizophrenia, and whether the drugs in question (cannabis, amphetamines and indeed a number of other substances), can truly increase an individuals risk of developing this condition. I will thus now consider the variety of explanations postulated to explain why there is such a close association between drug use and schizophrenia.

Chapter 3

Why is there an excess of substance use in schizophrenia?

3.1 Reasons given by people with schizophrenia for their substance use and the subjective effects of this use

As will be a theme in subsequent sections, early studies tended to lump together use of a variety of substances, and employed relatively unstructured interviews to ascertain motivations for any substance use. Frequently no comparator group (e.g. non-schizophrenic substance misusers) was included. One such example is the 1989 study by Test *et al.*, in which schizophrenic substance misusers stressed the reasons for their substance misuse as being anxiety reduction, relief of boredom, and as a means for social contact.[128] In 1995 Muesser *et al.* employed more sophisticated methodology.[129] Firstly they used structured interviews. Additionally however, they also compared the strength of endorsement of various potential drives for use of alcohol, cannabis or cocaine between people with a history of schizophrenia with and without a history of past or present abuse of each of these substances. By this approach they were able to demonstrate that the relationship between substance use disorders and expectancies displayed strong substance-specific expectations; namely, alcohol expectancies were related to alcohol disorders but not to drug disorders, whereas marijuana and cocaine expectancies were more strongly related to drug than to alcohol use disorders. It was also notable that motivations for use of the substances varied between alcohol and use of marijuana or cocaine (the latter two substances being considered together in this section of the paper). Whereas socialization, coping, and pleasure-enhancement motives were all strongly related to a history of alcohol use disorders, only coping motives were strongly related to a history of drug abuse (an explanation of what these groupings of motivations represent is detailed in Table 3.1). This study thus emphasises the point that it is important to consider that differences

may exist in the motivations to use different substances. While this is a phenomena that has been recognised in the substance misuse literature in general,[130] it can be forgotten when considering the reasons why associations exist between use of particular substances and schizophrenia.

Given the above considerations, in the following section I will restrict my summary of self report data to those obtained in relation to a specific substance. The additional criteria which studies must meet are comparable to those employed in the reviews ascertaining the prevalence of use of various substances in the schizophrenic population; specifically, they must use standardised criteria for diagnosing schizophrenia, at least 80% of the population sampled must have a diagnosis of schizophrenia and subjects included in the study must be aged 16-65. It is also important to note that only non-experimental studies are summarised here. By this I mean that the methodology employed in the studies included in this section centred on asking people with schizophrenia what effects they attributed to use of a particular substance, in the absence of that substance being accutley consumed. Experiment studies, undertaken by administering a given substance to people with schizophrenia and then rating the effects will be discussed in a subsequent section (Section 3.2.1)

Enhancement motives	**Coping motives**	**Social motives**
1. Because you like the feeling 2. Because it's exciting 3. To get high 4. Because it's fun 5. Because it makes you feel good	1. To relax 2. To forget your worries 3. Because you feel more self-confident or sure of yourself 4. Because it helps when you are feeling nervous or depressed 5. To cheer up when you're in a bad mood	1. As a way to celebrate 2. Because it's what most of your friends do when you get together 3. To be sociable 4. Because it is customary on special occasions 5. Because it makes a social gathering more enjoyable

Table 3.1.
Items on the Cooper *et al.* Drinking Motives Questionnaire (DMQ);[131] these motivational categories were employed by Muesser *et al.* in their 1995 paper, also being utilised to ascertain motivations for cannabis and cocaine use
Note: Each item is self-rated on a scale from 1 (never/almost never) to 4 (almost always).

3.1.1 Cannabis

Data ascertaining both the reasons for cannabis use and subjective effects of this use (ascertained by self-report outwith the context of acute consumption) in people with schizophrenia were recently reviewed by Dekker *et al.*[132] They identified five studies, each of which fulfilled my inclusion criteria. A supplementary literature search undertaken by myself revealed only one additional relevant study; this was undertaken by Schaub *et al.* in Switzerland and was published in 2008.[133] On reviewing these studies the most common reasons given for use of this drug were to increase pleasure (a major motivator in all studies and reported by >95% in two),[134, 135] to relax/decrease anxiety (>80% in four studies)[133-135] and out of a need to be more sociable (33-81%).[134-137] Use out of a desire to decrease psychotic symptoms was a relatively rarer motivation than those just described; nevertheless, it was reported as a drive to cannabis use by 40% of people questioned in two studies.[134, 135] The methodology employed in a German study by Lambert *et al.* was slightly different from the studies outlined above, but it still fulfilled inclusion criteria.[138] In this study, rather than the proportion of people volunteering/indicating support for particular statements being ascertained, people with schizophrenia were asked to state their single most important reason for using cannabis. When this methodology was employed the motivations outline above constituted three of the top four reasons given.[138] A further study of note included patients younger than my inclusion criteria (at least one patient was 15). In this study again the top three reasons given for cannabis use were to increase pleasure/get high, to relax and to be more sociable; these reasons were in fact very similar to those given by a non-psychotic cannabis using comparison group.[139]

A small number of studies were also identified ascertaining the subjective effects people with schizophrenia reported on using cannabis (patients being questioned outwith the context of acute consumption). In these studies the majority of patients did indeed report that they believed use of cannabis did improve mood and/or reduce anxiety/have a calming effect.[134, 136, 140] In each of these studies it was also reported however that this was believed to be at the cost of worsening psychotic symptoms.

3.1.2 Alcohol

A limited number of studies have investigated the subjective effects that people with schizophrenia report on using alcohol and the reasons they give for use of this substance. Again in these studies these attributions/reported effects were explored outwith the context of acute use of the substance. Noordsky *et al.* reviewed the early work in this area and found it somewhat contradictory.[141] Whereas Alpert and Silvers (1970) found that patients reported alcohol reduced the discomfort caused by hallucinations,[142] Kesselman *et al.*'s patients reported that it worsened their symptoms.[143] This was in contrast to the report of Dixon *et al.*, who found that while patients with schizophrenia and comorbid alcohol or drug disorder reported that alcohol decreased depression and anxiety, and increased calm and trust, they reported that it had no effect on hallucinations and suspiciousness.[140] Noordsky *et al.*'s own study consisted of interviewing 66 outpatients with schizophrenia or schizoaffective disorder who used alcohol with a structured interview specifically designed to ascertain subjective responses to alcohol.[141] Nineteen of these 66 subjects had a

current, and 38 a lifetime, alcohol use disorder (DSM-III-R abuse or dependence). Improvement in anxiety, tension, dysphoria, apathy, anhedonia and sleep difficulties was reported by over 50% of the sample. Similar numbers reported that alcohol worsened (31%) or relieved (26%) psychotic symptoms. Lifetime alcohol use disorder was strongly associated with reporting positive effects of alcohol, this being the case for the reported effects of alcohol on both psychotic and non-psychotic symptoms. Clarifying the beneficial effects that alcohol was reported to have on psychotic experiences, Noordsky *et al.* state that patients often reported that alcohol relieved the dysphoria associated with these symptoms rather than the symptoms themselves; to illustrate they quote one patient as saying: 'Drinking doesn't make the voices go away, but they do not bother me as much'.

The only subsequent study investigating this issue in a structured but non-experimental manner was undertaken by Pristach *et al.*[144] In this study 42 people with an established diagnosis of schizophrenia, 23 of who had a past or current alcohol use disorder (DSM-III-R abuse or dependence) were interviewed. Participants were asked about their reasons for drinking and the effects they experienced both in the month before their first episode of illness and in the month before their current admission. The most commonly given reasons for alcohol use were to be more sociable, to celebrate, to relieve depression, and to forget problems and worries. Subjects with a history of an alcohol use disorder were more likely to report using alcohol to alleviate depressive symptoms or alleviate worries, whereas they were less likely to cite increased sociability as a reason. Interestingly, in this study more subjects once again reported that alcohol consumption worsened hallucinations and delusions (12 and 13 subjects respectively) than reported that it helped them (4 and 7 respectively). In this

study those with a history of alcohol abuse or dependence were *much more* likely to report an increase in suspiciousness and paranoia with drinking.

It is thus the case the effects of alcohol use which people with schizophrenia report when questioned outwith the context of acute use of the substance remain inconsistent. Some themes do emerge however. Most obviously, and in-keeping with studies undertaken in the general population,[145] the most common reasons given for drinking are to socialise, improve mood, and reduce anxiety. People with schizophrenia rarely report that alcohol reduces psychotic symptoms; indeed, greater numbers tend to report that it *worsens* these symptoms..

3.1.3 Tobacco

Reasons given for smoking by the general population include tension reduction/relaxation, relief from boredom, socialization, stimulation/to aid concentration and addiction.[146, 147] Given the particularly strong association between schizophrenia and smoking however, it is clearly important to establish if the same reasons for smoking are reported in this population. Thankfully, a number of studies have specifically addressed the reasons that people with schizophrenia give for smoking. Two such studies, which did not include a control group of non-schizophrenic smokers, reported that the most prevalent reported reasons for smoking in schizophrenia bore marked similarities to those in the general population. Specifically, they included relaxation, habit, settling nerves, sedative effects, control of negative symptoms (which could be regarded as comparable to the need for 'stimulation'), and addiction.[148, 149]

Clearly, if there are different subjectively reported motivations for smoking in those with and without schizophrenia, these are most likely to be identified by studies which include a non-schizophrenic control group. Two such studies were identified. While these did find that the reasons given for smoking by people with and without schizophrenia were similar, the emphasis placed on different perceived benefits did differ between the two groups. Specifically, the study of Gerpegui *et al.* reported that patients more frequently reported the effects of cheerfulness, agility, concentration, and calmness.[150] Similarly, the study of Barr *et al.* reported that patients with schizophrenia placed greater emphasis than controls on the perceived benefits of pleasure derived from the act of smoking as well as psychomotor stimulation.[151] The latter construct indicates a desire to improve concentration and psychomotor energy, which clearly overlaps with the perceived benefits of concentration and agility reported by Gurpgui *et al.* It is thus the case that while the motivations for smoking reported by those with and without a diagnosis of schizophrenia are similar, there is a difference in the emphasis placed on specific factors between the two groups.

3.1.4 Stimulants and ecstasy

A small number of studies have ascertained the reasons that people with schizophrenia give for using amphetamines, but none were identified addressing reasons for use of cocaine or ecstasy. In the study of Fowler *et al.* the main motivations given for amphetamine use were drug intoxication and dysphoria relief;[98] similar motivations were cited by Baker *et al.*, though it is important to note that only 38% of the subjects included in this study actually had a diagnosis of

schizophrenia.[152] In an Australian study however the subjective benefit of a reduction in negative symptoms was also volunteered.[153]

3.2 Theoretical explanations for the association between schizophrenia and substance misuse

In section above I have outline the reasons that people with schizophrenia themselves give for their substance use. This is informative, but given that these data are entirely subjective it clearly has major limitations. Indeed, the limitations innate to self-report data are likely particularly prescient when considering reasons for substance misuse, given that denial and rationalisation are believed to play such a considerable role in the maintenance of these behaviours.[154] A comprehensive review of the most prominent theories postulated to explain this association, together with a critical evaluation of the evidence underlying them, is thus necessary. In the following section I will review these data. The three dominant theories postulated to explain the association are detailed below:

1. Schizophrenia causes substance use (self medication)
2. Substance misusers are at greater risk of developing schizophrenia
3. Common causality- the same people are at increased risk of substance misuse and schizophrenia

As was discussed in Section 3.1, it is easily conceivable that the association between substance use and schizophrenia differs for different substances. This being the case,

when possible data relating to each of the substances detailed earlier as of particular interest to the current study will be considered separately.

3.2.1 Schizophrenia causes substance misuse (the self medication hypothesis)

When asked why they use substances people with schizophrenia frequently report that it is for relief of sympotms. In theory, this drive could arise from a desire to alleviate either symptoms arising from the condition itself, or side-effects consequent to the use of medications prescribed with the intention of providing relief from the symptoms of schizophrenia. Thus, both of these possibilities will be explored. This discussion will begin with consideration of the possibility that having schizophrenia results in people having a greater tendency to self-medicate with *any* drugs of abuse. Given that the effects of different drugs are pharmacologically diverse however, I will then consider this possibility for each of the drugs of abuse considered in this study; these are specifically tobacco, alcohol, cannabis, stimulants (amphetamine and cocaine) and ecstasy.

How can the self medication hypothesis be explored?

The central argument of the self medication hypothesis is that people use drugs to alleviate symptoms. This hypothesis has been particularly associated with Khantzian, who proposed it in 1985 in a formulation largely based on psychodynamic theory.[155] Khantzian believed that a drug users' choice of drug is a result of an interaction between the psychopharmacological properties of the drug and the affective states from which the addict was seeking relief, meaning that the drugs

chosen by an individual could be predicted on the basis of their psychiatric disorder. As initially formulated this theory suggested that all people with schizophrenia would be driven to use the same drugs. It was subsequently refined however, with greater emphasis placed on the possibility that it was specific symptoms rather than the entire disorder which provided the drive to use specific drugs.[156]

Given the above, if we are to be able to ascertain if the drive to use substances does indeed derive from an attempt to address specific symptoms, then clearly we need to know what these symptoms are. Thus, before considering this potential explanation further, it is important to characterise what are the symptoms of this condition. These are outlined in detail in Table 3.2, though in practice are most commonly categorized as either *positive* symptoms, (essentially delusions and hallucinations), and *negative* symptoms, (symptoms such as lack of motivation, an inability to experience pleasure, and blunted affect).

Either *at least one* of the syndromes, symptoms, and signs listed under (1) below, *or* at least two of the symptoms and signs listed under (2) should be present for most of the time during an episode of psychotic illness lasting for at least one month (or at some time during most of the days). (1) At least one of the following must be present: (a) thought echo, thought insertion or withdrawal, or thought broadcasting; (b) delusions of control, influence, or passivity, clearly referred to body or limb movements or specific thoughts, actions, or sensations; delusional perception; (c) hallucinatory voices giving a running commentary on the patient's behaviour, or discussing the patient among themselves, or other types of hallucinatory voices coming from some part of the body; (d) persistent delusions of other kinds that are culturally inappropriate and completely impossible (e.g., being able to control the weather, or being in communication with aliens from another world). (2) *Or* at least two of the following: (a) persistent hallucinations in *any* modality, when occurring every day for at least 1 month, when accompanied by delusions (which may be fleeting or half-formed) without clear affective content, or when accompanied by persistent overvalued ideas. (b) neologisms, breaks, or interpolations in the train of thought, resulting in incoherence or irrelevant speech; (c) catatonic behaviour, such as excitement, posturing or waxy flexibility, negativism, mutism, and stupor; (d) "negative" symptoms, such as marked apathy, paucity of speech, and blunting or incongruity of emotional responses (it must be clear that these are not due to depression or to neuroleptic medication)

Table 3.2
ICD-10 general diagnostic criteris for schizophrenia

As will become apparent, the self medication hypothesis is notoriously difficult to prove or disprove. It may be expected that if it can be demonstrated that use of a particular drug does result in reduced experience of a particular symptom then this provides support for self-medication. This seems fairly logical, but is complicated by the fact that the acute and chronic effects of use of a particular drug may be quite different. Examples of this abound, but it is possibly best illustrated by the well recognised fact that though the acute effects of alcohol are anxiolytic, when used chronically alcohol is associated with anxiety disorders.[157] Nevertheless, it may be the case that short term benefit is worth longer term worsening of symptomatology. Indeed, persistence of substance-taking behaviours in the face of overt evidence of harm is a central feature of addictions, constituting the essence of one of the DSM-IV and ICD-10 criteria for the diagnosis of dependence.[23, 158] It would be expected that perceived or experienced short term benefits of substance use must play a significant role in these ongoing behaviours. Thus, despite the proviso outlined above, investigation of the possibility that the pharmacological effect of drugs of abuse may provide even temporary relief from the experience of positive, negative and other symptoms of schizophrenia is clearly necessary.

An alternative way to investigate the self medication hypothesis is to establish if the symptoms experienced by those who have both schizophrenia and a substance misuse problem are significantly different from those with schizophrenia alone. It may be expected, for example, that if a specific drug of abuse genuinely alleviates the experience of a particular symptom, then people with both schizophrenia and habitual use of this drug would experience a lesser magnitude of this particular symptom than those with schizophrenia alone. Again however, the fact that this is genuinely the reason for an association between use of a particular substance and schizophrenia is

very difficult to support or disprove. It could equally be argued that the association of more severe illness with a greater level of drug use is because people with a more severe illness have a *greater need* for some relief or, and completely contrary to the self medication hypothesis (and as will be discussed later), that this level of substance use has actually contributed to the severity of the illness experienced. The self medication hypothesis will now be considered for each of the substances of particular interest to this report.

3.2.1.1 Cannabis

As reviewed in Section 3.1, people with schizophrenia who used cannabis reported a number of positive effects (e.g. relaxation, increased pleasure and increased sociability), though there was some acknowledgement that use of this drug could result in worsening of psychotic symptoms. Delta-9-tetrahydrocannabinol (THC) has long been recognised as the principle active ingredient in cannabis,[159] and a number of studies have experimentally investigated the effects of this drug in normal subjects. In addition to THC however it is now also known that a further cannabinoid present in cannabis, cannibidiol, may also have an impact on the mental state (see below). This illustrates the heterogeneity of what is known as cannabis, and the fact that different strains with different THC/cannabidiol ratios can potentially have different effects. Given this, in the passage below I will review the psychiatric effects of each of THC, cannabidiol, and the combination of the two substances as is found in naturally occurring cannabis.

Effects of acute exposure

It is widely accepted that cannabis produces euphoria and relaxation, perceptual alterations, time distortion, and the intensification of ordinary sensory experiences, such as eating, watching films, and listening to music.[160] It is also accepted that cannabis use can cause unpleasant side effects, most commonly anxiety and panic attacks.[160] Though these effects are accepted as almost common knowledge however, modern studies that have actually investigated the acute effects of cannabis use in the general population are few. This is understandable; given that cannabis is a controlled substance which can potentially cause harm such studies are difficult to conduct experimentally. Thus, much of these data rely on self-report. It would be expected that such reports would have the greatest chance of being accurate if the nature of the subjective experience was ascertained shortly after exposure. Reflecting this expectation, Verdoux *et al.* undertook a naturalistic study with 79 cannabis-using university students.[161] Employing an experience-sampling method, they aimed to achieve recording of both cannabis use and symptom experience at the times they were actually occurring. They reported that within this non-clinical population there was a positive association between cannabis use and the experience of unusual perceptions. Interestingly, even though subjects with high psychosis vulnerability were more likely to report unusual perceptions and feelings of thought influence in periods of cannabis use, the above association was observed even when psychosis proneness was included into the statistical model. The investigators also reported that the effects of cannabis were time-limited and restricted to the 3 hours surrounding its consumption, with no evidence that use of cannabis is increased following the occurrence of psychotic-like experiences.

A recent study investigated the acute effects of cannabis use in an indisputably experimental manner.[162] This was achieved by assessing individuals 10-15 minutes after smoking a cannabis 'spliff' (from participants own cannabis supply). In this study it was again reported that cannabis use reliably increased the experience of psychotic-type symptoms across all users; as in the previous study this effect was particularly pronounced in people with increased psychosis-proneness (determined on the basis of high scores on the Schizotypal Personality Questionnaire). The idea that underlying psychosis proneness may influence the experience of cannabis use has actually gained increasing credence in this research area, and will be discussed further below.

It is more practicable to experimentally investigate the effects of THC administration than those of the native compound. A number of studies have evaluated the consequences of THC exposure in the general population, assessing this by self-report as well as formal cognitive/psychomotor testing. The most influential was that of D'Souza *et al.*, in which THC was administered by intravenous infusion.[163] This study suggested that though THC administration did result in the transient experience of euphoria, it also increased anxiety and resulted in positive-type symptoms which included suspiciousness, paranoid and grandiose delusions, conceptual disorganization, and illusions. Subjects also manifested negative-type symptoms, which including blunted affect, reduced rapport, lack of spontaneity, psychomotor retardation, and emotional withdrawal. Other studies have shown similar effects of THC administration,[164, 165]and additionally demonstrated that use of the drug impairs executive function and inhibits motor control.[165, 166] It has also become increasingly apparent that the adverse effects associated with THC use may only

become evident at higher doses of the drug,[166] and that habitual cannabis users may develop a degree of tolerance to these effects.[167]

As noted above, though the effects of drug exposure in the normal population are informative, it is conceivable that these could be altered in people with schizophrenia. One study has however managed to experimentally determine the effects of THC administration in people with schizophrenia. This was again undertaken by D'Souza *et al.*, and consisted of the administration of either 0mg, 2.5mg or 5mg of THC intravenously, followed by clinical, neurochemical and neuropsychological assessment for a period of 200 minutes afterwards.[168] This study found that THC transiently exacerbated a range of positive and negative symptoms, perceptual alterations, cognitive deficits, and medication side effects associated with schizophrenia. The effects on learning and memory were even more pronounced than had been observed when the same methodology was employed on a group of well control subjects. In addition, subjects with schizophrenia seemed to be more susceptible than controls to the effects of THC on the experience of positive symptoms, though this did not reach statistical significance. Cannabis administration did not produce any obvious "beneficial" effects; specifically, there was no suggestion that it was associated with a reduction in anxiety. The results of the study were thus consistent with other studies suggesting that cannabis has a negative influence on the symptomatology of schizophrenia, and did not provide evidence that would support the self-medication hypothesis as an explanation for the co-occurrence of schizophrenia and substance misuse.

As was acknowledged by D'Souza *et al.* in the above paper, it is conceivable that though THC does not have beneficial effects on schizophrenia symptomatology, other substances present in cannabis may. Though THC is generally accepted as the

principle active ingredient in cannabis, it is only one of many compounds present in the substance. Indeed, in recent years there has been renewed interest in another compound long recognised as being present in cannabis called cannabidiol (CBD). As early as the 1970s, it was suggested that cannabidiol may reduce the anxiety associated with THC administration,[169] an effect that was reproduced by Zuardi *et al.* in 1982.[170] The distinct effects of cannabidiol compared to THC have also been demonstrated using functional imaging techniques.[164, 171] It has also been demonstrated that when administered with THC to a normal population, as well as having anxiolytic effects cannabidiol can also attenuate the psychotogenic effects of the latter drug.[170, 171] Additionally, even if it is administered in the absence of THC cannabidiol can have anxiolytic effects.[172]

In keeping with the above findings, it has recently been suggested that cannabidiol may have beneficial effects on schizophrenia symptomatology, and that it may even have antipsychotic properties.[173] Indirect evidence of such an effect has been provided by a study which demonstrated that the experience of psychotic-type symptoms was in fact rarer in people who consumed cannabis with higher ratios of cannabidiol to THC.[174] It is important however to establish if there is direct evidence for the cannibidiol having beneficial effects in people with schizophrenia; clearly if this were the case it would support the possibility that people with schizophrenia may indeed consume cannabis for the potential benefits that one of the components of this substance can confer.

Though there are a number of annedotal reports of cannabidiol having beneficial effects for psychotic symptoms in schizophrenia,[173, 175] to date only one randomised controlled trial employing cannabidiol in the treatment of psychotic patients has been undertaken. Only preliminary reports have been published from this

trial at the time of writing. Over a four week period the effects of CBD and amisulpride were compared in the treatment of acute schizophrenic and schizophreniform psychosis.[176] This showed that CBD significantly reduced acute psychotic symptoms after 2 and 4 weeks of treatment when compared to baseline. In this trial CBD did not differ from amisulpride except for a lower incidence of side effects. Though this is the only trial of the clinical use of cannibidiol in schizophrenia, when combined with evidence that cannabiol has beneficial effects in animal models predictive of antipsychotic activity,[177] it does suggest that cannabidiol may have the potential to improve the symptoms of schizophrenia.

In summary, there is little evidence from the few experimental studies that have investigated the effects of cannabis use on symptomatology that it provides relief from the symptoms of schizophrenia. It seems even less likely that THC, the substance primarily responsible for the psychoactive effects of cannabis, has any beneficial effects on symptomatology associated with the condition. It is conceivable however that cannabidiol, a substance which was long thought to be an inactive component of cannabis may indeed confer some potentially beneficial effects; so much so that it has been trialled as an antipsychotic. This underlines the importance of viewing cannabis as an entity with a variety of components, and considering that the relative proportions of these various components can influence the effect a user experiences from cannabis use. It also raises the issue that different strains of cannabis may have different effects, and that skunk (a variety of cannabis with a very high THC:CBD ratio) may have particularly propsychotic effects. As mentioned above, this is an area of active research.[174]

Non-acute effects

A further potentially useful source of information on the effects of cannabis use on symptomatology experienced in schizophrenia is to compare the symptoms experienced by subjects with schizophrenia who use the drug compared to those who don't. Such observational study is inevitably vulnerable to innumerable confounders and the difficulty of disentangling cause and effect. It may be expected however that if cannabis use confers benefits people who use the drug would experience a milder profile of symptoms. Conversely, a detrimental effect could mean that users are more impaired. The association of cannabis use with positive, negative and depressive symptoms will be considered separately.

A number of cross-sectional studies have compared positive symptom expression in people with schizophrenia who use cannabis to those who do not. Interestingly however this is not an area which has been subject to systematic review; this likely reflects the difficulties in both aggregating data from studies with disparate methodologies and of differentiating what are and are not intoxication effects. Unsurprisingly given the propsychotic effects of cannabis consumption, these studies generally report increased positive symptoms in people with schizophrenia who consume cannabis;[178, 179] this is not a universal finding however, [53, 180]and a report to the contrary (i.e. that people with schizophrenia who consume cannabis exhibit reduced psychotic symptoms) does exist.[181]

In 2006 Potvin *et al.* published a meta-analysis of case-control studies comparing ratings of negative symptoms (as measured by the SANS) in people with schizophrenia who did and did not use cannabis. On pooling results from the three studies eligible for conclusion, they determined that those people with schizophrenia who used cannabis did experience significantly fewer negative symptoms than

abstinent subjects.[182] Additionally, Potvin *et al.* undertook a meta-analysis comparing the weight of depressive symptoms in people with schizophrenia who used cannabis compared to those who did not.[183] Though only two studies were identified explicitly addressing this question,[82, 184] they did not suggest that there was a difference in depressive symptoms between the two groups. This is further evidence that cannabis is not effective in relieving dysphoria/low mood when used by this population.

Synthesis of the above data suggests that use of cannabis by people with schizophrenia is likely associated with a greater weight of positive symptoms, is associated with a lesser load of negative symptoms, and is not associated with any difference in depressive symptoms. It could be argued from these data therefore that cannabis may serve to attenuate negative symptoms. Given the nature of these cross-sectional data however, such a conclusion would seem a step too far; it is of course equally eminently possible that the association occurs in the opposite direction i.e. people with schizophrenia with fewer negative symptoms are more likely to use cannabis.

Impact of cannabis use in individuals with the extended phenotype of schizophrenia

The consequences of cannabis use by people with the extended phenotype of schizophrenia may also be informative. The concept of the extended schizophrenia phenotype derives from the observation that the relatives of people with schizophrenia frequently exhibit subclinical, psychotic-type symptoms bearing marked similarities to the symptoms of schizophrenia. These include positive symptoms, such as unusual belief systems and perceptual experiences, and negative symptoms in the form of social withdrawal, anxiety, and depression.[185] These are known as 'schizotypal symptoms', and the extended phenotype of schizophrenia as 'schizotypy' (discussed

further in Section 4.2.2.3). Schizotypy exhibits developmental as well as genetic links to schizophrenia, with elevated rates of schizotypal traits being present in both those destined to develop schizophrenia as well as in relatives of individuals with the condition. It is generally regarded as being a milder phenotypic expression of schizophrenia.[186] The utility of studying individuals such as these derives from the fact that the impact of cannabis use can be ascertained in individuals who exhibit schizophrenia-like characteristics, but are not subject to the potential confounders of chronic illness and medication.

It could be speculated that though cannabis use may make established psychotic symptoms worse, it is actually beneficial when these are expressed in a milder form. Given that there are high rates of schizotypal symptoms in people destined to develop schizophrenia,[187] this could initiate the association observed in those with established schizophrenia. That an association between schizotypy and cannabis use exists has been demonstrated in cross-sectional studies in the general population.[188] Interestingly however, and as has already been discussed (above), Verdoux *et al.* reported that in the general population increased psychosis-proneness was associated with people being *more* likely to report unusual perceptions and *less* likely to report pleasurable experiences on using the drug.[161] Supporting these findings, Barkus *et al.* also reported that those with higher ratings of schizotypy were more likely to experience psychotic symptoms on exposure to cannabis and were also more likely to report unpleasant, dysphoric/amotivational-type after effects following use of the drug.[189, 190] Similarly, in a prospective study of a cohort at clinically high risk of schizophrenia, (help-seeking adolescents and young adults considered to be at heightened clinical risk for or prodromal to a nonaffective psychosis), longitudinal assessments showed participants to have significantly more perceptual disturbances

and worse functioning during epochs of increased cannabis use.[191] These findings are similar to those in studies of established schizophrenia, and suggest that in the context of schizotypy/schizophrenia prodrome cannabis use is once again associated with both the induction of psychotic symptoms *and* general unpleasant effects; once more this is difficult to reconcile with the self-medication hypothesis. A single experimental study provides further support that psychosis-prone individuals are particularly vulnerable to the psychotogenic effects of cannabis.[162] It demonstrated that following administration of THC these individuals do indeed experience enhanced psychotomimetic states compared to those who are less psychosis prone. The induction of additional pleasant or unpleasant effects was however not ascertained in this study.

Summary

On considering the studies reviewed above, data supporting the self-medication hypothesis as an explanation for the association between schizophrenia and cannabis use are weak. Though one constituent of the drug (cannabidiol) may have beneficial effects, use of the substance as a whole worsens both anxiety and psychotic symptoms. These effects likely also occur in the extended phenotype of schizophrenia. Interestingly, it seems likely that the negative effects of cannabis use have become more pronounced as the concentrations of THC in cannabis have increased. There is no experimental evidence that any of the constituents benefit negative symptoms, suggesting that the suggestion of such an association by case-control studies is more likely attributable to people with schizophrenia with fewer negative symptoms being more likely to use the drug.

3.2.1.2 Alcohol

As discussed in Section 3.2, common reasons people with schizophrenia give for alcohol use are to reduce anxiety and elevate mood, with relatively fewer reporting that it alleviates psychotic symptoms; indeed, it is in fact more commonly reported that it makes the latter symptoms worse. It is of course the case that self-reported reasons for behaviour are potentially unreliable, being prone to problems such as recall bias. As discussed above, when considering substance use this unreliability is likely magnified even further, being influenced by the denial and rationalisations so much a part of these conditions. Clearly it is thus desirable to obtain experimental data, utilising robust methodology to characterise the objective effects of alcohol use in schizophrenia. If these effects can be compared to those in a normal population, then any differences may offer some insight in to why people with schizophrenia are particularly prone to use alcohol.

The acute effects of alcohol use in the normal population have been well characterised.[192] At low doses (1-2 drinks, but it varies among individuals), alcohol tends to produce relaxation, reduced inhibitions, impaired concentration, slowed reflexes, reduced reaction time, and reduced concentration. As consumption increases these effects are magnified, with the onset of slurred speech and difficulty in walking at blood alcohol concentration levels of 0.15-0.2%. At 0.3% most people will be on the verge of unconsciousness or be comatose, and death is possible at levels of 0.35-0.4%.

Only a single study could be identified which used experimental methodology to investigate the effect of alcohol on the experience of symptoms of schizophrenia. This was a randomized, double-blinded, placebo-controlled controlled study, which

compared the effects of alcohol exposure in 23 patients with schizophrenia (treated with antipsychotics and without a diagnosis of an alcohol use disorder) to that in 14 healthy controls.[193] Neither patients nor controls had any history of an alcohol use disorder. Relative to healthy subjects, people with schizophrenia reported greater euphoria and stimulatory effects in response to alcohol. In the people with schizophrenia alcohol also produced small transient increases in positive psychotic symptoms and perceptual alterations but did not affect negative symptoms. On cognitive testing alcohol also impaired several aspects of immediate and delayed recall, vigilance and distractibility; although people with schizophrenia tended to perform worse on these measures, there was no significant group by alcohol dose interaction.

D'Souza *et al.* conclude that their results do not support the 'self-medication' hypothesis of alcohol and substance use in schizophrenia, as alcohol did not reduce any of the core symptoms of the illness. They do acknowledge that exclusion of alcohol-abusing subjects opens up the possibility that a subset of individuals who may derive these benefits from alcohol were not part of the study; it is notable however that their finding do seem to parallel those from self-report studies which did include such groups. While alcohol did not relieve psychotic symptoms, D'Souza *et al.* do suggest that the increased euphoric and stimulatory responses to alcohol observed in schizophrenia patients compared to controls may increase their vulnerability to alcohol problems. These findings suggest the possibility of a 'shared vulnerability' to alcohol problems and schizophrenia (as proposed by Chambers *et al.*, and which will be discussed later); while they speculate that brain reward circuitry dysfunction may explain these increased effects, D'Souza *et al.* do acknowledge that treatment with antipsychotic drugs could also play a role.

Studies investigating the association between chronic alcohol use and scores on schizophrenia rating scales will be discussed below. The manner in which studies were undertaken means that these data are most appropriately considered with studies exploring the associations between substance use and outcomes of schizophrenia. Also relevant to discussion in this section however is the limited evidence base exploring the acute effects of alcohol on symptom expression through the utilisation of observational methodology. This consists of a single study undertaken by Alterman *et al.*, which utilised nursing notes and interviews with nursing staff to characterise the acute effects of alcohol consumption in inpatients with schizophrenia who became intoxicated with alcohol during the course of their inpatient care.[194] Unspecified changes in sleep pattern, mood and behaviour were reported in 22 out of 25 patients. It is also reported that 'hallucinations' occurred in over a quarter of patients. Unfortunately the symptomatology experienced by patients who use alcohol in this study is too poorly characterised to add substantially to data provided by D'Souza *et al.* No studies were identified exploring the effects of alcohol in people with the extended phenotype of schizophrenia.

3.2.1.3 Smoking

Acute effects

As detailed in section 3.1, tension reduction/relaxation effects are repeatedly reported by smokers as beneficial effects of tobacco use. Nicotine is the component of tobacco generally regarded as responsible for its psychoactive effects,[195] and consequently would be expected to produce these effects in experimental conditions.

It is somewhat surprising therefore that meta-analysis of placebo-controlled laboratory studies of the subjective effects of nicotine reported that it actually decreased relaxation and increased tension/jitteriness.[196] Though this could potentially be explained by participants not being tolerant to tobacco, such an explanation is in fact not feasible as this effect was observed in smokers as well never-smokers.

It is also widely believed that smoking tobacco has beneficial effects on attention and memory.[197] The evidence supporting these purported cognitive benefits is rather stronger; for example, a number of studies have reported that in nicotine-dependent smokers, nicotine administration is associated with improved performance on tasks that require vigilant attention.[197] Additionally, and regardless of smoking status, nicotine administration also has been reported to improve reaction time.[198]

The above data provide some interesting insights. Firstly, it establishes that though smoking does not result in relaxation, it may indeed convey some cognitive benefits in the general population. Secondly, it also demonstrates that the benefits that users perceive from smoking can bear little relation to the experimentally derived effects of use of the drug; i.e. non-experimental self-reported accounts of the effects of substances need to be treated with considerable scepticism. Importantly however, while experimental data elucidating the effects of smoking in the general population are interesting, it is of course the case that the effects experienced by people with schizophrenia could potentially differ substantially. It is thus once again the case that if we are to ascertain whether the self-medication hypothesis does have any validity in explaining the association between smoking and schizophrenia it will be essential to establish (via experimental means), what the effects of smoking actually are *in this specific population*. Furthermore, studies which specifically investigate if the effects

of tobacco use/nicotine administration do differ between those with and without schizophrenia will be particularly informative; if such a difference does exist then it may also go some way to explaining why people with schizophrenia have an increased level of tobacco use. A number of studies have addressed exactly these issues; those which assess the effects of nicotine administration in people with schizophrenia, either with or without a comparator group of non-schizophrenic controls, will be discussed below.

Studies have utilised a variety of experimental designs to ascertain the effects of nicotine administration in people with schizophrenia. Smoking status has ranged from schizophrenic non-smokers to schizophrenic smokers who have been abstinent overnight. Consistent with findings in the non-schizophrenic population, smoking by people with schizophrenia has been shown to improve sustained attention, spatial accuracy and verbal memory. It also increases prepulse inhibition, a form of startle plasticity which has been reliably demonstrated to be reduced in people with schizophrenia.[199] Smoking thus has a normalising effect on this abnormality. Even more interestingly, in studies including a non-schizophrenic control group it was demonstrated that the attentional effects are greater in schizophrenic than other smokers,[200] and this may be particularly true for response inhibition.[201]

In addition to cognitive benefits, there is also evidence that smoking cigarettes may provide some relief from the negative symptoms experienced by people with schizophrenia. This was suggested by a study of Smith *et al.*, which demonstrated that the smoking of high-nicotine cigarettes resulted in a reduction in negative symptoms without affecting positive symptoms.[202] This effect was not observed in participants who smoked de-nicotinized cigarettes. It has been suggested that this effect may be a

consequence of nicotine raising dopamine levels in the nucleus accumbens and prefrontal cortex.[199]

There is also evidence that smoking may help reduce unpleasant side effects of neuroleptic medication, an effect which may arise through a number of mechanisms. Firstly, there are reports that smoking increases the metabolism of antipsychotics, an effect which would obviously result in reduced side affects (as well, of course, as reduced efficacy).[203] There have also been reports however that the benefits of smoking on antipsychotic side effects may also be more specific. It has been reported, for example, that application of nicotine patches results in a reduction of antipsychotic induced rigidity and akathisia,[204, 205] it being suggested that nicotine-induced dopamine release may be responsible for this effect.[199] In keeping with these potential benefits a relationship between antipsychotic treatment and smoking has long been recognised, McEvoy *et al.* demonstrating in 1995 that patients with schizophrenia smoke more after starting haloperidol.[206]

Non-acute effects

An observational, non-experimental, methodology which could yield insights in to the effects of tobacco use on schizophrenia symptomatology is to compare this symptomatology in people with schizophrenia who smoke and do not smoke. An example of a study employing such an approach is that of Zabala *et al.*, who reported that first-episode psychosis patients who are nicotine users had better cognitive functioning in the areas of attention and working memory than patients who were not nicotine users.[207] No significant differences were detected in sociodemographic and clinical data between the two groups, and they attributed these differences to

beneficial effects of smoking. Obviously, this form of study design is highly vulnerable to unidentified confounders, but it is interesting that similar associations are reported with smoking to those in experimentally designed studies outlined above.

Impact of smoking in individuals with the extended phenotype of schizophrenia

The impact of smoking on symptomatology in people with the extended phenotype of schizophrenia may also be informative. As previously emphasised, these data have the particular advantage of being able to demonstrate associations without the confounds of chronic illness and medication. A number of different approaches have been employed in exploring the relationship between the extended phenotype of schizophrenia and smoking, these focusing on individuals sharing genetic and/or symptomatic characteristics with people with schizophrenia. The increased rates of smoking in those sharing familial characteristics with people with schizophrenia has been most convincingly demonstrated in a twin study investigating smoking rates in twins of people with schizophrenia. This study found that unaffected co-twins had a frequency of ever daily smoking (88%) similar to that in male schizophrenia probands (83%) and higher than male twin controls (66%). The OR of ever daily smoking for the co-twin of a schizophrenic proband were 3.7 times greater than the odds of being a regular smoker among twins from pairs in which neither has schizophrenia.[208]

Other studies have focused more explicitly on the relationship between schizotypy and smoking. These have reported both that smokers are more likely to report schizotypal symptoms,[209]and that a significant relationship exists between smoking status and self-reported levels of schizotypy. Indeed, the latter has been reported in both the general population and in relatives of people with schizophrenia.[210] There are thus data to support the suggestion that schizophrenia

spectrum disorders (as well as the condition itself) are associated with an increased risk of smoking. What has not been ascertained however is what the experimental effects of nicotine administration are in the extended phenotype of schizophrenia, and if use of the drug by this population does confer benefit. Thus, though the data outlined above do demonstrate that schizotypy itself is associated with schizophrenia, they do not inform us if this relationship s driven by the beneficial effects of tobacco use on the extended phenotype. Thus, though the positive relationship between schiztypy and smoking *may* be driven by the fact that those who are more schizotypal have a greater need for the beneficial effects of tobacco use, the association could equally be driven by the fact that the two are independently attributable to one or more common risk factors.

Nevertheless, as the current study is concerned with people at high genetic risk of schizophrenia (which is, of course itself associated with schizotypy), the finding that schizotypy itself is associated with smoking is certainly interesting. It is conceivable that, just as schizophrenia patients might smoke in order to self-medicate cognitive deficits present after the onset of psychosis, so those at elevated genetic risk of the condition might smoke more in an attempt to use nicotine to self-medicate their premorbid cognitive dysfunction. There is certainly substantial evidence that such deficits exist in this group,[211] indicating that the high risk group under investigation in the current study may already be at elevated risk of using tobacco to alleviate cognitive deficits that they already posses, even in the absence of frank psychosis. Thus, it is conceivable that even in the currently well high risk group that is the focus of the current study, individuals are already using smoking as a means to self medicate subclinical abnormalities.

Summary

It is thus the case that the data detailed above demonstrate a number of reasons for an association between smoking and schizophrenia, each of which likely contributes to the high prevalence of smoking in the condition. On considering these data the possibility that self-medication may play a role in bringing about the association between smoking and schizophrenia cannot be discounted. A drive to ameliorate pre-existing cognitive deficits present in those destined to develop schizophrenia may place them at an elevated risk of smoking even before illness onset; following onset of psychosis the beneficial effects of cigarette smoking on worsening cognitive deficits, negative symptoms and the effects of antipsychotic treatment may magnify this drive.

3.2.1.4 Stimulants and ecstasy

Given the centrality of the observation that stimulants could provoke psychotic symptoms in people without schizophrenia (see Section 2.2), the suggestion that the association between amphetamine use and schizophrenia may be driven by self-medication may seem counter-intuitive. Indeed, as previously discussed, it is indisputably the case that drugs such as amphetamines have a particularly psychotomimetic effect in people with schizophrenia and clearly worsen psychotic symptoms.[125] A number of researchers have however suggested that stimulant drugs may have beneficial effects on the negative symptoms of schizophrenia, and a number of trials have been undertaken investigating these effects. These trials have generally employed modafanil, a drug regarded as having similar but milder effects to

amphetamines and possessing little abuse potential.[212] A small number of studies have utilised amphetamine however.

Angrist *et al.* administered 0.5mg/kg of *d*-amphetamine to 26 clinically stable patients with predominantly 'deficit state' schizophrenia who were not receiving antipsychotic treatment at the time of the experiment.[213] Over the next three hours, though a minority of patients did exhibit clinical deterioration, overall a statistically significant decrease in negative symptoms as measured by the BPRS was observed. These findings were not confirmed in a subsequent similar study, though it was the case in this later study that some diminution of negative symptoms was observed in the subset of patients with more severe negative symptoms.[214] This led the authors of the latter study to speculate that for amphetamine to effect a change in negative symptoms, then these symptoms must be relatively severe; this explanation was indeed compatible with their own data and the previously published studies they reviewed. That amphetamine may confer some benefit in patients with schizophrenia is also suggested by a study reporting improved performance on the Wisconsin card sort test (WCST, one of the most commonly used tests of executive function) in schizophrenia subjects administered the drug.[215] Additionally, studies demonstrating the beneficial effects of modafanil on cognition in schizophrenia are also in-keeping with these findings.[216] Adding to these studies, Kirrane *et al.* reported that amphetamine administration improved visuospatial working memory in a sample of people with schizophrenia-spectrum personality disorders (predominantly schizotypal personality disorder); this effect was greater than that seen in a comparator group of normal controls.[217]

As was discussed in relation to cannabis, some insight may be provided on the effect of stimulant use on symptoms of schizophrenia by comparing symptom profiles

in people with schizophrenia who do and do not use these drugs. Unfortunately however no studies could be identified specifically comparing positive, negative or depressive symptom severity in schizophrenic patients who used amphetamines to those who did not; in all studies which could potentially have provided this information amphetamine users were grouped together with users of other drugs. No experimental studies have been undertaken examining the effects of cocaine on symptoms of schizophrenia. It has been reported however that schizophrenic patients who had recently used cocaine exhibited fewer negative symptoms than those who had not.[218] Additionally, three studies assessed positive symptom severity in schizophrenic subjects shortly after admission with an acute psychotic exacerbation. These reported that estimates of positive symptom severity are similar in schizophrenic patients who used cocaine to those who did not.[219-221] Meta-analysis reports that people with schizophrenia who use cocaine experience fewer negative symptoms but similar levels of depressive symptoms when compared to abstinent patients.[182, 183] Smelson *et al.* have reported that there is no difference on cognitive testing between people with schizophrenia who use cocaine compared to those who do not.[222] Two studies undertaken by Serper *et al.* have however reported that comorbid patients do exhibit memory impairments compared to non-using patients with schizophrenia.[223] No studies could be identified examining the effects of MDMA on the symptoms of schizophrenia.

Data on the effects of stimulant use in people with schizophrenia spectrum (personality) disorders are limited. In addition to the study of Kirrane *et al.* detailed above and undertaken in people with predominantly schizotypal personality disorder, Satel and Edell did report that cocaine users who experience transient paranoia while

intoxicated exhibited more traits of psychosis-proneness than individuals who did not have transient cocaine-induced paranoid symptoms.[224]

In summary therefore, experimental data are compatible with the possibility that though stimulant use (either amphetamines or cocaine) can temporarily relieve negative symptoms, it results in a worsening of positive symptoms of schizophrenia. Cross-sectional data in this area are sparse, but what does exist is also compatible with the former possibility. As was discussed in relation with cannabis however, cause and effect is impossible to disentangle from these cross-sectional data, which limits their usefulness. Nevertheless, taken as a whole the existing data are compatible with the possibility that people with schizophrenia do indeed use stimulant drugs in an attempt to relieve negative symptoms.

3.2.1.4 Synthesis of data examining the viability of the self-medication hypothesis as an explanation for the association between substance misuse and schizophrenia

As reviewed above, there are considerable data relevant to consideration of the validity of the self-medication hypothesis as an explanation for the association between schizophrenia and substance use. On reviewing these data, two principles stand out as fundamentally important in establishing if evidence supports this hypothesis: firstly, it is important to look beyond a conceptualisation of schizophrenia as simply psychotic symptoms and also to consider the cognitive, affective and motivational features of the condition; and secondly, the diverse pharmacological effects of drugs of abuse does justify considering these substances separately. When this is done a number of conclusions can be drawn:

1. None of the substances investigated appear to have any significant effect in attenuating positive psychotic symptoms. Conversely stimulants, cannabis and possibly even alcohol worsen the experience of psychotic symptoms. Indeed, people with schizophrenia/on the schizophrenia spectrum appear to be particularly sensitive to the psychotomimetic effects of these substances.
2. Stimulants and possibly also tobacco smoking may result in a mild attenuation of negative symptoms.
3. Tobacco smoking and possibly also stimulant use may transiently benefit a number of cognitive deficits, such as attentional and memory impairment.
4. Alcohol may transiently elevate mood and relive anxiety, and it is possible that people with schizophrenia are particularly susceptible to the euphoric and stimulant effects of this drug.

It is thus the case that overall the existing data suggest that self-medication, if this is interpreted as relief of psychotic symptoms, is not a feasible explanation for the association between substance misuse and schizophrenia. Nevertheless, particular substances may have some beneficial effect on symptoms associated with the broad phenotype; evidence for this seems to be particularly strong for tobacco improving the attentional deficits associated with the condition.

3.2.2 Substance use causes schizophrenia or influences the course of the condition

Schizophrenia is the archetypal form of insanity, and throughout times and cultures has inspired intense fear in the general population.[225] Given that this fear of insanity is such a core feature of the human condition, it is understandable that the introduction of psychoactive drugs in to societies, particularly if they are believed to provoke psychotic symptoms, is frequently associated with widespread concern that use will lead to massively increased rates of schizophrenia. Such a response followed widespread use of both cannabis and LSD in the 1960s,[226, 227] and remains a preoccupation with the popular press.[228] Clearly schizophrenia cannot be *entirely* attributable to drug use, given that it is recognised to occur in both people and societies with very low or even non-existent levels of use of these substances. Nonetheless, it is conceivable that use of a particular drug does *contribute* to the risk of developing the condition without this use being an invariable prerequisite for the condition to occur. Such a model of contribution to risk would both be compatible with the occurrence of the condition in the absence of any drug use and also be a potential explanation for the increased prevalence of substance use in schizophrenia; as people who use drugs are at increased risk of developing the condition, so drug use and schizophrenia co-occur more commonly than would be expected by chance. As discussed in Section 3.2.1, it is an undeniable fact that drugs such as amphetamines and cannabis can provoke psychotic symptoms in the normal population, and that people with schizophrenia are particularly susceptible to these effects. Thus, the possibility that drug use can increase the risk of develop schizophrenia (as well as the experience of transient psychotic symptoms) does demand serious consideration.

In examining the evidence for the possibility of drug use contributing to risk of schizophrenia, each of the drugs of abuse that are the focus of this study will be considered individually. Data will primarily be derived from epidemiological and cohort studies, but reports characterising chronic drug-induced psychosis will also be considered. As will be discussed, the boundaries between the concepts of drug induced psychoses and schizophrenia have become increasingly blurred. In addition to a role in *causation* of schizophrenia, this section will also consider evidence that substance misuse modifies the *course* of schizophrenia. Given the fact that substance misuse is so common in established schizophrenia, consideration of the latter issue is clearly of considerable importance. This section will thus comprise the following subsections:

1. Evidence that various substances increase risk of developing schizophrenia: clinical observational studies
2. Evidence that various substances are associated with increased rates of schizophrenia: population-based studies
3. Impact of use of various substances on the course of schizophrenia

3.2.2.1 Evidence that various substances increase risk of developing schizophrenia: clinical observational studies

3.2.2.1.1 Cannabis

The possibility that cannabis may contribute to the aetiology of schizophrenia has been considered more than any other drug, this being the subject of substantial media interest and numerous journal editorials. That such an association should be postulated is unsurprising; as has already been discussed, this drug is more commonly used than any other illicit drug, rates of use are further elevated in schizophrenia, it is known to provoke psychotic symptoms, and people who have schizophrenia are particularly susceptible to this psychotogenic effect. That proving (or indeed disproving) a role in *causality* has been so difficult once again reflects the obstacles encountered in a research area that relies almost entirely on observational data. A consensus has emerged however that cannabis does contribute to the risk of schizophrenia (as well as precipitating transient psychotic episodes), and the data from which this evolved will be summarized below. This evidence takes four main forms: cross-sectional data that schizophrenia is associated with cannabis use (see Section 2.1.1); observation of a protracted cannabis-induced psychosis with similarities to schizophrenia; evidence from cohort studies; and evidence from population data. The second and third of these bodies of evidence will be discussed below, with population data being discussed in a subsequent subsection. Particular emphasis will be placed on characterising *who* is most vulnerable to the risk-

modifying effects of cannabis. Additionally, I will also discuss the related issue that cannabis impacts on the course of schizophrenia in an additional subsection.

Support for a distinct cannabis-induced psychosis

That persistent cannabis use has the potential to produce protracted psychotic episodes in addition transient psychotic symptoms has long been suggested.[229, 230] Clearly, if this could be established to be the case then this psychosis could be characterised and its features compared to those of schizophrenia; if clearly distinguishable it would constitute a discrete diagnostic entity, if not it may provide evidence that cannabis contributes to the aetiology of schizophrenia. A small number of studies have compared persistent psychoses believed to be attributable to cannabis intake to schizophreniform psychoses in those who have not used the drug. Differences that have been reported are that cannabis-induced psychoses exhibit more hypomania and agitation and less affective flattening and auditory hallucinations,[231] or more violence and panic but less thought disorder and loss of insight.[232] In the face of these inconsistent reports, other researchers have reported that symptom profiles in the two conditions are actually very similar.[233] Nevertheless, one fundamental difference between the two conditions is consistently described; that is that whereas (even in these more protracted episodes) the former condition is transient, with symptoms generally resolving in days,[234] the latter condition is characterised by its chronicity.

The observation described above is reflected in clinical practice by the fact that a diagnosis of drug induced psychosis is generally only made when a psychosis develops shortly after drug use and resolves when drug use ceases i.e. *the condition is largely defined by its transience*. Indeed, this is formalised in the diagnostic criteria of

both DSM IV and ICD-10; DSM-IV suggests that a diagnosis of substance-induced psychotic disorder should be reconsidered if symptoms last for longer than a month, while in ICD-10 the diagnosis is deemed inappropriate if symptoms persist beyond six months. Thus, in clinical practice, if a drug-induced psychosis does not resolve it ceases to be a drug induced psychosis. Despite the obvious circularity of this argument, it does reflect the generally accepted belief that there is in fact insufficient evidence to propose that 'cannabis psychosis' exists as a distinct entity; instead, persistent psychotic symptoms occurring in an individual who has used cannabis are generally regarded as schizophrenia. Indeed, though the reasoning behind this interpretation does sound a little like sophistry, there is in fact evidence to support it; persistent psychoses in people who have used cannabis are indistinguishable from schizophrenia, and a large follow up study of people diagnosed with cannabis psychosis demonstrated that over a mean follow-up period of 5.9 years, 44.5% of the sample went on to receive a diagnosis of schizophrenia-spectrum disorders.[235] It is thus the case that the observations outlined above cannot tell us that cannabis *causes* psychosis, as it may simply be that those ultimately destined to develop schizophrenia are simply more vulnerable to more protracted psychotimetic effects on using the drug the drug. Clearly therefore investigation of an aetiological role for cannabis necessitates other research methodologies; evidence deriving from them will be discussed below.

Longitudinal studies

Just as the association between cannabis consumption and transient psychotic symptoms does not mean cannabis causes a chronic psychosis, so cross sectional studies reporting an association between cannabis use and schizophrenia tell us

nothing of causality. Instead, to address this question studies must ascertain if cannabis consumption when well is associated with the *subsequent* onset of the condition.

A number of researchers have applied longitudinal methodology to samples from the general population to address this question. This was first undertaken using a large historical, longitudinal cohort study of all Swedes conscripted between 1969 and 1970.[236] As Sweden mandates military service, this included 97% of all males aged 18-20 years. The relationship between self-reported cannabis use at the time of conscription and psychiatric hospitalization for schizophrenia in the ensuing 15 years was examined. A dose-response relationship was observed, with individuals who reported having used cannabis more than 50 times being six times more likely than non-users to be diagnosed with schizophrenia in the follow-up period. Adjusting for other risk factors did reduce the strength of this association, but nonetheless the relationship remained significant; after these adjustments people who had used cannabis 1 to 10 times were 1.5 times more likely to develop schizophrenia, while those who had used it 10 times or more were 2.3 times more likely. These data have subsequently been reanalysed, with the follow-up period extended to 27 years.[237] This re-analysis also improved on the earlier report in a number of other ways. Most importantly it increased number of potential confounding variables included in the analysis, additional factors such as other drug use, urbanicity, IQ and social integration also being incorporated in to the statistical model. Though reduced, the adjusted odds ratio for cannabis use and schizophrenia remained significant, those with a history of any use of cannabis having a 1.5, (CI 1.1-2.0), times risk of developing schizophrenia compared to never users. Indeed, this elevated risk was also robust to the exclusion of subjects who developed schizophrenia within 5 years of

cannabis conscription, making it highly unlikely that the observed association was attributable to cannabis use arising as a consequence of prodromal manifestations of psychosis. Once again, in these analyses a dose dependent relationship between cannabis exposure and the subsequent development of schizophrenia was evident.

The relationship between adolescent cannabis use and schizophrenia has also been investigated using prospective methodology. Two of the most notable of these studies were birth cohorts undertaken in New Zealand, in Dunedin and Christchurch. The former study achieved complete follow-up in 759 of 1037 of the originally recruited participants, these people being assessed intensively on risk factors for psychotic disorders from birth.[238] Of particular note the research protocol of this study included assessment of psychotic symptoms at age 11 years, (i.e. before the onset of cannabis use), and also distinguished between early- and late-onset cannabis use. A significant relationship was found between cannabis use by age 15 years and an increased risk of both psychotic symptoms and actual schizophrenifrom disorders by age 26 years. Though controlling for other drug use did not affect this relationship, adjusting for psychotic symptoms reported at age 11 years did. Though the relationship between cannabis use and psychotic symptoms remained significant, that between cannabis use and schizophreniform disorder did not. While it could be argued that this casts some doubt on cannabis having an aetiological role, raising the possibility that the association between cannabis use and schizophrenia could be driven by self medication of prodromal symptoms, such an explanation seems unlikely. Instead, it is generally regarded as more likely that this loss of significance reflects the small number of psychotic disorder outcomes observed in the sample.[239] Such an interpretation is strengthened by findings in the Christchurch study, a second birth cohort study which also collected data on cannabis use and psychotic symptoms

at various timepoints.[240] Similarly to the Dunedin study, this found that a history of cannabis dependence was associated with increased psychotic symptoms at age 18 and 21. Additionally however, it also determined that the presence of psychotic symptoms at the age of 18 appeared to inhibit, rather than encourage, subsequent cannabis use. This strengthens the case for a causal link between cannabis use and the development of psychotic symptoms, being incompatible with the possibility that this association is driven by 'self-medication'. Though these data are compelling, a clear weakness of the latter study is its reliance on psychotic symptoms rather than diagnosed disorder as the outcome measure.

Several other cohort studies have longitudinally investigated the association between cannabis use and subsequent psychosis, and a number of reviews have been undertaken synthesising these data. Among the most robust and comprehensive of these reviews is that of Moore *et al.*[241] They searched for all relevant studies published up until September 2006. Studies were included in the review if they were population-based longitudinal studies, or case-control studies nested within longitudinal designs. As well as schizophreniform disorders, a variety of other diagnostic outcomes were permissible for a study to be included. Amongst these were 'non-affective or affective psychoses' and the even broader outcome of 'psychotic symptoms'. The potential vagueness of the latter outcome was limited by the clarification that caseness on the basis of 'psychotic symptoms' required the presence of either delusions, hallucinations or thought disorder.

Moore *et al.* identified 7 studies sufficiently homogeneous to be included in the meta-analysis. These are detailed in Table 3.3, this being reproduced from their published meta-analysis. Moore *et al.* report that there was no evidence to support the presence of publication bias, and that the unadjusted results of all studies reported

evidence of an increased risk of psychosis in people who used cannabis compared with non-users. These associations were reduced, but nevertheless persisted, in six of the studies after adjustment for confounding factors. On pooling these data, there was an increased risk of a psychotic outcome in individuals who ever used cannabis (OR=1·41, 95% CI 1·20–1·65). Six of the studies also provided dose response data. On pooling these data findings were consistent with a dose-response relationship between self-reported frequency of cannabis use and the risk of subsequently developing psychotic symptoms or a psychotic disorder, with the greatest risk in people who used cannabis most frequently (OR=2.09, 95% CI: 1.54, 2.84). Three studies utilised the narrower definition of casesness representing psychotic disorder rather than simply exhibiting psychotic symptoms;[237, 238, 242] on pooling data from these three studies an increased risk of psychotic outcomes in individuals who had ever used cannabis was again seen (OR=2·58, 95%CI 1·08–6·13).

The conclusion of the meta-analysis of Moore *et al.* would seem to provide robust evidence for the role of cannabis in the aetiology of schizophrenia. This position is further strengthened by the fact that these conclusions are very similar to those from a number of earlier systematic reviews and meta-analyses.[243-245] Only one significant cohort study of this issue has been published since the review of Moore *et al.* (see McGrath *et al.*, below) and it seems unlikely that further cohort studies will add substantially to the evidence base. It is the case however that an association seen in an observational study does not necessarily reflect a causal relation. Thus, despite the seemingly robust evidence for cannabis contributing to risk for schizophrenia, a number of alternative explanations for the observed associations could potentially be proposed.

Firstly, it could be argued that in a number of studies the association between cannabis consumption and schizophrenia could potentially be driven by intoxication effects. The thrust of this argument would essentially be that a history of cannabis use is associated with psychotic symptoms because it predicts subsequent cannabis use, and the transient psychotic symptoms induced by this are mistakenly diagnosed as a psychosis outcome. It is the case however that the majority of the studies included took steps to exclude this possibility. Though it could conceivably be difficult to exclude this effect completely in people who are smoking daily, it is nonetheless highly unlikely that cannabis intoxication would result in a diagnosis of schizophrenia. Thus, though this possibility may justify caution in the interpretation of some of the studies included in the review, it is not tenable that this effect resulted in the outcomes seen in, for example, the Swedish conscript study.

Other alternative explanations for the results observed in the cohort studies reviewed centre on reverse causality and residual confounding. The exclusion of people with psychosis at baseline makes reverse causation (i.e. psychosis gives rise to substance misuse) unlikely. As discussed above, the methodology of the Christchurch study means that this possibility is particularly untenable in it.

It is certainly the case that in six of the seven studies included in the review, (the exception is the study of Wiles *et al.*),[246] the increased risk of psychosis in cannabis users compared to non-users was robust to the inclusion of a comprehensive list of confounding factors. These included likely candidate confounders, such as use of other drugs and markers of premorbid disturbances that are commonly observed in patients with schizophrenia. Nevertheless, it is also the case that the resulting reductions in effect sizes were frequently substantial, and the pooled effect size reported by Moore *et al.* could be regarded as relatively modest. This does raise the

possibility that the association is attributable to residual confounding; i.e. it is due to one or more known or unknown variables being inadequately controlled for in the analysis. While this is an unavoidable possibility in cohort studies, two approaches have been implemented to minimise its potential influence.

The first of these approaches is the utilisation of more searching statistical methods to control for confounding factors. This was undertaken by Fergusson and colleagues, who applied a sophisticated structural equations modelling design that accounted for both observed and nonobserved confounding factors to examine the association between cannabis use and psychotic symptoms in a re-analysis of data from the Christchurch study.[247] In addition they extended the follow up period of the cohort in this analysis, data on psychotic symptoms being acquired at the additional age point of 25. Consistent with the earlier study, this analysis determined that daily users of cannabis had rates of psychotic symptoms that were between 1.6 and 1.8 times higher ($P<0.001$) than non-users. An alternative approach to reduce residual confounding is the use of sibling pairs analysis. This was recently employed by McGrath *et al.*, and focused primarily on the importance of age of onset of cannabis use on the rate of subsequent development of psychosis.[248] It will be discussed in more detail in the appropriate section below, but also demonstrated an association between cannabis and psychosis and that the association appeared to move from cannabis use to symptoms of psychosis, rather than vice versa.

A further alternative explanation that has been proposed to explain the relationship between cannabis use and schizophrenia is that rather than cannabis use being a risk factor for schizophrenia *per se*, it simply precipitates the condition among vulnerable individuals destined to develop the condition. That is, among persons who would have developed the disorder regardless of whether they used cannabis or not.[249]

Indeed, the association between cannabis use and younger age of onset of psychosis does have considerable support, being reported in several independent studies.[44, 250-252] Though such an explanation could explain the findings of increased rates of psychosis among cannabis users in studies with relatively short periods of follow up, it is however not a feasible explanation for those studies in which subjects were followed up for many years. In the Swedish conscript study for example, men were recruited at age 18-20 and followed up for 27 years i.e. until aged approximately 38. By this time it would be expected that the vast majority of people destined to develop schizophrenia would have done so. As a difference in rates of schizophrenia between cannabis exposed and unexposed groups was clearly apparent, an explanation that cannabis simply brings forward expression of illness is clearly not tenable.

In summary therefore, the data from longitudinal epidemiological studies of general population samples indicate that cannabis use is associated with elevated risk of subsequent psychotic symptoms and illnesses including schizophrenia. Despite considerable torturing of these data this association stubbornly persists, and this body of research provides some of the strongest evidence to date that cannabis use is associated with an increased risk of schizophrenia.

	Setting and sample size	Baseline screening	Cannabis measure and age	Exposure n (%)	Follow-up and attrition*	Outcome n (%)	Confounders adjusted for	Main results	Dose–response effects
CHDS [240, 247]	Birth cohort, Christchurch, New Zealand (n = 1055)	SCL-90 for past month symptoms Ages 16, 18, 21	Use since prior measure, ages 16, 18, 21, 25 Frequency of use past 1 year: None, <1/month >1/month, >1/week, daily	Age 18: 427 (42%) Age 21: 473 (47%) Age 25: 444 (44%)	Up to 7 years Attrition 17%	Ages 18, 21, 25 Past month symptoms from SCL-90	Past psychotic symptoms and cannabis use, past psychiatric disorders, other substance dependence, peer affiliations, childhood adversity, life events, IQ, personality traits, social, demographic and family variables Also used fixed effects model for non-observed fixed confounding factors	Ever use: † Crude OR = 1·9 (1·6–2·2) Adjusted OR = 1·3 (1·0–1·6) Frequency of use (adjusted): <1/month OR = 1·1 (1·1–1·2) >1/month OR = 1·3 (1·1–1·4) >1/week OR = 1·4 (1·1–1·7) Daily OR = 1·6 (1·2–2·0) Test for trend p <0·001	Evidence from linear term for frequency of use
Dunedin [121, 238]	Birth cohort, Dunedin, New Zealand (n = 759)	DISC-C used for psychotic symptoms at age 11	Used cannabis >3 times Ages 15, 18	Age 15 29 (3·8%) Age 18 236 (31%)	8–11 years Attrition 4%	DIS at age 26 used for past year: Psychotic symptoms ≈ 25% Schizophreniform disorder 25 (3·3%)	Stratified analyses adjusted for sex, socioeconomic status, other drug use, psychotic symptoms at age 11 Whole sample analysis adjusted for confounders above and also IQ	Whole sample: †Schizophreniform disorder Crude OR = 3·1 (1·5–6·6) Adjusted OR = 2·9 (1·2–7·0) *Cannabis use by age 15‡: Schizophrenia symptoms* Adjusted β = 6·6 (0·9), p<0·001 *Schizophreniform disorder* Adjusted OR = 3·1 (0·7–13·3) Cannabis use age 15–18‡: *Schizophrenia symptoms* Adjusted β = 1·0 (0·4), p<0·01 *Schizophreniform disorder* Adjusted OR = 1·4 (0·5–3·7) Cannabis use by age 18 Stratified by COMT genotype§ Adjusted results for schizophreniform disorder Val/val OR = 10·9 (2·2–54·1) Val/met OR = 2·5 (0·8–8·2) Met/met OR = 1·1 (0·2–5·4)	Not studied
ECA [253]	Case–control nested within adult population based cohort study, USA (n = 2295)	DIS interview for psychotic symptoms	DIS interview for lifetime ever use and daily use 'Never used' included use of cannabis <5 times Age 18–49	Ever use: 381 (21%) Daily use: 87 (4·8%)	1 year Attrition 20%	DIS interview for any self-reported psychotic experience 507 (11·4%)	Age, gender, school attendance, educational level, marital status, employment status, baseline mental health problems (excluded if psychotic symptoms), other drug and alcohol use	Ever use: Crude OR=1·3 (1·0–1·7) Adjusted (excluding other drugs) OR=1·3 (1·0–1·7) Daily use: Crude OR = 2·4 (1·6–3·6) Adjusted (including other drugs) OR = 2·0 (1·3–3·1)	Stronger effect for daily use than ever use, although CI overlap
EDSP [254]	Adult population based cohort, Munich, Germany	SCL-90-R used for paranoid and psychoticism	Ever use >5 times Frequency at heaviest: None, <1/month, 3–4/ month, 1–2/week, 3–	Ever use 320 (13·1%)	3–4 years Attrition 16%	M-CIDI used for: Broad psychosis (≥1 symptom) 424 (17·4%)	Age, sex, socioeconomic status, urbanicity, childhood trauma, other drug use, smoking, alcohol use, predisposition to psychosis	***Broad psychosis (Ever use)*** Crude OR = 1·8 (1·4–2·4) Adjusted OR = 1·7 (1·1–2·5)	Evidence from 6-level frequency of use variable examined as linear

	(n = 2437)	subscales Scores summed, and identified as 'predisposed to psychosis' if score >90th percentile	4/week, daily Ages 14–24			Narrow psychosis (≥2 symptoms) 174 (7·1%)		***Narrow psychosis (ever use)*** Adjusted OR = 2·2 (1·5– 3·3) Broad psychosis (Frequency) Crude OR = 1·2 (1·2–1·4) Adjusted OR (but not for other substance use) = 1·2 (1·1–1·3)	term (broad psychosis only reported)
NEMESIS [242]	Adult population based cohort, Netherlands (n = 4045)	CIDI for psychotic symptoms 47% of subjects with symptoms on CIDI re-interviewed with SCID	Lifetime ever use and cumulative frequency from baseline to follow-up, summed as lowest, middle and highest levels Age 18–64	Ever use: 312 (7·6%)	3 years Attrition 30%	BPRS for: Any psychotic symptoms (BPRS>1) 38 (0·94%) Pathology level symptoms (BPRS>4) 10 (0·25%) 'Need for care' from CIDI, SCID, BPRS 7 (0·17%)	Age, sex, ethnicity, marital status, education, urbanicity, discrimination, and other drug use Subjects with any lifetime ever psychotic symptoms at baseline excluded	Ever use: *Any symptoms* Crude OR = 3·3 (1·5– 7·2) Adjusted OR = 2·1 (0·8–5·7) *Pathology level symptoms* Crude OR = 28·5 (7·3–110·9) Adjusted OR = 16·9 (3·3–86·1) *Need for care* Crude OR = 16·2 (3·6–72·5) Adjusted OR = 10·5 (1·8–63·2) Cumulative frequency: *Any symptoms* Adjusted OR = 1·7 (1·0–2·7) *Pathology level symptoms* Adjusted OR = 3·7 (2·0–7·0) *Need for care* Adjusted OR = 3·5 (1·6–7·4)	Evidence from 3-level frequency of use variable examined as linear term
NPMS [246]	Adult population based sample, UK (n = 2413) Over-sampled for baseline mental disorder	PSQ used for past year psychotic symptoms	Used in past year but not dependent Dependent past year Age 16–74	Used 109 (4·5%) Dependent 57 (2·4%)	18 months Attrition 32%	PSQ for psychotic symptoms since initial interview 134 (4·4%)	Psychotropic drugs and therapy, IQ score, marital status, urbanicity, support group, life events, smoking, alcohol, age, gender, CIS-R score at baseline	Used past year Crude OR = 1·1 (0·5–2·5) Adjusted OR = 0·7 (0·3–1·7) Dependent Crude OR = 3·4 (1·5–7·7) Adjusted OR = 1·5 (0·6– 3·9)	Not studied Suggestion of increasing effect for dependent compared to non-dependent
Swedish conscripts [236, 237]	Adult population based conscript cohort, Sweden (n = 48 481)	Psychologist/ psychiatrist interview for ICD-8 diagnoses	Ever use Frequency: None, 1 time, 2–4 times, 5–10 times, 11–50 times, >50 times Age 18–20	Ever use 5391 (10·8%) >50 times: 731 (1·5%)	27 years No data on attrition available	Admissions with ICD8/9 clinical diagnosis of schizophrenia/ schizoaffective disorder 362 (0·7%)	Other drug use, IQ, social personality traits, other diagnoses at conscription (excluded if psychotic at baseline), place of birth, childhood behaviour, family history, alcohol use, family income, paternal occupation, tobacco use, paternal age	Ever use: Crude HR = 2·2 (1·7–2·8) Adjusted HR = 1·5 (1·1–2·0) Frequency of use: Crude HR = 1·4 (1·3–1·5) Adjusted HR = 1·2 (1·1–1·3) Stratified by age of first use: First use age ≤15 Adjusted HR for cumulative frequency = 1·2 (0·9–1·4) First use age >15	Evidence from 6-level frequency of use variable examined as linear term

	Adjusted HR for cumulative frequency = 1·2 (1·1–1·4)

*Attrition based on proportion of subjects lost to study from baseline cannabis assessment to outcome assessment at follow-up. †Additional data kindly provided by study authors of these studies. ‡Results adjusting for other drug use not presented as uncertain validity (large increase in CI for schizophreniform disorder, indicating possible collinearity or problems related to small numbers); §val[158]met. β = linear regression coefficient. BPRS = Brief psychiatric rating scale. CIDI = Composite international diagnostic interview. CIS-R = clinical interview schedule-revised. DIS = Diagnostic interview schedule. DISC-C Diagnostic interview schedule for children. DSM = Diagnostic and statistical manual of mental disorders. HR = hazard ratio, 95% confidence intervals in parentheses. ICD = International classification of diseases. M-CIDI = Munich version of CIDI. OR = odds ratio, 95% confidence intervals in parentheses. PSQ = Psychosis screening questionnaire. SCID = Structured clinical interview for DSM-III-R. SCL-90 = Symptom checklist 90. SEM = Structural equation modelling

Table 3.3

Studies included in the meta-analysis undertaken by Moore *et al.* which investigated if cannabis use was associated with subsequent psychotic mental health outcomes

Who is most at risk?

I. The influence of age of first cannabis use on risk of schizophrenia

A number of studies have suggested that the association between cannabis and subsequent psychotic illness appears to be highly dependent on the age when drug use begins. For example, in the Dunedin Birth Cohort study it was reported that whereas initiation of cannabis use by the age of 18 years doubled the odds of developing a schizophreniform disorder by the age of 26 years, initiation by 15 years quadrupled this risk.[238] Though it has been suggested that this association may simply have arisen because initiation of cannabis use at a younger age is associated with greater cumulative exposure, such an explanation was not supported by a cross-sectional study undertaken by Stephanis *et al.*[255] Their study investigated the association between self-reported cannabis use and positive and negative dimensions of psychosis (rather than schizophrenia itself). They demonstrated that self-reported first use of cannabis below age 16 years was associated with a much stronger effect than first use after age 15 years, this association existing independent of life-time frequency of use. Though studies in which data are acquired retrospectively must be interpreted with some caution (given their vulnerability to recall bias), when combined with findings from the Dunedin study this report does suggest that the relationship between early cannabis use and psychosis does indeed exist independent of total quantities used.

Prospective cohort studies (such as the Dunedin study) reduce vulnerability to recall bias. As discussed above however, even in these studies there can be substantial threats to the validity of the findings. Primary among these is the potential for residual confounding, this being repeatedly raised as a concern when the relationship between

cannabis use and subsequent schizophrenia has been investigated utilising this methodology.

Primarily in response to these concerns about the possibility of residual confounding, elucidation of the association between cannabis use and age of onset of psychotic symptomatology has most recently been attempted by McGrath *et al.* using sibling pair analysis.[248] They argue that this model of study design enables the association between cannabis use and psychosis-related outcomes to be examined while reducing the influence of these factors, since differences are less likely to be attributable to shared genetic and environmental exposures. Thus, the application of this approach nested within a prospective birth cohort will reduce the influence of unmeasured residual confounding. If a significant association between cannabis use and psychosis related outcomes was not detected in sibling pairs, they argue, it would seriously weaken the argument that cannabis use was a risk-modifying factor for psychosis-related outcomes.

In the study of McGrath *et al.*, the researchers were particularly interested in the association between early cannabis use and subsequent psychosis-related outcomes. The researchers found that for the total cohort (including many more subjects than just the sibling pairs), those with duration since first cannabis use of six or more years (implying first use of cannabis by about age 15) had a significantly increased risk of nonaffective psychosis. This finding corresponds with the previously discussed studies investigating the impact of adolescent cannabis use on the subsequent development of psychosis. The sibling pairs analysis was limited to scores on the Peters *et al.* Delusions Inventory (PDI), an instrument used to measure delusional-like experiences in clinical and community populations. For each pair, the authors calculated difference scores for duration since first cannabis use and PDI total

score. The association between time since first cannabis use and PDI score remained statistically significant in the sibling subset analysis. Though a weakness of this study is that age of first cannabis use was again determined retrospectively, this study does provide further support for the hypothesis that early cannabis use is a risk-modifying factor for psychosis-related outcomes in young adults.

Despite the establishment of this association, it is not know why the effect of cannabis consumption is greater in those who begin cannabis use early in adolescence. While it has been argued that it could be because they are more likely to become heavily dependent on cannabis, this is not supported by the study undertaken by McGrath *et al.* Instead, it seems more likely that the significance of this early use of cannabis may instead arise from the fact that it is occurring at a time when many neurobiological and hormonal changes are taking place. Cannabis use in this time of rapid biological change may be more likely to lead to alterations in neurobiology that increase psychosis risk.[256, 257]

II. High risk groups

As detailed in Section 3.2.1.1, individuals from the general population with a greater propensity to experience psychotic symptoms experience a more psychotogenic effect on consuming cannabis. A number of studies have taken these investigations further, and investigated if people at increased risk of schizophrenia are at greater risk of actually developing psychosis if they consume the drug. These studies take a number of forms. In one manifestation the high risk group is determined on the basis of being symptomatic, clinically compromised, help-seeking and at imminent risk of psychosis, but not yet floridly psychotic. This group is labelled as ultra-high risk, and an example of such studies are those conducted in the Personal

Assessment and Crisis Evaluation (PACE) clinic in Melbourne, Australia.[258] In an alternative approach the high risk group is determined at an earlier stage, and on the basis not of symptoms but of a genetic propensity for schizophrenia. These are generally called high risk studies, and the current study is one such example. A third approach has also been employed. In this predisposition to psychosis is determined by the degree of expression of psychotic-type symptoms at the point of entry in to the study; in contrast to ultra-high risk studies these individuals are from the general population (e.g the study of Henquet *et al.*,[254] previously mentioned in the review of longitudinal studies above).

The only previous study of cannabis use and risk of subsequent schizophrenia in a population at high genetic risk that could be identified was a previous analysis of data from the Edinburgh High Risk Study by Miller *et al.*[259] Though this study did report an association between illness onset and frequent cannabis use just prior to onset, an association was not reported between early cannabis use and subsequent schizophrenia. It is notable however that the focus of this earlier analysis was predominantly on cannabis use in the period immediately preceding the development of psychosis, and relatively broad categories of cannabis exposure were employed. Given that these could have obscured an association between early cannabis use and schizophrenia, a re-analysis of these data will be included in this report.

Philips *et al.* used data from the aforementioned PACE clinic to investigate the association between cannabis use and the development of a first psychotic episode in a group of 100 young people at ultra-high risk of psychosis.[260] They had either subthreshold psychotic symptoms or a combination of a first-degree relative with a psychotic disorder and recent functional decline. Though thirty-two per cent of the cohort developed an acute psychotic episode over the 12-month period following

recruitment, cannabis use or dependence in the year prior to entry to this study was not associated with a heightened risk of developing psychosis. While the authors concluded that cannabis use did not appear to contribute to the onset of psychosis, they acknowledge several limitations to the study design, including a low level of cannabis use in the sample, and the lack of monitoring of cannabis use. It is also the case of course that analysis of cannabis use was limited to that in the year prior to recruitment. Given that considerable data have stressed the importance of age of first cannabis exposure in determining risk, it may be that what is essentially an extended prodromal period is already too late in the development of psychosis to observe the risk modifying effects of the drug. In-keeping with this possibility, an American study focussing on a similar population found that a *lifetime* history of cannabis abuse or dependence was associated with transition to psychosis.[261]

A number of studies have identified individuals at elevated risk of psychosis within a general population sample on the basis of exhibiting psychotic or psychotic-type symptoms. An example of this third form of study is van Os *et al.*'s Dutch general-population based longitudinal study.[242] In this study vulnerability to psychosis was determined by meeting DSM-III-R diagnostic criteria for a psychotic disorder (though not necessarily requiring treatment), and incident psychosis by having a psychotic disorder requiring treatment. They reported that the difference in risk of psychosis at follow-up between those who did and did not use cannabis was much stronger for those with an established vulnerability to psychosis at baseline than for those without one. Comparable findings were reported by Henquet *et al.*, in their study of 2437 adolescents and young adults in Munich.[254] Similarly to the Dutch cohort, individuals who reported psychotic symptoms at baseline were much more

likely to experience psychotic symptoms at follow-up if they used cannabis than were their peers who did not have such a history.

III. Genotype

Taken in its entirety, the data outlined above do indicate that those with a genetic vulnerability to psychosis are more sensitive to the deleterious effects of cannabis. This makes intuitive sense; given that the vast majority of young people who use the drug do not become unwell, it is only tenable for cannabis to have a causal role in psychosis if some individuals are more genetically vulnerable to its effects. The first example of a genetic by environment interaction predisposing to schizophrenia was described by Caspi *et al.*, in a further investigation of the Dunedin birth cohort.[121] It was demonstrated by these researchers that a functional polymorphism in the catechol-O-methyltransferase (COMT) gene moderates risk of the development of schizophrenia on exposure to cannabis.

The enzyme produced by the COMT gene plays an essential role in the breakdown of dopamine in the prefrontal cortex. In 1996 Lachman *et al.* reported that a common genetic polymorphism in humans resulting from a G to A missense mutation in this gene generated a valine (Val) to methionine (Met) substitution at codon 158 (Val158Met).[262] This altered gene product has less enzymatic activity and consequently is associated with slower break down of dopamine.[263] The functional polymorphism of the COMT gene an individual posseses is believed to have particular significance for the efficiency of prefrontal cortex functioning, possession of the high activity COMT Val allele having been associated with impaired memory and attention.[264]

Caspi *et al.* have shown that COMT moderates the influence of adolescent cannabis use, with at least a fivefold increased risk of developing schizophreniform disorder in cannabis users homozygous for the high activity Val allele.[121] In contrast, homozygosity for the M*et* allele offered relative protection (odds ratio 1.1), whereas the risk for heterozygotes was intermediate (odds ratio 2.5). These researchers also demonstrated that was no correlation between the COMT genotype and cannabis use, indicating that the COMT genotype does not influence cannabis consumption.

The COMT-variation-cannabis-use interaction has since been investigated experimentally. Henquet *et al.* gave study volunteers either THC or a placebo, and noted that carriers of the COMT Val allele were more likely to develop impairments of memory and attention than carriers of the Met allele.[265] Those with the homozygous Val genotype were also more sensitive to the effects of THC on psychotic symptoms, but this was dependent on their pre-existing proneness to psychosis. Still further support for the association of this polymorphism with psychotic symptoms has come from the application of an experience sampling technique. This was utilised to collect data on cannabis use and occurrence of symptoms in daily life in patients with a psychotic disorder and healthy controls.[266] Carriers of the COMT Val allele, but not Met homozygotes showed an increase in hallucinations after cannabis exposure, conditional on prior evidence of psychometric psychosis liability. These data thus support Caspi *et al.*'s initial report that genetic variables moderate the effect of cannabis exposure, a finding which constitutes the first example of a genetic by environmental interaction predisposing to schizophrenia.

3.2.2.1.2 Alcohol

Though overshadowed by cannabis research in recent years, consideration of the possibility that alcohol use may play a role in the aetiology of schizophrenia is not entirely novel. Indeed, such speculation has long been supported by the observation that chronic alcohol consumption may occasionally result in the generally self-limiting psychotic disorder known as alcoholic hallucinosis. In this condition auditory hallucinations and delusions of reference and persecution are prominent, though other schizophrenic symptoms such as thought disorder and passivity are generally absent.[267] Symptoms can appear relatively suddenly, are experienced outwith the withdrawal period (though may first arise during it), and are experienced regardless of whether the patient is drinking or abstinent. In one case series it was reported that men who developed alcoholic hallucinosis were significantly younger at the onset of alcohol problems, consumed more alcohol per occasion, developed more alcohol-related life problems and had higher rates of drug experimentation.[268] Case series of prognosis have reported that only a few (5-10%) continued to have symptoms for six months or more if abstinence was maintained.[269] Renewed drinking did however tend to bring about a return of hallucinations.

The data outlined above clearly demonstrate that alcohol consumption can result in psychotic symptoms, and that in the minority of people who develop them they can be chronic. This raises the possibility that heavy alcohol consumption may precipitate an attenuated form of schizophrenia in vulnerable individuals. This possibility was however undermined by the findings of family and genetic studies, which failed to demonstrate a greater prevalence of schizophrenia in relatives of

patients with alcoholic hallucnosis.[270] This was taken to imply that genetic predisposition for the two conditions were independent from each other,[271] and there was thus little potential for alcoholic hallucinosis to shed light on the pathophysiology of schizophrenia. Instead, the basis of alcoholic hallucinosis was presumed to be subtle alcohol-induced damage or dysfunction, this possibly located in the temporal lobes.[272] Once this distinction between alcoholic hallucinosis and schizophrenia was apparently established, interest in alcohol as a risk factor for schizophrenia, (as well as alcoholic hallucinosis itself), became almost non-existent. As illustration of this, only a handful of either treatment or imaging studies have been undertaken in relation to alcoholic hallucinosis in the last decade. The limited imaging data relating to the condition will be discussed in Section 4.4.

Despite waning interest in alcoholic hallucinosis, a small number of studies have investigated if a history of alcohol abuse/dependence is associated with the subsequent development of schizophrenia. The largest study to explore this was undertaken by Lewis *et al.*, and used data from the Swedish conscript survey linked to the Swedish National Register of Psychiatric Admissions.[273] This is the same database previously discussed in relation to its application to exploring if a similar relationship existed with cannabis.[237] Lewis *et al.* report that there was an increase risk of schizophrenia in those with an ICD-8 diagnosis of alcohol abuse at age 18, this persisting after controlling for numerous confounders present at the point of initial assessment (including, for example, non-specific psychiatric symptoms and drug abuse). Similar findings were also reported in a subsequent study using Israeli data, though in this analysis drug and alcohol use disorders were analysed together.[274]

It is thus the case, on the basis of the limited data available, that alcohol use disorders do seem to be present in people destined to develop schizophrenia at a rate

greater than one would expect by chance. One potential explanation is that this is a consequence of self medication for prodromal features of schizophrenia. In contrast to the detailed analyses undertaken to exclude this possibility as an explanation for the comparable association seen with cannabis, such detailed analyses have not been undertaken for alcohol. It is interesting however that in the study of Lewis *et al.* the association between alcohol and abuse and schizophrenia remained significant even after controlling for non-specific psychiatric symptoms. The other possible explanation is that excessive or very early use of alcohol does indeed constitute a risk factor for schizophrenia. There is little additional published data to support this, but investigation of this possibility will be a focus of the current study.

A role for alcohol (as well as the other substances under investigation in this study) in the aetiology of schizophrenia is easily conceptualised within the framework of the stress-vulnerability theory of schizophrenia.[275] Indeed, this theory could also provide a model by which alcoholic hallucinosis and schizophrenia can be regarded as related disorders, despite findings from the aforementioned genetic and family studies suggesting that they are quite distinct. By the stress-vulnerability model, alcohol interacts with other risk factors for schizophrenia to increase an individual's vulnerability to developing the condition. In this context the finding that those with alcoholic hallucinosis have no family history of schizophrenia is actually not anomalous. As was previously discussed by Picchioni and Murray, this model would predict that as a consequence of heavy alcohol consumption some individuals (likely those with fewer susceptibility genes for schizophrenia) develop alcoholic hallucinosis, generally a temporary and self-limiting phenomena. Those with a greater predisposition for schizophrenia however, on exposure to similar or even lower levels of alcohol, may go on to develop a condition indistinguishable from classical

schizophrenia.[276] Thus, alcohol consumption could potentially both give rise to alcoholic hallucinosis in some individuals, and in others be a risk factor for schizophrenia.

3.2.2.1.3 Tobacco

Though illicit drugs have long been suspected as potentially contributing to the risk of schizophrenia, the possibility that such an association could exist with tobacco smoking has received little attention. This is likely attributable to the fact that tobacco is not associated with overt psychotomimetic effects. It is the case however that tobacco is the most commonly abused drug in people with schizophrenia and it does seem theoretically possible that repeated activation by nicotine of the mesolimbic system over a long time could precipitate the onset of psychosis in vulnerable individuals. It is also the case that increased rates of smoking *predate* the onset of schizophrenia (see Section 3.2.1.3), and that a positive association exists between the number of cigarettes smoked and the risk of developing the condition.[274] Though the latter association was not observed in the Swedish conscript study,[277] a study in the general population has demonstrated a prospective association between smoking and the subsequent self report of incident psychotic symptoms.[246] Additionally, a small study in an ultra-high risk cohort also suggested that cigarette smoking was associated with transition to schizophrenia, though this may have been confounded by cannabis use.[261] No data were identified suggesting that smoking had an adverse effect on the course of schizophrenia and, as reviewed in Section 3.2.1.3, there is some suggestion smoking has cognitive benefits in the condition. It is thus the

case that the possibility of causative association between smoking and schizophrenia must be considered cautiously and has sparse support in the published literature; it will however not be dismissed in my analysis of the high risk dataset.

Diagnosis of a psychotic disorder caused by tobacco use is theoretically possible within ICD-10, but no reports of such an effect were identified by a thorough literature search.

3.2.2.1.4 Other drugs

As was previously discussed in section 2.2, the association between amphetamines and psychosis was central to the formulation of the dopamine hypothesis of schizophrenia. That a relationship exists between these drugs and schizophrenia was reinforced by Section 2.1.4, which demonstrated that cross-sectional studies have generally reported that people with schizophrenia use amphetamines at a greater rate than the general population. As was done with cannabis however, consideration of the possibility that amphetamines contribute to risk for *schizophrenia* requires two further pieces of information: Firstly, how does the psychosis induced by amphetamines compare to schizophrenia; and secondly, does use of amphetamines increase the subsequent risk of schizophrenia. These issues will be considered for amphetamines below. Additionally, the same issues will also be considered for cocaine and MDMA (ecstasy).

Amphetamines

The psychosis associated with amphetamine use has been of great interest to schizophrenia researchers since its recognition. These early researchers soon recognised that it had a closer resemblance to schizophrenia than comparable conditions produced by other drugs. This led to it being regarded it as a 'model psychosis', with the potential to provide insights into the processes giving rise to schizophrenia.[75] Even in these early case series however it was reported that amphetamine psychosis and schizophrenia did display some differences; specifically, the researchers state that visual hallucinations are more prominent in the former condition, while thought disorder is absent. Interestingly, and as was the practice in the studies outlining cannabis psychosis, in three cases in which psychotic symptoms persisted, individuals were diagnosed as having schizophrenia. In the 'amphetamine psychosis' cases by contrast symptoms did not persist beyond 10 days.

Numerous other studies were undertaken in the 1960s and 1970s characterising amphetamine psychosis. They argued both in support of and against the presumption that many similarities exist between amphetamine psychosis and schizophrenia.[76] Over time however a consensus has emerged that though symptoms of acute amphetamine psychosis and schizophrenia are similar, visual and tactile hallucinations are indeed more common in the former condition.[278]

In recent years a derivative of amphetamine, methamphetamine, has been increasingly popular in America.[279] This drug is created by the addition of a methyl group to amphetamine, resulting in a product with greater lipophilicity than the parent drug. It can consequently achieve increased central nervous system concentrations and thus has an augmented relative potency.[278] Widespread use of this drug has been accompanied by concerns that it may be particularly neurotoxic, and may be

particularly liable to precipitate psychosis. Though large scale recreational use of methamphetamine is a relatively recent phenomenon in the West, it has a much more established history in Japan. In that country the potential for methamphetamine to produce persistent psychotic symptoms has long been recognised. Specifically, it has been reported that while the majority of cases of methamphetamine psychosis do resolve within a month, 11% persist beyond this,[280] and 5% had residual symptomatology many years later.[281] These chronic psychoses display considerable clinical overlap with schizophrenia, though affective flattening and alogia may be less pronounced.[278] Additionally, it does seem that in some cases in which resolution of psychotic symptoms does occur, the experience of psychological stress results in relapse in the absence of further drug exposure.[282] This phenomenon is believed to represents a sensitisation effect. Both of these findings indicate that use of methamphetamine can indeed result in sustained abnormalities, these persisting long after use of the substance has ceased.

In practice, and as was discussed in relation to cannabis, schizophreniform conditions which persist for a sustained period of time are eventually diagnosed as schizophrenia. Underpinning this practice is the central belief that drug induced psychoses are essentially most characterised by their transience. As discussed above however it does seem that, in vulnerable individuals at least, amphetamine (and particularly methamphetamine) use does have the potential to cause a more chronic psychotic condition which may be difficult to distinguish/indistinguishable from schizophrenia. The brain structural changes consequent to (meth)amphetamine use which potentially underpin this will be discussed in a later chapter, but these findings do provide support for the supposition that amphetamine/methamphetamine use does indeed have the potential to increase the risk of developing chronic psychoses. Indeed,

such a perspective is given further support by a recent study of Australian methamphetamine users. This reported that among individuals with no known history of schizophrenia, 18% had experienced a clinically significant psychotic symptom in the past year.[283]

Cocaine

Cocaine and amphetamines are both psychomotor stimulants, with broadly similar actions at the synaptic level (such as, for example, blocking reuptake of dopamine released from the meso-limbo-cortical dopamine terminals).[284] It is unsurprising therefore that cocaine, similar to amphetamine, can also induce psychotic symptoms. The development of frank psychosis appears to be more sporadic than is the case with methamphetamine, but symptomatology is similar. As was the case with methamphetamine, a variety of schizophrenic-type symptoms are reported but tactile and visual hallucinations are more pronounced than is the case in schizophrenia.[285] Protracted psychotic symptoms were more likely in those people with a prior history of psychiatric illness.

MDMA

MDMA (ecstasy) is a further amphetamine derivative, which became increasingly popular as an illicit substance in the UK in the 1990s.[286] Its effects on the central nervous system predominantly involve serotonin.[287] The potential for MDMA to induce psychotic symptoms has been recognised since shortly after its use became commonplace, at which time it was also suggested that in some individuals the experience of psychotic symptoms could be protracted.[286] Landbaso *et al.* recently published data on a cohort of 32 individuals who presenting with hallucinatory-

delusional symptoms after repeated consumption of ecstasy (and who did not consume other drugs).[102] At baseline schizophreniform symptoms such as hallucinations, conceptual disorganisation and blunted affect were common. Over the six month follow-up period these symptoms all improved substantially, though remained present to a mild extent in some individuals. When combined with case reports of persistent psychoses following MDMA use,[286, 288] it does seem that, (in vulnerable individuals at least), this drug does have the potential to cause protracted psychotic episodes. As always in this sort of research it is impossible to exclude the possibility that these individuals may have developed persistent psychotic symptoms irrespective of use of MDMA. The frequency of co-occurrence does however serve to make this explanation unlikely.

3.2.2.2 Evidence that various substances are associated with increased rates of schizophrenia: Findings from population-based studies

As reviewed above, data associating cannabis with the subsequent development of psychosis have come predominantly from cohort and experimental studies. There is an alternative approach to investigation of the link between substance use and schizophrenia however; namely to examine changes in population rates of psychosis and schizophrenia and to compare these to known trends in use of a specific substance. If it is indeed the case that exposure to a particular drug is a significant risk factor for schizophrenia, then an increase in levels of consumption of that drug by a given population would be expected to be associated with a

comensurate increase in psychosis incidence. If such an association was not seen, then this could call in to question the veracity of this putative association. Data investigating the association between population levels of particular drugs and psychosis incidence will now be discussed.

Association between population levels of cannabis use and incidence of psychosis/schizophrenia

It has been reported that cannabis use increased dramatically in many Western societies in recent decades, though this increase may have dropped off in recent years. Even in the face of such general trends however, and as discussed in Section 2.1, levels of substance use can vary greatly between different nations, or even regions within these nations. For investigation of associations between cannabis use and incidence of schizophrenia to be meaningful therefore, these trends must be investigated at relatively local levels.

In 1998 Boutros *et al.* reported that in Connecticut a rapid increase in new schizophrenia admissions coincided with a peak period for drug-related admissions.[289] This is the first study that I could identify which explored the association between these variables. It was based on first admissions data for drug abuse and schizophrenia/paranoid disorders from all Connecticut state hospitals from 1965 to 1983. A clear increase in schizophrenia/psychosis admissions is evident, beginning in the early 1970s and peaking in 1979. The authors suggest that increased drug use from the late 1960s may have contributed to the increase in first admissions of patients diagnosed with psychotic disorders some years later. Unfortunately, the authors do not explore associations between admission rate for use of *specific* drug classes and admissions for psychosis. Thus it is unclear which specific drugs were

responsible for the rise in drug abuse admissions that occurred concurrent with the rise in admissions for schizophrenia/paranoid disorders. It would be expected however, (on the basis of current clinical practice at least), that the proportion of these drug admissions specifically attributable to cannabis use *per se* would be relatively small. Conversely, cannabis was responsible for the bulk of the general increase in illicit drug use observed in the American population during this period.[290] Thus, it is conceivable that the increase in admissions for psychotic disorders and the increase in admissions for drug abuse occurred somewhat independent from each other, though both were attributable to the general increase in drug use in the American population; i.e. the increase in opiate use resulted in the increase in admissions for drug abuse, whereas the increase in cannabis use contributed to the increase in admissions with psychosis. Unfortunately, this possibility is not explored in the paper.

Subsequent studies have examined the association between levels of use and incident cases of psychosis specifically for cannabis. In one such study, Degenhardt and colleagues utilised Australian data acquired from birth cohorts dating from 1940 to 1979. During this period there was both a steep rise in both the prevalence of cannabis use and a marked decrease in the age of initiation of use. This was not accompanied by a corresponding increase in the incidence of schizophrenia, leading the authors to conclude that cannabis did not appear to be causally related to the incidence of schizophrenia.[291]

A more recent study by Ajdacic-Gross challenged the conclusion outlined above.[292] This study was based on an analysis of admissions for psychosis in Zurich between 1977 and 2005. In their overall analysis of the data, they report that first admission rates of patients with psychotic disorders were constant in men over this time period, and actually showed a downward trend in women. On looking at the data

stratified by age however, they demonstrate that distinct patterns are apparent in particular age groups. Specifically, they report that though in males there is actually a slightly decreasing trend in first admission rates in most age groups until the early 1990s, at this point this trend reverses for the youngest male age group, (those aged 15–19 years). Subsequently, after a 2-3 year time delay, this reverse is also seen in the 20–24 year olds. By contrast, a comparable trend reversal is not seen in the youngest female age groups, nor is it apparent in older age groups of either sex. In endeavouring to explain this pattern, the researchers discuss the fact that during the 1990s cannabis availability in Zurich increased substantially; specifically, lifetime prevalence of cannabis use in 15–16 year old teenagers rose from 15% (boys) / 5% (girls) in 1990 to 41% / 30% in 1998, the rise in consumption of the drug being most pronounced in this group. They discuss the fact that as young men are heavier consumers of cannabis than young women, the increase in psychosis admissions that is observed is occurring in exactly that group experiencing the most dramatic rise in cannabis consumption during this period. They conclude that these data provide further support for an association between cannabis consumption and first admissions with psychosis.

Hickman and colleagues looked at the same question using UK data, and reported results that are more equivocal.[293] No reliable data on changing rates of cannabis use over the period of interest are available in the UK. The authors instead relied on a single national survey in which people of different ages reported when they had first and most recently used cannabis and their approximate frequency of use between these points; from these data time trends in use by age could be constructed. The authors report that large increases in cannabis use occurred between the early 1970s and the late 1990s, but that the biggest increases among young people were

more recent. High-quality data on psychosis incidence were also relatively recent, and therefore a possible prior influence of cannabis on this incidence could only be modelled. Despite these limitations, the authors do report that to date an increase in incident rates of schizophrenia corresponding to the increased rates of cannabis consumption has not been observed. On modelling the relationship between the two factors they do suggest that this apparently contradictory finding may be explicable by the increases in psychosis attributable to cannabis use being less substantial, and consequently less noticeable, than some had assumed. Nevertheless however, they go on to say that if a truly causal relationship did exist then this would lead to larger increases in schizophrenia incidence, unlikely to be missed by reliable surveillance, by around 2010. They emphasised the importance of such an increase being detected by robust surveillance systems. They did also emphasise however that even if such an increase in incident cases of schizophrenia were not observed, this did not rule out the possibility that cannabis played an aetiological role. It is conceivable that a change in other factors (e.g. better obstetric care) could have masked or diminished the recent and future projected increases in schizophrenia occurrence due to cannabis. This could of course further explain why no change in overall schizophrenia trends has been observed despite increases in general population rates of cannabis consumption.

The most recent study addressing this issue was undertaken by Frisher *et al.*, utilising retrospective analysis of data from the General Practice Research Database for 183 practices in England, Wales, Scotland and Northern Ireland.[294] They investigated if a substantial rise in UK cannabis use from the mid-1970s was associated with changes in the annual incidence and prevalence of schizophrenia and psychoses from 1996 to 2005. They reported that between 1996 and 2005 the incidence and prevalence of schizophrenia and psychoses were either stable or

declining, and concluded that the specific causal link between cannabis use and the incidence of psychotic disorders was not supported.

In conclusion therefore, the findings from studies that have compared changes in population rates of psychosis and schizophrenia to known trends in cannabis use have been inconclusive. It must of course be remembered however that the ability to test this hypothesis is entirely dependent on the availability and quality of data on rates of cannabis use and psychosis in the population. Often these data are rudimentary, and of dubious quality. Additionally, and as discussed by Hickmen *et al.*, potential confounding factors abound. Thus, that any conclusion drawn from the data outlined above must be interpreted in a guarded manner.

Association between population levels of other drug use and incidence of psychosis/schizophrenia

As is apparent from the above, data exploring the relationship between population levels of cannabis use and incident rates of schizophrenia are sparse. Data exploring this relationship for alcohol and other illicit drugs are however even scarcer. I will now discuss these limited data; obviously the provisos discussed in interpretation of the studies outlined above apply equally to these studies.

A single study has investigated the association between the levels of alcohol consumption in a society and rates of hospitalisation for schizophrenia.[295] This was ascertained using American data spanning the years 1934 to 2005, and reported in letter form in 2009. The correlation between admission rates for schizophrenia and per capita absolute alcohol consumption was explored separately for beer, wine and distilled spirits. For first admission rates this association was explored separately for men and women; for total admission rates only combined male and female data were

available. To clarify the direction of the association, (i.e. the temporal order of changes in alcohol consumption levels and incident cases of schizophrenia), lag correlation analysis was employed.

Cawood and Bartko report that for distilled spirits the correlations were positive, significant, and always highest at zero lag years; this was the case both in males and females and for both first and total admissions. Correlation values for beer were positive and significant, but lower than those for distilled spirits. Those for wine were inconsistent. The findings suggest that alcohol use is a tenable risk factor for schizophrenia, acting close to the onset of schizophrenia, and that this association is stronger for distilled spirits than beer. The authors suggest that the greater effect from spirits than beer may be because consumption of concentrated alcohol overwhelms first pass metabolism, resulting in rapidly peaking high blood alcohol levels with damage to brain cells and neural connectivity in areas implicated in schizophrenia. Beer drinking involves the continuous administration of more dilute alcohol; thus, even if more total alcohol is consumed the process outlined may not occur, resulting in a weaker association with schizophrenia.

No data could be identified exploring the relationship between population levels of either smoking or amphetamine use and schizophrenia. In their study examining this association in relation to cannabis use, Ajdacic-Gross *et al.* did consider the possibility that an increase in ecstasy use was contributing to the increase in schizophrenia in young males observed in Zurich.[292] Overall however, and as outlined in the passage above, they placed greater emphasis on the possibility that these changes were attributable to increased levels of cannabis use.

Summary

In conclusion therefore, data demonstrating an association between changes in levels of alcohol/illicit drug use and a consequent effect on schizophrenia incidence are sparse. Those studies which have been undertaken are vulnerable to innumerable potential confounders, both positive and negative. It is therefore difficult to use these data to either confirm or refute suggestions that use of specific substances does affect the risk of the subsequent development of schizophrenia.

3.2.2.3 Impact of use of various substances on the course of schizophrenia

An additional way in which the association between drug use and schizophrenia can be ascertained is by investigating the impact of these substances on the course of the illness. Given the increased prevalence of use of a variety of licit and illicit substances in schizophrenia, information on the impact that such behaviours have on the course of the illness is of course important in itself. Such data can also however provide indirect evidence supporting or refuting the possibility that these substances contribute to the aetiology of condition. Specifically, if an environmental exposure was genuinely a risk factor for the condition, then it may be expected that continued exposure to that factor could have a detrimental impact on outcomes such as scores on symptom rating scales or relapse of psychosis. Conversely, if use of the substance had no influence on outcomes, then this could be interpreted as implying that a role in aetiology is less likely. Clearly interpretations such as these do have limitations. Specifically, it does remain conceivable that a factor which impacts on the

course of schizophrenia only exerts its detrimental influence once schizophrenia is established; conversely, a factor could genuinely increase risk of developing the condition, but have a benign effect on its course once schizophrenia is established. Additionally, it is also conceivable that substance misuse could have a detrimental impact on outcomes simply through being a predictor of non-compliance with medication. While accepting these provisos is important however, it does nonetheless remain the case that data examining the impact of substance use on the course of schizophrenia do remain important in achieving a comprehensive understanding of the relationship between these behaviours and the illness.

In Section 3.2.1 studies investigating the cross-sectional association between use of a variety of substances and symptom profile in schizophrenia was investigated. This demonstrated that substance misuse was generally associated with a greater severity of symptoms, which cast some doubt on the possibility that self-medication was driving the association between substance misuse and the condition. The current review will be limited to those studies investigating the *longitudinal* association between drug use and schizophrenia outcomes. Clearly such studies can much more reliably inform us that the use of drugs by people with schizophrenia is indeed impacting on the outcomes oserved. Initially I will briefly review data examining the impact of non-specific substance use on schizophrenia outcomes. Greater emphasis will however be placed on the impact that use of specific substances has on the course of the illness. In all of these reviews only studies which employed outcome measures directly relevant to illness severity (i.e. scores on symptom rating scales or relapse of psychosis/necessity for readmission) will be included. This is because other measures of adverse outcomes (for example, rates of violent offending), could be attributable to substance use *per se*, rather than directly reflecting symptom severity.

A small number of studies have compared readmission rates in substance misusing schizophrenic patients to those without such comorbidity. In one such study, readmission rates in 11 of the former patients were compared to those in 11 of the later over a period of at least two years.[296] All patients were receiving antipsychotic treatment through depot preparations, thus ensuring medication compliance. The mean number of readmissions in the substance misusing group, (in which cocaine, alcohol and cannabis were the substances predominantly used), was 2.5; this compared to 0.5 in the group which did not abuse substances. Active substance abuse was associated with significantly ($p<0.001$) higher readmission rates to the hospital because of symptom recurrence.

Subsequent studies have reported results comparable to those of Gupta *et al.* In an Australian prospective study, for example, Hunt *et al.* reported that substance use was associated with a shortening of mean time to next admission from a median of 37 months to 10 months.[297] The patients in this analysis were once again all reported as being compliant with medication (defined as regularly taking prescribed medication >75% of the time). Drake *et al.* have expanded further on these findings, reviewing studies exploring the impact of abstinence from alcohol/drug use on the course of schizophrenic illness. They report that if abstinence is achieved, then this is accompanied by a decrease both in scores on symptom rating scales and rates of hospitalisation.[298]

A small number of studies have addressed the impact of substance misuse on schizophrenic illness specifically in first episode subjects. The single study in which 80% or more of the subjects included had a diagnosis of a schizophrenia spectrum disorder found that substance misusers had higher scores on symptom rating scales at

a median follow up period of 14 months.[62] Studies in which fewer than 80% had a diagnosis of a schizophreniform disorder (though the vast majority did have this diagnosis), reported greater rates of psychotic relapse in the substance misusing group.[299, 300]

3.2.2.3.1 Impact of cannabis use on the course of schizophrenia

A number of studies have specifically looked at the impact of cannabis use on the course of schizophrenia. In one of the earliest reports, Negrete *et al.* reported that during a 6-month observation period subjects who used cannabis presented with a significantly higher degree of delusional and hallucinatory experiences than those who did not.[178] They also made a higher average number of visits to the hospital during the same period. A higher frequency of relapse in continuing cannabis users was also reported in a Spanish study published in 1994.[301] In the same year this issue was addressed using prospective methodology and attempting to control for confounders in a highly influential paper by Linszen *et al.*, who assessed 93 patients with a recent onset schizophrenic illness on a monthly basis for a year.[302] The 24 patients who were cannabis users both experienced earlier psychotic relapse, and had more frequent relapses in the year of follow-up than the patients who did not use the drug. Additionally, the authors reported a dose-response relationship between cannabis use and relapse; daily users relapsed earlier and more often than less than daily users who, in turn, relapsed sooner and more often than the patients who did not use cannabis. These relationships persisted even after statistical correction for

premorbid adjustment and use of alcohol and other drug use during the follow-up period.

Zammit *et al.* published a systematic review of the effect of cannabis use on outcomes of psychotic disorders in 2008.[303] Studies were included if they were longitudinal studies of people with psychosis, or case–control studies nested within longitudinal designs, where cannabis use was measured at a time prior to the outcome being measured. Studies were not excluded if they included individuals with affective psychoses, but in all studies the vast majority of individuals had schizophreniform disorders. As well as the outcome measures of relapse/readmission and scores on symptom rating scales, outcomes less directly related to symptom severity (e.g. harm to self or others, non-compliance with treatment) were also included.

Zammit *et al.* identified 13 studies meeting their inclusion criteria, of which only 7 looked only at people with schizophrenia (or schizophrenia-spectrum disorders). The seminal study of Linzen *et al.*, (mentioned above), was excluded as it was believed that the methodology employed could have resulted in the inclusion of individuals increasing or initiating cannabis use secondary to the outcome studied (i.e. relapse resulting in cannabis use rather than vice-versa). Given this concern, cannabis users were determined by cannabis use at baseline rather than during the follow-up period. Though it is probably the case that most cannabis users will continue to use the drug over the follow up period, given that some may stop using the substance this could result in a reduction in the strength of any associations seen. The variety in outcome and exposure measure definitions used, as well as the content of statistical results presented, meant that it was not possible to pool data in a meta-analysis.
In the reviewed studies, cannabis use was consistently associated with a greater risk of relapse or rehospitalisation. Associations with scores on symptom rating scales were

less consistent, but cannabis use was generally associated with higher positive symptom ratings. In the three studies in which the issue was addressed, cannabis use was reported as being associated with poorer treatment compliance. The reviewers discuss the issue of confounding at some length. They believe the two most likely sources of this to be use of alcohol and other drugs, and baseline illness severity and level of functioning (the latter, they report, could potentially lead to reverse causation effects). Of the 13 studies included in the review only 3 make any adjustment for use of other substances, and only five adjust for baseline illness severity. These studies did report a significant association between cannabis use and relapse/rehospitalisation. Though less emphasised in this review, an important additional factor which could confound the association between cannabis use and relapse/worsening symptomatology is of course treatment non-compliance. This was controlled for in the 1994 study of Martinez-Arevalo *et al.*, in which the association between cannabis use and relapse remained robust.[301] Generally however treatment non-compliance was regarded as a poor outcome in and of itself, rather than a factor directly responsible for the association between cannabis use and relapse/worsening of symptoms. The studies undertaken in patients on depots (described above) are clearly particularly informative in this regard; they clearly demonstrate that even in the context of full treatment compliance substance misuse can precipitate relapse.

3.2.2.3.2 Impact of alcohol use on the course of schizophrenia

In one of the earliest studies of the impact of alcohol use on outcomes of schizophrenia, Drake *et al.* reported in 1989 that alcohol use was strongly associated

with rehospitalisation.[304] In this prospective study of 115 patients with established schizophrenia, 68% of heavy users of alcohol returning to hospital at least once in the year following recruitment to the study, compared to 27% of abstainers. Similar findings have also been reported by Osher *et al.* in another American study.[305]

As in studies investigating the impact of cannabis use, in the reports outlined above no correction was made for the possibility that the association between alcohol use and poor outcomes was mediated by medication non-compliance. For this reason a 1999 report by Gerding *et al.* is particularly significant.[306] This study employed methodology similar to that utilised by Gupta *et al.* in their investigation of the impact of cannabis on schizophrenia relapse, including only patients being treated with depot preparations. This obviously ensured medication compliance, enabling the effects of alcohol use to be ascertained free of an important potential confounder. In keeping with the studies reviewed above, Gerding *et al.* reported that patients with alcohol dependence were more likely to be admitted during the two years of the study, had a greater total number of admissions and when they were admitted these admissions were longer. It does seem that the association between alcohol use and relapse of schizophrenia is indeed a robust finding, and exists independent of the effects of medication non-compliance.

3.2.2.3.3 Impact of tobacco use on the course of schizophrenia

Both the acute effects of tobacco on schizophrenic symptoms and studies comparing symptom load in schizophrenic smokers and non-smokers were reviewed

above. No longitudinal studies were identified investigating the impact of tobacco use on psychotic relapse. A single two-year prospective study has however reported that cigarette smoking at baseline was a significant predictor of suicidal behaviour.[307]

3.2.2.3.4 Impact of use of other drugs on the course of schizophrenia

Cross-sectional studies investigating the association between stimulant drugs and schizophrenia symptom severity were outlined in Section 3.2.1.4. Few studies have investigated the longitudinal association between use of these substances and schizophrenia outcomes however.

As previously discussed, given the association between substance use and medication non-compliance, studies in patients treated with depot antipsychotic preparations are of particular importance. Approximately half of the subjects in the aforementioned study of Gupta *et al.* of patients receiving depot antipsychotics used cocaine, indicating that this drug is indeed associated with psychotic relapse.[296] An additional study undertaken in people with schizophrenia treated with depot antipschotics provides even more compelling data however. It compared rates of hospitalization in depot treated subjects who specifically abused cocaine to people without this comorbidity.[308] It demonstrated that patients maintained on depot neuroleptics who abuse cocaine are hospitalized at significantly higher rates than nonabusing patients similarly medicated. Though comparable data for amphetamines are not available, given their comparable acute effects, similar findings would be expected for this class of drug. It thus seems to be the case, despite the claims that amphetamines can have beneficial effects on the symptoms of schizophrenia (Section

3.2.1.4), that in reality recreational use of these drugs (as well as cocaine) has an adverse effects on outcomes in the condition. The effects of MDMA (ecstasy) use on the course of schizophrenic illness has not been specifically investigated.

3.2.3 Common causation

The final possibility that has been proposed to explain the association between substance misuse and schizophrenia is common causation; i.e. the same risk factors give rise to both conditions. This hypothesis argues that if disorders are predominantly the result of a set of risk factors and these are the same or similar for two disorders, then 'comorbidity' reflects the fact that the pathways by which people develop one disorder are the same as those by which they develop another.

There is much to suggest that this hypothesis may have some relevance from the data already discussed. For example, one compelling reason is that, as is evident from Section 2.1, people with schizophrenia misuse a diverse range of substances rather than the condition being consistently associated with a particular pharmacological group of licit or illicit drugs. Indeed, the drugs used by an individual tend to reflect (though levels of use exceed) general patterns of drug use in the population in which the patient resides. This is at odds with both the self medication and drug use as aetiological factor hypotheses. If all excessive drug use was to be explained by the former hypothesis, then it would be expected that drugs with particular pharmacological or symptomatic effects would predominantly be used, which does not seem to be the case. Similarly, if the latter were the complete explanation the

association, the drug use/schizophrenia relationship would again be expected to be particularly strong with particular substances as only specific drugs would be expected to have an effect on aetiology.

Thus, on considering the above, it does seem that people with or destined to develop schizophrenia may have an innate vulnerability to drug use in general. The common factors which might potentially contribute to the development of both disorders are biological, personality and social/environmental variables. These will each be discussed in turn.

3.2.3.1 Biological

Genetic

There is a good deal of evidence to suggest that genetic factors contribute to an individual's risk of schizophrenia [309] and to substance use disorder.[310, 311] It has also been reported that a genetic propensity for substance misuse can be shared across substances.[312, 313] A handful of studies have specifically addressed the question of whether schizophrenia and substance misuse share a common genetic vulnerability, the primary method employed in elucidating this being examination of family history. For such a link to be supported, studies would be expected to find that patients with schizophrenia have more relatives with substance use disorders than people in the general population or that people with substance use disorder are more likely to have family members with schizophrenia. The studies that have been conducted to date have however been conflicting. Though one study did report that dually diagnosed patients are more likely to have family members with substance use disorders than

patients with schizophrenia alone,[141] other studies have not found this to be the case.[314, 315] In summary, while it is possible that genetic vulnerability may contribute to the development of comorbid substance use in some patients, the available evidence does not appear to support the idea that the high rates of comorbidity observed occur as a result of a common genetic basis for both disorders.

Neuropathology

As reviewed in Section 2.3, the dopamine hypothesis retains an important role in our understanding of the genesis of schizophrenia. Central to this theory, even in its most recent incarnation, is the idea that functional hyperactivity of a network of dopamine neurones projecting to the nucleus accumbens plays an important role in the genesis of psychotic symptoms.[110] It is the case however that this system, the mesolimbic dopamine system, has also been central in our understanding of the development of drug dependency. Indeed, it is now firmly established as the major neural substrate for the reinforcing effects of drugs of abuse (see Section 1.2 and Koob & Le Moa[316]) The fact that the core features of both substance dependence and psychotic symptomatology rely on the same neural substrate offers a parsimonious explanation for the observation that the two conditions co-occur more often than would be expected by chance. It has led to speculation that the excess of substance misuse observed in schizophrenia may actually arise as a consequence of the neuropathology associated with the condition impacting on the neural circuitry mediating drug reward and reinforcement. As a consequence, this theory would suggest, people with schizophrenia are biologically more vulnerable to the rewarding effects of drug abuse and thus at increased risk of addictive behaviour.

This hypothesis was first coherently formulated by Chambers *et al.*[317] They emphasised the failings of the self-medication hypothesis of comorbidity, and proposed that schizophrenia and substance use are actually independent manifestations of the same disease. Utilising findings from human and animal studies, they hypothesized that abnormalities in hippocampal-cortical function in schizophrenia impair the inhibitory hippocampal projections to the nucleus accumbens, resulting in reduced inhibitory control over dopamine-mediated functional hyperresponsivity to dopamine release. In this model, dysregulated neural integration of dopamine and glutamate in the nucleus accumbens resulting from frontal and hippocampal dysfunction could lead, in subjects without prior drug exposure, to neural and motivational changes similar to those in long-term substance use. Thus, the dopamine dysregulation innate to having/being at risk of developing schizophrenia also renders these people are particularly susceptible to substance misuse problems. Rather than substance misuse arising as a result of self-medication, Chambers *et al.* argue, a predilection to drug use is in fact a primary disease symptom.

Impairment in cognitive functioning.

The possibility that people with schizophrenia use substances for their cognitive benefits was discussed in Section 3.2.1. Impairment in cognitive functioning has also been proposed as a common factor that may increase risk for both substance use disorder and schizophrenia.[318] Such impairment could reflect genetic factors, early environmental factors, or a combination of both. The evidence is mixed that cognitive deficits are predictive of later development of substance use disorders; low IQ has been associated with increased risk in some studies,[319, 320] but not in others.[321, 322]

Conversely, there is strong evidence that cognitive impairment increases risk of developing schizophrenia.[323] Thus the possibility that cognitive impairment is a common risk factor for both conditions cannot be excluded, and should ideally be controlled for in prospective studies investigating putative associations between substance misuse and the subsequent development of schizophrenia.

3.2.3.2 Personality

Blanchard *et al.* proposed in 2000 that the prevailing understanding of comorbid substance use in schizophrenia was constrained by a failure to examine individual differences in personality, stress and coping.[324] They proposed that it was these enduring individual differences, rather than transitory psychotic symptomatology, that were likely to place an individual at a sustained increased risk of substance misuse. The essence of this theory is that certain personality traits are associated with both schizophrenia and substance misuse, and that it is consequent to this association that the two conditions co-occur more commonly than would be expected by chance. In constructing this theory Blanchard *et al.* focussed primarily on the two personality dimensions they reviewed as having considerable empirical support for being associated with substance misuse in the general population; namely, negative affectivity/neuroticism (NA/N) and disinhibition/impulsivity (DIS/IMP).

NA/N is characterized by the experience of general negative mood states. [325, 326] It is associated with decreased tolerance for stress; lowered threshold for experiencing negative affects; the tendency to dwell upon and magnify mistakes, frustrations, and disappointments; and to have negative appraisals of self and

others.[326] Conversely, individuals high in DIS/IMP are oriented towards feelings and sensations of the immediate moment and are less concerned by future implications of behaviour. Impulsivity, irresponsibility, risk taking, norm rejection, and danger seeking have been identified as core features of DIS/IMP.[327] NA/N and DIS/IMP have been found to be largely independent personality dimensions, each with its own unique correlates.[325, 327] Both of these personality traits have been found to be associated with the development of substance use disorders in longitudinal studies.[328-330]

For Blanchard *et al.*'s theory to hold, both of these personality dimensions would also have to be over-represented in people with schizophrenia. On considering NA/N first, it has indeed been demonstrated that people with schizophrenia exhibit high negative affectivity, and that this is a stable characteristic.[331] This alone of course does not exclude the possibility that this elevated negative affectivity simply arises as a consequence of having a schizophrenic illness (rather than been associated with increased risk of it). Emphasising the primacy of this characteristic however, elevated neuroticism has also been found to be a feature of preschizophrenic adolescents,[332] and the healthy relatives of people with schizophrenia.[333] Interestingly, and of particular relevance to the current study, anxiety-type symptoms were also noted to be prominent in high risk subjects even at the point of recruitment in to the EHRS (when subjects were all clinically well).[334] That the association between negative affectivity and substance misuse remains relevant in the context of schizophrenia has also been specifically investigated. Evidence to support this was reviewed in Sections 3.1 and 3.2.1 and is further outlined by Blanchard *et al.*[324]

Data identifying disinhibition/impulsivity as a risk factor for substance misuse problems are particularly robust, being supported by numerous independent

longitudinal studies.[335, 336] The available data once again suggest that a comparable association exists with schizophrenia, longitudinal methodology demonstrating that impulsive, undercontrolled behaviour in childhood does characterize individuals destined to develop schizophrenia.[337, 338] Once again, such an association also receives some support from the EHRS. In this study the related characteristics of delinquent-aggressive behaviour in adolescents at risk of schizophrenia was found to be a significant predictor of later schizophrenic illness.[339] As was the case with negative affectivity and schizophrenia, it is important to establish that that disinhibition/impulsivity remains associated with substance misuse in the specific context of schizophrenia. The available evidence does suggest that this is indeed the case,[340] and rates of substance misuse are certainly increased in people comorbid for schizophrenia and antisocial personality disorder.[341]

3.2.3.3 Social and environmental factors

A number of social and environmental factors that could potentially underpin both disorders have also been hypothesized. Economic and social disadvantage, for example, are widely accepted as being associated with both substance abuse and schizophrenia. Thus, (as some studies reviewed in Section 2.1 did), it is important to match cases and controls for these criteria when comparisons are made of prevalence of substance misuse in people with schizophrenia to that in other groups. Another possible mechanism linking the two, rarely considered, is traumatic early childhood experience. We know that members of the general population who report physical or sexual abuse in childhood are more likely to abuse substances in adulthood [342] and

that, for some, childhood abuse can also contribute to psychosis.[343] Scheller-Gilkey *et al.* compared 70 patients with schizophrenia and a history of substance abuse with 52 patients without a history of substance abuse and found that the former had significantly higher scores on a measure of childhood traumatic events and on a PTSD scale.[344]

3.3 Synopsis of data investigating relationship between drug use and schizophrenia

The wealth of data outlined above confirms that, as may be expected from what is known about substance misuse in the general population, the reasons why a particular individual with schizophrenia has a substance misuse problem are complicated. Self medication and shared vulnerability factors may both play a role, and the extent of this role would be expected to vary both between individuals and between different substances. Nevertheless, evidence that some substances may actually contribute to risk of developing the condition does remain compelling. The evidence for such an association is strongest for cannabis, the drug which likely also has the least evidence of there being any desirable consequences for use in schizophrenia. Conversely, there is evidence that tobacco smoking may ameliorate some of the cognitive deficits seen associated with schizophrenia, and there is little evidence that it increases risk of the condition. Other substances fall somewhere in the middle; alcohol for example may transiently reduce the anxiety associated with schizophrenia, and so use may be driven by a degree of self medication. Evidence that

alcohol may contribute to the risk of schizophrenia is relatively weak, but the possibility is notably under-explored.

In considering whether use of a particular substance could contribute to the risk of developing schizophrenia it is important to consider the consequences that that drug can have for brain structure. If use of a drug can give rise to structural brain changes then it both makes it more biologically feasible that it can give rise to a psychiatric illness such as schizophrenia. This may be particularly the case if the structural abnormalities seen are similar to those associated with the condition. Additionally, if the brain structural abnormalities consequent to use of a particular substance associated with increased risk of schizophrenia (e.g. cannabis) can be characterised, then this may give clues to the nature of the pathophysiological process by which use of the substance confers this risk. Data addressing these issues will be reviewed in the following chapters.

Chapter 4

Review of structural imaging findings

4.1 Structural imaging findings in substance misuse

The data investigating reasons for the relationship between substance abuse and schizophrenia were discussed in Chapter 3. As was apparent from this review, there is considerable evidence that cannabis use contributes to the risk of developing schizophrenia. Evidence supporting such an association for other substances is weaker, but is nonetheless conceivable for amphetamines and possibly also cocaine, ecstasy and alcohol. As discussed in Section 3.2.2 demonstration of these associations is however fraught with difficulties; furthermore, it is also unlikely that further cohort studies of a similar design, (cohort studies being the most robust study design that can feasibly be employed in humans to demonstrate these associations), will significantly advance our understanding of these relationships.

If substance use does result in an increase in psychosis risk, then this must be mediated by biological changes arising as a consequence of substance use. Logic dictates that underpinning these biological changes must be abnormalities in brain structure or function. Though it is of course conceivable that these could be undetectable with currently available technology, brain imaging does at least have the potential to detect these abnormalities. Thus, while Type II errors remain a distinct possibility, demonstrating that habitual use of drugs of abuse was indeed associated with structural brain changes could substantially enhance our understanding of this process. It would strengthen the case that use of these substances increased an individual's risk of psychiatric pathology, and potentially suggest the mechanism or mechanisms by which this occurred. Furthermore, if these abnormalities transpired to be similar to those seen in schizophrenia, then this may inform our understanding of how use of this substance may result in an increased risk of this condition.

As ever, a number of provisos must first be acknowledged however. As will become apparent from the discussion that follows, much of the imaging data in this area are from cross-sectional studies. Consequently, they can tell us little about causation. It is frequently assumed that any differences observed when imaging findings in substance-misusing populations are compared to those in controls arise entirely as a consequence of the effects of substance use on the brain. This is obviously not the only feasible explanation for this association however, and the possibility that structural abnormalities predate substance misuse and are themselves contributing to the risk of substance misuse must of course be considered. In teasing apart these possibilities, longitudinal studies are of particular value.

An important further consideration is multiple substance use, an issue which is increasingly the rule rather than the exception. Given its prevalence, it has the potential to be an important confounder of any apparent specific substance/structural imaging abnormalities which are observed. It is thus important that any studies which are included in the following discussion have ascertained the extent to which this is an issue in their study populations, and state whether or not it has been controlled for.

With these considerations in mind, I will now review data examining the structural brain imaging abnormalities associated with habitual use of the drugs of abuse commonly encountered in the UK. I will begin with a brief review of imaging findings associated with drug use in general, and then go on to discuss findings in relation to specific substances.

4.1.1 Imaging findings associated with increased risk of alcohol/drug use

It is widely acknowledged that substance use disorders tend to aggregate in families, and that genetic factors play a major role in this. For example, family, twin, and adoption studies have convincingly demonstrated that genes make a substantial contribution to the development of alcohol dependence, with heritability estimates ranging from 50 to 60 percent for both men and women.[345] Though less well researched, it has also been reported that there is a strong genetic basis to cannabis abuse and dependence,[346] and the concept of there being a general genetic propensity to drug use has increasingly gained credence.[347] Though specific factors may well differ for different substances of abuse, these genetic studies do therefore suggest that some of an individual's risk of developing an addiction problem is innate, and present from birth. This being the case, it may be that some of this increased risk is reflected in abnormalities of brain structure which are associated with an innate propensity to substance use (and are present in the absence of the effects that use of these substances subsequently have on the brain). As relatives of people with substance misuse problems share many of the same genetic characteristics, then these abnormalities would also be expected to be apparent in the relatives of people with substance misuse problems. Thus, the most obvious way to identify if brain structural abnormalities are associated with a propensity to drug use (while avoiding the possibility that substance misuse by these individuals is itself contributing to any imaging abnormalities seen), would be to image individuals who are relatives of people with substance misuse problems but have never used/only used at low levels the substance in question. This approach does have a major flaw however. Namely, selecting individuals on the basis of not exhibiting problematic use of a substance

raises the obvious concern that they *do not* share the propensity of their affected relatives to problematic use of the substance in question (so explaining why they have not developed a problem). Consequently they may not actually display any of the imaging abnormalities postulated to be associated with problematic use of the substance. If this was the case they would obviously tell us little about the imaging characteristics *which are* associated with substance use.

One way potential way around the above problem is to utilise identical twins, but this is itself fraught with its own difficulties, and no such studies could be identified. An alternative approach, analogous to that employed in the EHRS in relation to schizophrenia, is to scan people who are at risk of substance use, but have not yet reached the age at which this behaviour would be expected to begin. If they are compared to individuals without this propensity, then this may indeed inform us of the imaging findings associated with this innate predisposition. This model of study does also has its own potential pitfalls; for example, could any abnormalities seen actually be consequent to antenatal substance exposure, to which these individuals are likely to be at greater risk? Nevertheless, this study design does have the potential to provide useful information.

A small number of studies have indeed employed the latter approach, all of which have focussed on individual at high risk of alcohol dependence. These studies have exclusively been undertaken by two research groups, Hill *et al.* in Pittsburgh and Benegal *et al.* in Bangalore. Hill *et al.*'s initial study compared amygdala and hippocampal volume in 17 male individuals at high risk of alcohol problems on the basis of family history to 17 matched control subjects with no such history.[348] Mean age of the former group was 17.6, and the latter group 17.3. After controlling for current alcohol consumption (as well as a number of other variables), they reported

that the at-risk subjects exhibited significantly reduced volume of the right amygdala. No difference was observed in total grey or white matter volumes, the left amygdala or either hippocampi. In subsequent studies of the same data set, cerebellar and orbitofrontal cortex volumes were compared between the two groups. They reported a tendency for cerebellar volume to be increased in adolescents and young adults at high risk for alcohol use disorders,[349] and that right orbitofrontal cortex volume was significantly reduced in this group (in the context of their being no difference in total orbitofrontal volume).[350] Hill *et al.* also discuss that age regression of total grey matter volumes suggested a slower reduction in grey matter volumes among the offspring of alcoholics during adolescence. The authors suggest that this may indicate a delay in grey matter pruning or slower maturational increases in white matter.
The studies of Benegal *et al.* focussed on a slightly younger high risk group, mean age of high risk subjects being approximately 15. In contrast to the report of Hill *et al.*, Benegal *et al.* reported that cerebellar volume was *decreased* in their study of high risk alcohol-naive subjects which employed both region of interest and voxel-based morphometric analyses.[351] This study also noted that when compared to controls, high risk subjects also had decreased grey matter volume in the thalamus, superior frontal gyrus, and cingulate gyrus. A subsequent study by the same group compared corpus callosum morphology in the high and low risk subjects.[352] Total corpus collosal area, and that off the genu and isthmus regions were significantly smaller in high-risk than low-risk subjects after controlling for age and intracranial area.

The findings outlined above require replication, and the suggestion of some contradictory results in the findings of the two groups, (most notably in relation to cerebellar volume) suggests they may not be robust. Nevertheless, these studies do underline the importance of always considering the possibility that not all of the

abnormalities detected in the brains of alcoholics are necessarily a consequence of alcohol exposure. Given the lack of data, the relevance of these findings to users of other drugs is a matter of speculation.

4.1.2 Imaging findings associated with general drug use

In the review below I will discuss data relating to the use of specific drugs of abuse. Prior to this there are however a number of studies of drug use in general/multiple substance use which are important to mention. Firstly, is a 2006 study of adolescent recreational of users of both alcohol and cannabis undertaken by Yucel *et al.*[353] This employed linear regression analyses to establish if use of alcohol and cannabis use were predictive of hippocampal, amygdala and whole-brain volumes. They reported that use of cannabis and alcohol at an earlier age were independently predictive of larger amygdala volumes. By contrast, longer duration of cannabis use was predictive of smaller hippocampal volumes. Secondly, a case controlled study was undertaken comparing total volumes of grey and white matter, frontal grey and white matter, both lateral ventricles, and cerebral spinal fluid in 16 male substance abusers (mean age 38.8) to 16 controls.[354] Substance abusers used a variable combination of cannabis, cocaine, methamphetamine and heroin; however, neither substance abusers nor control subjects were currently abusing alcohol or had any previous history of alcohol abuse or dependence. Substance abusers had significantly less frontal white-matter volume percentage than controls, but there were no significant differences in any of the other brain volumes measured. The authors acknowledge that the cross-sectional nature of the study means that it can not

distinguish whether the difference in frontal lobe white matter is explained by a direct neurotoxic effect of drug use on white matter, a pre-existing abnormality in the development of the frontal lobe or a combination of both effects.

4.1.3 Associations between use of specific drugs and brain imaging abnormalities

Data examining structural imaging abnormalities associated with use of specific substances will be detailed below. A number of themes will recur in review of these studies. Firstly, concurrent use of other substances is a common feature, and the extent to which studies acknowledge/model for this does differ. Thus, the possibility that results are confounded by use of other substances must always be kept in mind. Second is the fact that the majority of studies that have been undertaken are cross-sectional in design. This means that though authors frequently assume that any abnormalities observed in association with substance use are *caused* by that substance use, the cross-sectional design (in association particularly with the findings of Benegal *et al.*, see above), means that this can be questioned. Where available longitudinal studies will be emphasised, given the important data they can provide on direction of causation.

4.1.3.1 Cannabis

Two reviews of imaging studies investigating structural and functional brain imaging findings in cannabis users have been published in recent years. The first of these was published by Quickfall and Crockford in 2006,[355] while the second was published by Martin-Santos *et al.* in 2009.[356] Both studies employed a comprehensive search strategy. While inclusion/exclusion criteria were relatively lenient in the Quickfall and Crockford study, they were more restrictive in the study of Martin-Santos *et al.* The specific inclusion/exclusion criteria employed in the latter study were that structural imaging studies had to: comprise subjects at least 18 years of age; match cases and controls for age, sex and handedness; not include participants with any other psychiatric or neurological disorder; and that participants did not have other substance use disorders. Martin-Santos *et al.* identified eight structural imaging studies evaluating the effects of chronic cannabis use. Five of these utilised volumetric methodology, while the remaining three were diffusion tensor imaging (DTI) studies.

To identify relevant studies published after the search undertaken by Martin-Santos *et al.* I employed search criteria comparable to that which they employed. Obviously the publication period was extended, studies published up to May 2010 being included. This methodology identified only two additional studies of relevance; a study by Mata *et al.* comparing cerebral gyrification in cannabis users to people with no history of illicit drug use,[357] and a study by Medina *et al.* comparing prefrontal cortex volume in cannabis users to controls.[358] These studies, together with those identified by Martin-Santos *et al.*, are summarised in Table 4.1 below.

Study	Method	Gender Users/ controls (M:F)	Mean age (S.D.) U/C	Image analysis	Level of drug use in users	Significant findings
Block *et al.* (2000)[359]	sMRI	9:9/ 6:7	22.3 (0.5)/ 22.6 (0.5)	Voxel-based ROI	Mean use 18 times/week. Excluded if history of dependence on alcohol or other illicit drugs	Nil, including a lack of difference in the specifically investigated hippocampi
Tzilos *et al.* (2005)[360]	sMRI	22/26	38.1 (6.2)/ 29.5 (8.5)	Voxel-based ROI	Mean (SD) of 20,100 (13,900) lifetime episodes of smoking	Nil, including a lack of difference in the specifically investigated hippocampi
Matochik *et al.*. (2005)[361]	sMRI	11:0/ 8:0	25.4 (5)/ 29.7 (4.7)	Voxel-based morphometry	Minimum duration of use 2 years, currently use four or more times per week	Increased density: Precentral and R thalamic grey matter L hippocampal and fusiform, R lentiform and brain stem white matter Reduced density: R parahippocampal grey L parietal white
Gruber *et al.* (2005)[362]	DTI	8:1/ 8:1	26 (3.6)/ 26.2 (3.1)	Voxel-based ROI	Lifetime use at least 4000 and tested positive for urinary cannabinoids	Nil
Jager *et al.*. (2007)[363]	sMRI	13:7/ 13:7	24.5 (5.2)/ 26.2 (3.1)	Voxel-based morphometry	Median lifetime use 1,900 joints; range 675–10,150	Nil (Analysis restricted to (para)hippocampal regions)
DeLisi *et al.* (2006)[364]	sMRI DTI	9:1/ 9:1	21.1 (2.9)/ 23.0 (4.4)	Semi-automated MRI Voxel-based ROI	Began use prior to age of 18 and had used more than 21 times in any single year	Nil, including a lack of difference in the specifically investigated amygdala-hippocampal complex No findings indicating impaired integrity of white matter tracts in cannabis users
Yucel *et al.* (2008)[365]	sMRI	15:0/ 16:0	38.8 (8.9)/ 36.4 (9.8)	Semi-automated ROI	Consumed >5 joints a day for >10 years	Decreased volume: L/R hippocampus L/R amygdala
Arnone *et al.* (2008)[366]	DTI	11:0/ 11:0	25.0 (2.9)/ 23.3 (2.9)	Tract-based spatial statistics	Daily use for a minimum of two years	Mean diffusivity increased in prefrontal regions of corpus callosum, suggesting impaired structural integrity of fibre tracts in this region.
Medina *et al.* (2009)[358]	MRI	12:4/ 10:6	18.1/ 18.0	Semi-automated ROI	Used in past month and >60 lifetime marijuana experiences	Only compared volume of prefrontal cortex in two groups. After controlling for lifetime alcohol use, gender, and intracranial volume, cannabis users did not differ from controls in PFC volume
Ashtari *et al.* (2009)[367]	DTI	14:0/ 14:0	19.3 (0.8)/ 18.5 (1.4)	Voxel-wise and fiber tractography analyses	At least 3 months drug free, had been using daily prior to that for at least one year (mean use 5.8 joints/day	Reduced fractional anisotropy and increased radial diffusivity in arcuate fasciculus (links fronto-temporal regions) bilaterally. [note: users drawn from offender population, controls from medical clinic]
Mata *et al.* (2010)[357]	MRI	23:3/ 25:19	25.7 (5.0)/ 25.8 (5.8)	Automated volumetry Cortical gyrification	Mean(SD) duration of regular use 8.4 (9.4) years, mean cumulative total joints 11618.9 (9386.6)	No difference in volume of frontal grey matter, parietal grey matter and temporal grey matter Cannabis users showed bilaterally decreased concavity of the sulci and thinner sulci in the right frontal lobe.

Table 4.1
Studies investigating structural brain imaging abnormalities in cannabis users

My discussion of the available data on structural imaging abnormalities associated with cannabis use will centre on the studies outlined above. In addition however, I will also include a number of studies not meeting these criteria, but of note for historical or other reasons.

The earliest study to employ a case-control design to compare structural imagine findings in subjects who did and did not use cannabis employed

pneumencephalography and was published by Campbell *et al.* in 1971.[368] Though it suggested the presence of enlarged ventricles in the cannabis using subjects, these findings were confounded by a number of variables; not least amongst these, for example, was the fact that many of the patients were initially referred with heterogeneous neurological complaints. As pneumencephalograpy was superseded by computer tomography in the 1970s, so this technology was applied to scanning the brains of cannabis users. Interestingly however, only one study could be identified employing this technology to anything even approximating a standard case-control design. This, a study undertaken by Hannerz and Hindmarsh, compared certain brain imaging characteristics in cannabis smokers to those from normal people who did not use the drug.[369] Specifically, the means of measures such as transverse diameter of the lateral horns and the largest diameter of the third ventricle were compared between the two groups. In contrast to the pneumencephalography findings Hannerz and Hindmarsh report that no differences were identified between cases and controls. The sole exception to these findings was one cannabis-using subject who reported daily alcohol abuse; in his case the researchers felt there was unquestionably pathology.

The next significant advance in neuroimaging was MRI, studies employing this technology being summarised in Table 4.1. With the exception of the three DTI and a single GI study, the reports included in the table compare either direct (e.g. semi-automated ROI) or proxy (e.g. voxel-based morphometry) measures of structure volume in cases and controls. This thus constitutes six relevant studies undertaken in adults investigating the impact of cannabis on brain structure volume. None of the studies reported significant differences in global measures of brain volume. In two of the studies however, significant regional differences are found. Firstly, Matochik *et al.* found that cannabis users had reduced grey matter volume in the right

parahippocampal gyrus, while this was conversely increased in the right thalamus.[361] More recently, Yucel *et al.* reported bilateral volumetric reductions in the hippocampal and amygdalar areas in a group of 15 chronic cannabis users compared to controls.[365] These findings do suggest that if brain structural abnormalities are associated with cannabis use they are most likely to be identified in the region of the hippocampus. Such speculations aside however, what is most apparent from these data is that, (in keeping with what was suggested by early CT studies), there is currently no consistent evidence for either global or regional volumetric differences between cannabis users and normal controls.

In addition to the volumetric studies, three of the studies in Table 4.1 compared DTI findings in cases and controls. DTI is a technique used to examine the integrity of white matter tracts, quantifying white matter structural integrity in terms of mean diffusivity (MD) and fractional anisotropy (FA). MD quantifies the magnitude of water diffusion in each image voxel, while FA quantifies the directionality and coherence of white matter fibre tracts. When boundaries to water diffusion are reduced, (i.e. white matter structural integrity is impaired), MD increases while FA decreases. Two of these studies found no differences between cannabis users and controls.[362, 364] The third study reported a significant increase in mean diffusivity, but no decrease in fractional anisotropy associated with cannabis use, in the prefrontal section of the corpus callosum.[366] Finally, the most recent study by Ashtari *et al.* reported reduced fractional anisotropy, increased radial diffusivity, and increased trace in the arcuate fasciculus (tracts underpinning fronto-temporal connectivity).[367] This study does need to be interpreted with considerable caution however, as there are important potential confounders; of note, cannabis users were dawn from an offender population (unlike the controls), and alcohol intake was not

controlled for. Importantly, these considerations meant it was in fact excluded from the review of Martin-Santos *et al.* Thus, and similarly to the findings from the volumetric/VBM studies demonstrating minimal differences in regional grey matter volumes, the DTI studies do not provide strong evidence of major cannabis effects on the integrity of white matter fibres.

Finally, there is the single study comparing gyrification in cannabis users and controls.[357] The main findings of this study were that cannabis users had bilaterally decreased concavity (more flattening) of the sulci, and thinner sulci in the right frontal lobe. The authors suggest that the most likely explanation is that cannabis use during adolescence may affect normal neurodevelopment, especially in the prefrontal cortex, leading to a pattern of decreased gyrification with less concave and thinner sulci. They do acknowledge however that multiple hypothesis testing means that the possibility that the results are attributable to a type I error cannot be excluded.

On considering the above data, it is apparent that at present there is no consistent evidence of brain structural abnormalities being associated with cannabis use. Where single studies have identified regional brain volume loss (e.g. Yucel detecting hippocampal volume loss), these have not been identified in other studies specifically investigating that brain region (e.g. Block and Tzolis), and must therefore be treated with scepticism. Unsurprisingly, this is also the conclusion reached by other groups who have reviewed these data. While meta-analysis may have the potential to reveal some subtle effects, the heterogeneous nature of the studies undertaken to date means this is not feasible at the present time.

4.1.3.2 Alcohol

4.1.3.2.1 Review of findings across age groups

Alcohol is Western society's favourite drug, and chronic, heavy use has long been recognised as resulting in catastrophic cognitive impairment. Given this, it is perhaps unsurprising that studies investigating the structural imaging consequences of alcohol abuse and dependence are considerably more numerous than those investigating these associations in people who abuse cannabis. Many of these studies have been undertaken in older alcoholics with very many years of alcohol dependence. The subjects included in these studies have also often been rather heterogeneous. This issue is particularly important in this area, as brain changes in those who have experienced the most catastrophic sequelae of alcohol dependence (e.g. Korsakoff's psychosis) are likely to be substantially different from those in people who have drunk heavily, but not experienced such a dramatic event. Thus, while findings in these older populations are clearly important, they may have limited relevance when considering the impact of alcohol use on structural imaging measures in younger people with much shorter alcohol use histories. Given these considerations, though important data arising from studies undertaken in older individuals will be considered in this review, its primary focus will be the impact of alcohol use on brain structure in people under the age of forty. These data are obviously of particular relevance to the current study, concerned as it is with a population in late adolescence/early adulthood.

The early history of brain imaging of alcohol dependent subjects was reviewed by Krill *et al.* in 1999.[370] In this review they discuss the heterogeneity of the studies

reviewed and the fact that differences in the manner in which the heavy alcohol using groups are defined, (e.g. mean consumption over a certain threshold per day versus fulfilment of DSM-IV criteria for alcohol dependence), may also produce fundamental differences in the populations being studied. Additionally, they note that sequelae resulting from uneven drinking patterns (e.g. binge drinking) have rarely been addressed, but that this may result in different pathology due to the higher blood alcohol levels attained during these periods. Current drinking status is also an important consideration, as some of the brain abnormalities associated with alcohol use are know to correct with cessation of drinking.

As reviewed by Krill *et al.*, the first studies to demonstrate brain shrinkage in alcoholics achieved this by employing pneumoencephalography to identify ventricular enlargement.[371] Following computerized tomography becoming available in the 1970s, these initial findings were confirmed and extended.[372, 373] Indeed, these CT studies demonstrated not only that alcoholics without overt clinical signs of brain damage had larger ventricles, wider cerebral sulci and wider Sylvian and interhemispheric fissures; additionally, application of a longitudinal study design demonstrated that with abstinence some resolution of these abnormalities could occur.[374]

With the introduction of MRI in the 1980s, more accurate determination of the structural abnormalities associated with alcohol use could be achieved. This modality has the potential to delineate discrete neuroanatomical structures, meaning that the regional changes giving rise to brain shrinkage can be elucidated. Initially MRI studies in alcoholics further confirmed the findings of CT studies, noting ventricular enlargement and that this was party reversible following abstinence.[375, 376]With

improved MRI resolution, so the capacity of this technique to measure and compare the volume of specific brain structures did begin to be utilised however.

Much of the MRI imaging work in alcohol dependence was undertaken by Sullivan and Pfefferbaum, who recently reviewed MRI findings in chronic alcoholism in a 2005 publication.[377] This review primarily addresses 'uncomplicated' alcoholism, by which is meant alcoholics free of the severe syndromes arising from alcohol-associated nutritional deficiencies or electrolytic imbalance. As well as Korsakoff's psychosis these clinically dramatic conditions include Marchiafava–Bignami disease, which primarily affects the corpus callosum and results in a disconnection syndrome; central pontine myelinolysis, which affects myelin in the central pons and can cause paraplegia; and alcoholic cerebellar degeneration, which results in severe ataxia of gait and posture. Sullivan and Pfefferbaum note that the application of quantitative MRI analysis techniques to scans from these 'uncomplicated' alcoholics reveals that both cortical grey matter [378, 379] and white matter sustain widespread volume loss,[380] which is greatest in the prefrontal cortex and white matter.[321, 381] They also report that in most brain regions and structures examined, an age-alcoholism interaction is observed, older alcoholics having greater volume shrinkage for their age than younger alcoholics. In one study, this interaction was shown to be independent of the possibility that older alcoholics had the opportunity to drink more alcohol for a longer time than younger alcoholics.[380]

As well as these cortical changes, Sullivan and Pfefferbaum also note that subcortical and brainstem structures are also affected in uncomplicated alcoholism. Interestingly, the brain structures affected are the same as those affected in the clinical syndromes outlined above (albeit to a lesser degree), and include the corpus callosum,[382, 383] pons,[384] cerebellar hemispheres, and vermis.[385] On considering the

brain abnormalities in Korsakoff's syndrome, the amnesia has traditionally been attributed to lesions of the thalamus and mammillary bodies,[386] but more recently was also related to hippocampal volume loss.[387] Similarly to the other specific syndromes, more subtle volume loss has also been detected in these regions in non-amnesic chronic alcoholics. Notably, this has been reported in the mammillary bodies,[388, 389] anterior hippocampi,[390] and thalamus,[377] as well as in other regions including the caudate, putamen and nucleus accumbens.[391]

Subsequent to the review of Sullivan and Pfefferbaum, Marinus Verbatun undertook a review of MRI findings in studies of those termed 'low to moderate' or 'social drinkers' published from 1997 to December 2006.[392] By his usage, the term 'social drinking' referred to people who were not dependent on alcohol, but could be consuming up to 40 drinks a week (the upper limit of consumption for inclusion in the review). The particular focus of the review however was on the effects of 'moderate drinking', which they defined as consuming up to 21 standard glasses of alcohol a week. Only seven studies were found fulfilling their inclusion criteria (necessitating, for example, data on age, number of drinks consumed etc.). These are summarised in Table 4.2. Findings were generally rather inconsistent. Nevertheless, in two studies a significant linear relationship was found between brain shrinkage and alcohol consumption, even when the latter was at non-dependent levels. Specifically, increasingly wider sulci and/or increasingly larger ventricles were reported as amount of consumed alcohol increased in two populations with mean ages of 57[393] and 75.[394] Other studies however (e.g. Kubota *et al.*[321]) reported that age was the main factor responsible for brain shrinkage, and that only heavy alcohol consumption (35 or more UK units per week) increased such normally occurring shrinkage significantly. On the basis of these (and a small number of other studies , included in Table 4.2), the

authors concluded that even the consumption of light and moderate doses of alcohol led to shrinkage of the brain, with *increases* in white matter volume but decreases in grey matter volume. On reviewing the included studies however, the central data supporting this conclusion comes from the observation of dose response relationships.[393-395] Extension of these findings to moderate drinkers is actually rather questionable, as many of the subjects driving these associations were actually drinking at 'heavy' rather than 'moderate' levels. Even more concerning, the conclusion drawn in relation to white matter is clearly at odds with that reported in studies of dependent subjects, summarised by Sullivan and Pfefferbaum. Furthermore, these conclusions are actually not supported by a number of studies included in the review, e.g. Kubota *et al.*[321] Despite these concerns, it is notable that a large American study (mean age 60.6) published after this review once again found a negative linear association between alcohol consumption and reduced brain volume (unfortunately, grey and white matter were not investigated separately); this study did indeed suggest that moderate alcohol consumption (8-14 drinks a week) may well be associated with reduced brain volume.[396] No other relevant recent studies were identified on undertaking a literature search, and given the current state of the literature it is my impression that the evidence that moderate alcohol consumption is associated with structural brain imaging abnormalities is equivocal at best.

In interpreting all the above findings, it is also important that the influence of age is not forgotten. All the discussed studies were undertaken in people who were middle aged or older, and the applicability of findings to younger individuals is questionable.

Authors	Gender M/F	Mean Age	Imaging modality employed, and how volume change identified	Number in each drinking category (drinks/week)				Association with increasing alcohol consumption (for males and females)			Notes
				Zero (0)	Light (<7)	Mod 7-21	Hvy >21	Vent. Vol.	White matter volume	Grey matter volume	
Mukamal *et al.*. (2001)[394]	1624/1752	75.1	MRI. Subjective grading of ventricular volume	1294	1131	304	136	M↑ F↑	-	-	
Kubota *et al.* (2001)[321]	1061/371	49.1	MRI. Widening of CSF space used as proxy for frontal lobe volume	667	157	362	246	M↑ F↑	-	-	
Ding *et al.*. (2004)[393]	761/1148	57.0	MRI. Subjective grading of ventricular volume	742	615	210	-	M↑ F↑	-	-	'Mod' category includes people drinking >21/week
Taki *et al.*. (2004)[397]	356/413	46.4	VBM	Numbers not specified per drinking category				-	M↓ lPoCG	M↓ rSFG, lMOG, lPrCG, lMIG, lCuneus	
Den Heijer *et al.*. (2004)[398]	260/249	73.0	MRI. Manual tracing of amygala and hippocampus	72	145	72	70	-	-	No assoc. (amyg. and hipp.)	
De Bruin *et al.*. (2005)[395]	44/47	49.7	VBM	-	30	32	34	-	M↑ rFG, R parietal region	M↓ rFG, R parietal region	Asscs. with mean lifetime alcohol intake
Anstey *et al.*. (2006)[399]	211/174	62.0	MRI. Automated tissue segmentation and manual tracing of ventricles, amygdala, hippocampus, corpus callosum	Numbers not specified per drinking category				M↑	M↓ F↓	M↑	No sig. assoc. with manually traced structures

Table 4.2.
Studies identified by Marinus Verbatun investigating structural imaging abnormalities in non-dependent drinkers.
Table has been modified from original publication, and additional relevant data extracted from the included publications.
Key: SFG: superior frontal gyrus; MOG: middle occipital gyrus; PrCG: precental gyrus; PoCG: postcentral guys; MIG: middle inferior gyrus; rFG: right frontal gyrus (Brodmann area 6)

Longitudinal studies

A substantial proportion of the longitudinal MRI studies investigating the impact of alcohol use on brain structure have again been undertaken by Sullivan and Pfefferbaum. Their controlled longitudinal MRI studies of alcoholics in recovery or relapse have revealed that with short-term (about 1 month) abstinence from alcohol cortical grey matter increases in volume. With longer-term abstinence (about 1 year), the third ventricle shrinks, but with relapse it expands and white matter shrinks.[400] Over a 5-year interval, the degree of excessive drinking in alcoholics is related to the degree of cortical grey matter loss, especially in the frontal lobes.[401] Additional

studies have suggested that cortical white matter volume may be particularly amenable to recovery with abstinence,[402, 403] but it remains vulnerable to further decline with continued drinking.[400] Although the mechanism for either volume loss or restoration with abstinence remains unclear, Sullivan and Pfefferbaum discuss that it probably involves changes in both myelination and axonal integrity in white matter and glial and dendritic changes in cortical neuropil.[377]

Diffusion Tensor Imaging

Sullivan and Pfefferbaum also discuss the DTI data for brain microstructural compromise in chronic alcoholism. As discussed in relation to cannabis, DTI is a technique used to examine the integrity of white matter tracts. Given the apparent propensity for alcohol consumption to be particularly associated with white matter volume loss, then it would be expected that this imaging technique would be sensitive to the detection of abnormalities consequent to alcohol consumption.

Sullivan and Pfefferbaum report that their studies using DTI in uncomplicated alcoholism found abnormally low anisotropy in regionally defined white matter of alcoholic men[404] and women.[405] As discussed in relation to cannabis, this indicates that white matter structural integrity is impaired in these regions. They note specific deficits in both men and women in the callosal genu and centrum semiovale, and additional deficits in the callosal splenium in men. Pericallosal white matter was also affected.[404] In women, the white matter abnormality identified with DTI went undetected with structural MRI,[405] emphasising the greater sensitivity of DTI to detection of abnormalities in these structures. In interpreting these results, Sullivan and Pfefferbaum once again emphasise the importance of an age-alcoholism

interaction, citing findings that older alcoholics have greater abnormalities for their age than younger ones in support of this.[377]

Arnone *et al.*, in their 2006 report, review DTI studies specifically investigating the corpus callosum.[366] By the time of this review data from two further studies were available, both of which were undertaken by or involved Pfefferbaum's group.[406, 407] This review confirms white matter pathology in the corpus callosum in association with alcoholism, studies consistently reporting reduced FA which is most pronounced in the genu followed by the splenium. Effects in the genu were more pronounced in women, whereas those in the splenium were restricted to men. As previously discussed, the finding of reduced FA suggests that the integrity of white mater tracts in these regions has been compromised. Given that the genu and the splenium of the corpus callosum connect left and right frontal sites and parietal and occipital sites respectively,[408] this localisation implies that it is connections between these brain regions which are compromised. It is notable that these findings are occurring in similar regions to those in which MRI reported deficits in alcoholics; though DTI is more sensitive to white matter deficits, it does thus seem that there is indeed overlap between the regions detected as abnormal with the two techniques.

Post mortem findings

Sullivan and Pfefferbaum also discuss MRI findings in alcoholism in relation to post mortem studies of people who have had the condition. Findings are consistent, these studies reporting white matter abnormalities in brainstem and subcortical structures, including the cerebellar vermis,[409] mammillary bodies,[410] hippocampus,[411] and corpus callosum, which thins [412] and atrophies.[413] Although white matter

pathology is reported more often than grey matter pathology, neuronal loss does occur but is restricted to the superior frontal cortex.[414-416]

4.1.3.2.1 Systematic reviews of structural brain abnormalities in young alcohol abusers

The mean age of all subjects included in the studies addressing structural brain imaging abnormalities in cannabis users was under 40. By contrast, in the studies undertaken in alcohol dependent/heavy alcohol users the mean age was generally at least late 40s, and often participants were in their 70s. The greater age of these subjects implies that they had many years of exposure to alcohol, and so findings from these studies may not be relevant to younger populations (such as are the focus of the current study). This issue is particularly relevant given the robust age-alcohol interaction which has been repeatedly stated.

Given the above, I felt it important to conduct a systematic review of studies specifically addressing structural imaging findings in younger age groups. Such studies should give a better indication of the 'pure' effects of alcohol on the brain, minimising the effect of confounders such as cerebrovascular disease and the influence of an age-alcohol interaction. Such a review is acutely needed; despite the studies investigating if structural brain abnormalities predate use of alcohol in high risk subjects (see above), data investigating the presence (or otherwise) of imaging abnormalities in younger populations of alcohol abusers/dependent subjects have not been synthesised. In undertaking such a review, the immediate question is what age constitutes young. Given that it has long been accepted alcohol-related brain changes

are evident by the fifth decade of life,[380] the cut off age I have taken for 'young' is 40. Furthermore, studies undertaken in people under 40 could either be in young adults or adolescents (i.e. people under the age of 18). As the effects of alcohol on the brains of these two age groups could potentially differ, my review will comprise of two components. The first will identify studies of alcohol abusing subjects (and controls) with a mean age of less than 40, but greater than 18. The second will focus on adolescents, participants in included studies having a mean age of less than 18.

Systematic review in young adults

Methods for systematic review

Methodology for both systematic reviews was identical other than the selection of age range of interest. Searches were performed on Medline (1970-May 2010), EMBASE (1970-May 2010) and PsychInfo (1970- May 2010). The following search terms were used: alcohol abuse OR alcohol dependence OR alcoholism OR alcohol drinking AND neuroimaging OR brain imaging OR computerized tomography, CT OR magnetic resonance imaging, MRI OR diffusion tensor imaging, DTI. Both free-text and expanded medical subject headings were used. Subject headings were adapted to the specific subject headings of the biomedical databases used. The search strategy was supplemented by inspecting the reference lists of included articles.

I was primarily interested in case-controlled studies (expecting these to be present in the greatest numbers); however, cohort studies are clearly of great value, and any identified will also be discussed. For inclusion studies had to utilise CT,

structural MRI, or DTI scanning methodologies. Additionally, subjects with specific neurological syndromes, even if directly related to alcohol use (e.g. Korakoff's syndrome), must have been excluded from the study and subjects must not have been chosen on the basis of cognitive deficits or other adverse sequelae of alcohol use. Similarly, studies were not regarded as eligible for inclusion if subjects had been identified by any specific clinical characteristics (e.g. antisocial traits); this was felt important as selection on the basis of such characteristics could potentially confound findings. Studies employing any generally accepted, quantitative form of image analysis technique directly comparing tissue volume/density or integrity in the two groups were acceptable; it was expected the majority of studies would utilise either ROI-based approaches or VBM. Inclusion criteria for case-controlled studies were inclusion of a control group of healthy volunteers. For inclusion in the review of young adults mean age of alcohol using subjects had to be greater than 18 but less than 40. For inclusion in the review of adolescent studies mean age had to be greater than 14, but less than 18. Studies undertaken only in people in these age ranges were identified by limiting searches to the appropriate age ranges. This had to be modified for different search engines, and in some search engines (e.g. in EMBASE), the only adult age limits available included all people aged 18-65.

Methods for young adult systematic review

Primary research studies were considered for inclusion if they were published as a peer-reviewed article in English and compared a sample of adults (aged 18-40) with an alcohol problem (abuse or dependence) with a group of healthy controls. Studies could either compare the groups cross-sectionally, or compare changes over time. If both models of analysis were included in a single study, both analyses will be

reported. Additionally, if different studies have used the same patient sample but investigated different brain structures, then both reports will be included.

Results for young adult systematic review

The search outlined above identified 1458 potentially relevant studies, abstracts of which were assessed for inclusion. On review of the abstracts, one-hundred and thirty articles warranted retrieval in full text form for evaluation. Study flow and reasons for exclusion are summarised in Figure 4.1. Unfortunately, there were substantial difficulties in obtaining some of the older studies, this being a problem predominantly for CT studies undertaken in the 1970s. Given that the structural brain changes that would be expected in young alcohol abusing subjects would be expected to be relatively subtle, it was thought to be doubtful that these studies would add much to the data provided by more recent MRI studies. It was also apparent from an earlier review of CT studies, that few of these would actually meet the inclusion criteria;[417] notably, only a single case-control study included in this review focused on the age group of interest, and this did not employ a quantitative methodology.[418] Thus, if difficult to obtain it was believed reasonable to exclude these older CT studies, a fact which do not believe to be significantly detrimental to the review as a whole.

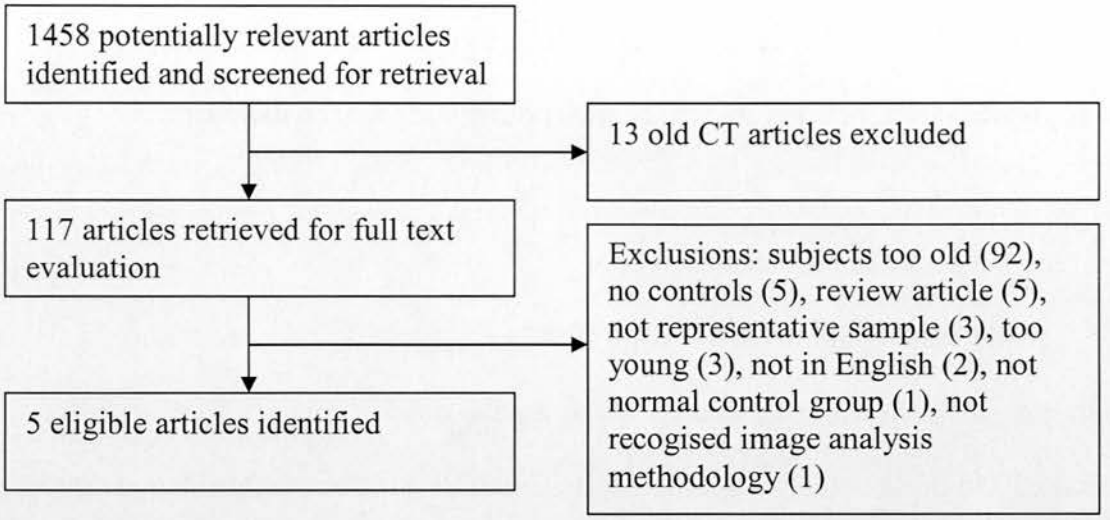

Figure 4.1
Study flow and reasons for exclusion in systematic review of structural imaging findings in adult problem drinkers aged under 40.

Five studies were thus identified which fulfilled the inclusion criteria. All were cross-sectional, and are summarised in Table 4.3. Common reasons for studies being excluded were: too old, difficult to obtain CT study; possibly relevant study but unobtainable; not employing quantitative structural methodology; mean ages of either alcohol using subjects and/or controls exceeding 40; duplicate publication of the same data; review article; and no control group/not normal controls. Structural data were extracted from all included studies and recorded along with a description of the image analysis technique employed, the type and unit of measurement (area or volume) and characteristics of the subject and control groups which possibly confounded any observed differences (including age, gender ratio and use of other substances).

Study	Imaging modality	Image analysis	Gender A/C (M:F)	Mean age (SD) A/C (M/F)*	Inclusion criteria	Drinking status	Use of other subs	Structures compared	Findings
Hommer *et al.* 1996[383]	MRI	Semi-automated ROI	13:14/ 10:9	M: 39.3(7.2)/ 37.1(5.6) F: 38.9(6.7)/ 35.9(4.7)	Inpatient or outpatient treatment for DSM III-R AD. No evidence psychotic or cognitive disorder	Abstained at least 10 days	Excluded if use of any other substance abuse disorder in past 6 months or any history of intravenous drug use	Cross-sectional area of corpus callosum	Corpus callosum area was significantly smaller in alcoholic women than control women. No significant difference was observed in alcoholic men. This association was unchanged when intracranial volume was controlled for.
Pfefferbaum *et al.*. 1997[381]	MRI	Semi-automated ROI	33:0/ 65:0	37.5(4.5)/ 32.9(6.5)	Hospitalised for alcoholism (meeting RDC for same)	4 weeks after admission	Excluded if met RDC for substance abuse other than alcohol within the past year	Total grey and white matter volumes; ventricular volumes; prefrontal, frontal, anterior superior temporal, posterior superior temporal and occipital regional volumes	Lateral and third ventricular enlargement. Significant grey matter deficit in fontal cortex. No differences in white matter volumes.
Agartz *et al.* 1999[419]	MRI	Semi-automated ROI	26:26/ 17:19	M: 36.9(6.2)/ 35.7(8.2) F: 37.4(5.6)/ 35.6(7.9)	Hospitalised for DSM-III-R AD No history psychosis, DTs or dementia	Abstained for at least 3 weeks	Excluded if used in preceding 6 months (except tobacco)	Hippocampal volume Non-hippocampal brain volume	R hippocampus smaller in AD men and women. L hippocampus smaller In AD women. Non-hippocampal brain volume only reduced in women
Fein *et al.* 2002[378]	MRI	Voxel-based ROI	24:0/ 17:0	38.7(9.0)/ 30.0(5.2)	Recruited from community, treatment naïve, but meeting DSM-IV-R criteria for alcohol dependence	Not stated. Likely drank shortly before scan	Excluded if history of substance abuse other than alcohol	15 cortical regions of interest, encompassing whole of cortex	After adjustment for ICV alcohol dependent had significantly reduced total cortical, posterior prefrontal, dorsolateral prefrontal, lateral parietal and mesial parietal grey matter volume. Only findings in posterior and dorsolateral prefrontal regions remained significant in analysis of age-balanced subset
Agartz *et al.* 2003[420]	MRI	Semi-automated ROI	40:14/ 17:3	38.3/39.3	Hospitalised for DSM-III-R AD No history psychosis, DTs or dementia	Abstained for at least 3 weeks	Excluded if used in preceding 6 months (except tobacco)	Volume of total grey and white matter, hippocampi and lateral ventricules Corpus callosum area	After adjustment for WBV reduced grey and white matter and corpus callosum area.

Table 4.3
Studies investigating structural imaging abnormalities in adult problem drinkers aged under 40

AD= alcohol dependence; DTs= delirium tremens

*If genders have been analysed separately, mean age is displayed separately for each gender. Otherwise mean age is combined.

The brain imaging abnormalities observed in uncomplicated alcohol dependence are generally regarded as predominantly a consequence of the toxic effects of the substance of the brain. It would be expected therefore that the extent of abnormalities seen would be a function of both duration of exposure and total amount of alcohol consumed. This being the case, it is unsurprising that the magnitude of abnormalities seen in the younger subjects included in this review are less pronounced than in reports from older populations. Nevertheless, even in these younger populations some themes do emerge. Specifically, it does seem that even in these younger individuals grey matter deficits may be seen in the prefrontal cortex (this possibly being particularly the case in the dorsolateral region).[378, 381] Volume reduction of the hippocampi is much more contentious; though Agartz *et al.* did detect this in one study, they did not detect it in another.[419, 420] While one possibility is that this inconsistency may reflect the different gender balance of these two studies, with women being particularly vulnerable to this effect, these conflicting findings do suggest that any abnormalities that do exist are subtle. Corpus callosum area was reduced in two studies, though this was a consistent finding only in women, again suggesting that they may be more susceptible to the effects of alcohol on the brain.

Systematic review in adolescents

Primary research studies were considered for inclusion if they were published as a peer-reviewed article in English and compared a sample of adolescents (aged 14-18) with an alcohol problem (abuse or dependence) with a group of healthy controls. A small number of studies specifically focused on binge drinking; these were also

included. As with the young adult systematic review, studies could either compare the groups cross-sectionally, or compare changes over time. If both models of analysis were included in a single study, then both can be included. Additionally, if different studies have used the same patient sample but investigated different brain structures, then both reports will be included.

Results for the adolescent systematic review

The search outlined above identified 209 potentially relevant studies, abstracts of which were assessed for inclusion. Thirty five articles were retrieved in full text form for evaluation. Study flow and reasons for exclusion are summarised in Figure 4.2. Five studies were identified which fulfilled the inclusion criteria; all were cross-sectional, and are summarised in Table 4.4. Examples of reasons for studies being excluded were: mean ages of either alcohol using subjects and/or controls exceeding 18, duplicate publication of the same data and study focusing on a high risk rather than alcohol abusing/dependent population. Structural data were extracted from all included studies and recorded along with a description of the image analysis technique employed, the type and unit of measurement (area or volume) and characteristics of the subject and control groups which possibly confounded any observed differences (including age, gender ratio and use of other substances). Before considering the findings of the systematic review, a small number of studies not meeting criteria for inclusion in the review but nonetheless of relevance to subsequent discussion must be considered.

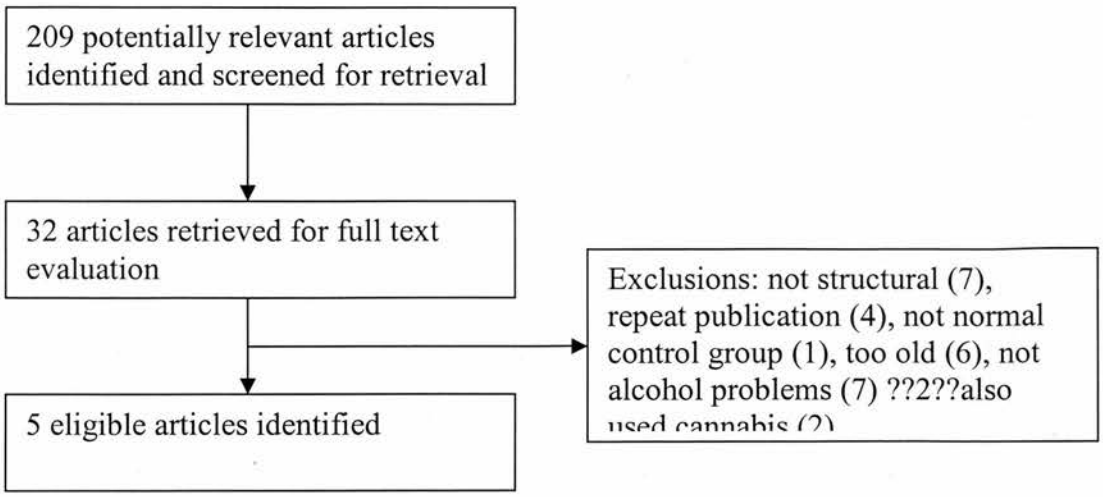

Figure 4.2.
Study flow and reasons for exclusion in systematic review of structural imaging findings in adolescent problem drinkers.

Firstly, in 2003 Tapert *et al.* reported findings from a small pilot study which used diffusion tensor imaging to investigate corpus callosum microstructure integrity among 8 teenagers with AUD and 8 non-abusing controls.[421] This was presented at a conference rather than being published in a peer reviewed journal, and so is not included in the table below. Nevertheless, this low powered study reported a trend towards lower FA in the splenium in the alcohol abusing group (0.79 ± 0.03 versus 0.83 ± 0.04, $p = .055$). This trend was weaker in the body of the structure, ($p = .084$) and not present in the genu ($p = .650$). A second study of note is that of McQueeny *et al.*, not eligible for inclusion in the review as the mean age of alcohol using subjects was 18.09.[422] Nonetheless, given the young age of the subjects (the majority of whom were actually under 18), it seems more appropriate to include it here than in the discussion of adult studies (above). This was a DTI study focusing on 14 adolescents with a history of binge drinking, which they defined as consumption of at least 5 or 4 alcoholic beverages (for males or females, respectively) in one sitting during the 3 months prior to imaging. Controls were 14 teens without a history of a binge drinking.

Neither group had a history of abuse or dependence on alcohol or any other drug. Binge drinkers had lower FA than controls in 18 white matter areas throughout the brain, including the corpus callosum, superior longitudinal fasciculus, corona radiata, internal and external capsules, and commissural, limbic, brainstem, and cortical projection fibers. They exhibited no areas of higher FA. In-keeping with the pilot study of Tapert *et al.*, this study suggests that exposure to large doses of alcohol during youth may compromise white matter tract coherence.

Two further studies compared white matter integrity in alcohol users who also used cannabis. Tapert *et al.*'s group found that when binge drinking individuals were compared to those who binge drink *and* use cannabis, abnormalities were actually *less* pronounced in the dual substance abusing group.[423] A subsequent report from the same group reported a rather mixed picture of FA abnormalities in dual substance using subjects compared to controls; in a number of predominantly left sided regions FA was reduced (e.g. left superior longitudinal fasciculus, left postcentral gyrus), while in several exclusively right-sided regions it was increased (e.g. anterior limb of internal capsule).[424] Data extracted from the five studies eligible for inclusion in this systematic review are summarised in Table 4.4. On considering the totality of data available on structural brain imaging in adolescents with alcohol use disorders it is apparent that studies are few, and that this limits the conclusions which can be drawn.

The few DTI studies in this population are characterised primarily by their inconsistencies, though it may be that adolescent drinkers do exhibit reduced FA in certain regions of the corpus callosum. The four volumetric studies also present rather contradictory data, though prefrontal white matter may be reduced in females, and reduced left hippocampal volume is reported in two studies each undertaken by independent researchers. Hippocamapal volume reduction was not reported in

individuals at high risk of alcohol use disorder,[348] suggesting that this abnormality may well be consequent to alcohol exposure rather than being a trait characteristic of people vulnerable to such conditions. As was previously discussed in relation to data from young adults, it may be the case that women are particularly vulnerable to these effects.

Study	Imaging modality	Image analysis	Gender A/C (M:F)	Mean age (SD) A/C (M/F)*	Inclusion criteria	Drinking status	Use of other subs	Structures compared	Findings
De Bellis *et al.* 2000[425]	MRI	Semi-automated ROI	5:7/ 10:14	17.2 (2.2)/ 17.0 (2.4)	Substance misuse diagnoses made using modified version of DSM-IV. 7 lifetime alcohol dependence, 5 lifetime alcohol abuse. Majority comorbid Axis 1 disorders, most commonly depression, conduct disorder or PTSD	No use for 2 weeks prior to scan	Majority (9) history of cannabis abuse or dependence. Smaller numbers had used other substances	Total grey and white matter volumes. Amygdala, hippocampal and lateral ventricular volumes. Corpus callosum area	Reduced L and R hippocampal volumes. No difference total grey and white matter volumes, amygdalar volumes or corpus callosal area.
De Bellis *et al.* 2005[426]	MRI	Semi-automated ROI	8:6/ 16:12	17.0 (2.1)/ 16.9 (2.3)	DSM-IV alcohol abuse or dependence. Majority had Axis I comorbidity, most commonly depression or conduct disorder	No alcohol use in 12 hours preceding scan	Majority of AUD group (11) also had history of cannabis use disorder. Small numbers had used other substances, but not within two weeks of scan	Total cerebral volume, total prefrontal cortex volume, grey and white matter prefrontal cortex volume, volume of thalamus, brainstem, right and left cerebellum	Reduced total prefrontal cortex and prefrontal cortex white matter volumes.
De Bellis *et al.* 2008[427]	MRI DTI	Voxel-based ROI	25:7/ 17:11	16.9 (1.2)/ 15.9 (1.1)	Recruited from treatment centres. Met DSM-IV criteria for lifetime alcohol dependence or current alcohol abuse. Majority had Axis I comorbidity, most commonly depression or conduct disorder	Mean (SD) number of days since last consumed alcohol 63.7(88.2)	Majority (24) of AUD group also had history of cannabis use disorder. Small numbers had used other substances	Corpus callosum divided in to seven regions: rostrum, genu, rostral body, anterior midbody, posterior midbody, isthmus and splenium	FA values in the CC rostral body higher in the AUD group than in the control group, though not significant after adjusting for age and sex ($p = 0.09$). Also higher in the isthmus in this group, which was significant after controlling for age and sex
Medina *et al.* 2008[428]	MRI	Semi-automated ROI	9:5/ 5:7	M: 16.6 (0.7)/ 16.6 (0.7) F: 17.1 (0.6)/ 16.5 (1.0)	Met DSM-IV criteria for alcohol abuse or dependence. No additional DSM-IV diagnosis (aside from mild to moderate conduct disorder)	Abstinent from use of alcohol or other drugs for 5 days	AUD group did not differ from controls in lifetime use of other drugs, recent marijuana use, or marijuana abuse/dependence criteria.	Prefrontal cortex volume. Total and divided into following regions: posterior, anterior dorsal, and anterior ventral. Also white matter volume divided in to same suregions.	Whole group comparison: no significant difference in prefrontal cortex volume (whole or white matter) between groups. On comparing gender subgroups however, females with AUD demonstrated smaller PFC volumes, while males with AUD had larger PFC volumes. The same pattern was observed for PFC white matter volumes.
Nagel *et al.* 2005[429]	MRI	Semi-automated ROI	9:5/ 5:7	16.8 (0.7)/ 16.5 (0.9)	Met DSM-IV criteria for alcohol abuse or dependence. No additional DSM-IV diagnosis (aside from mild to moderate conduct disorder	Abstinent from use of alcohol or other drugs for 5 days	AUD group did not differ from controls in lifetime use of other drugs, recent marijuana use, or marijuana abuse/dependence criteria	Whole brain grey and white matter volumes. Hippocampal volumes	Reduced left hippocampal volume

Table 4.4.

Studies investigating structural imaging abnormalities in adolescent problem drinkers.

AUD = alcohol use disorder; encompasses either alcohol abuse or dependence; AD= alcohol dependence

*If genders have been analysed separately, mean age is displayed separately for each gender. Otherwise mean age is combined

4.1.3.2.3 Summary of findings in younger populations of alcohol abusers

The main findings from these studies are that any brain abnormalities detected in young populations of alcohol misusers are subtle, this being in some contrast to the gross and repeatedly reproduced abnormalities seen in older populations. If any abnormalities are apparent in these younger populations they seem to be most detectable in the hippocampi and frontal cortex, and may be more obvious in women. On balance however, it is questionable if abnormalities would be expected even in these brain regions in younger populations of even quite substantial alcohol misusers. Given the subtle nature of any abnormalities which are/are not present, meta-analysis of these data would be very useful. Unfortunately however, once again the heterogenous nature of the studies which have been undertaken means that this is not possible.

4.1.3.3 Tobacco

As reviewed in Section 2.1, tobacco is the most commonly used drug of abuse by people with schizophrenia. It is also used by approximately one third of the general population, generally being consumed by smoking. The association between smoking and cardiovascular disease has long been established, and people who do smoke are at increased risk of cerebrovascular accidents. It is conceivable however that tobacco smoking may also be associated with structural brain abnormalities in the absence of an individual having a medical history of experiencing a stroke. To investigate this

possibility I undertook a systematic review of studies comparing magnetic resonance imaging scans in smokers to non-smoking controls. This review will be outlined below.

4.1.3.3.1 Systematic review of structural imaging findings in smokers

Methods

The methodology employed for this systematic review was comparable to that employed in the systematic reviews of the structural imaging effects of alcohol (outlined above). Searches were again performed on Medline (1970-May 2010), EMBASE (1970-May 2010) and PsychInfo (1970- May 2010). The following search terms were used: tobacco OR smoking AND magnetic resonance imaging, MRI OR diffusion tensor imaging, DTI. Both free-text and expanded medical subject headings were used. Subject headings were adapted to the specific subject headings of the biomedical databases used. The search strategy was supplemented by inspecting the reference lists of included articles.

I was again primarily interested in case-controlled studies (expecting these to be present in the greatest numbers); however, cohort studies are clearly of great value, and any identified will also be discussed. For inclusion studies had to utilise structural MRI or DTI scanning methodologies. Additionally, as before, subjects with specific psychiatric or medical syndromes must have been excluded from the study. Studies employing any generally accepted, quantitative form of image analysis technique directly comparing tissue volume/density or integrity in the two groups were acceptable; it was expected the majority of studies would utilise either ROI or VBM.

Inclusion criteria for case-controlled studies were inclusion of a control group of healthy volunteers.

Primary research studies were considered for inclusion if they were published as a peer-reviewed article in English and compared a sample of adults (aged 18-65) with nicotine dependence (being delivered through smoking) with a group of healthy controls. Studies could either compare the groups cross-sectionally, or compare changes over time. If both models of analysis were included in a single study, both analyses will be reported. Additionally, if different studies have used the same patient sample but investigated different brain structures, then both reports will be included. Studies were excluded if subjects had experienced a cerebrovascular accident (stroke or TIA), as investigating the association between smoking and cerebrovascular disease was not the objective of this study. This review was concerned with studies ascertaining if non-ischaemic structural abnormalities were detectable in the brains of adults who smoked. As white matter intensities are generally considered to reflect covert vascular brain injury,[430] studies quantifying the relative concentrations of these abnormalities in smokers and non-smokers were considered to be beyond the scope of this review. This was decided while acknowledging accumulating (though conflicting) data that white matter hyperintensities are prominent in the brains of young people with no history of cerebrovascular disease but major mental illness.[431]

Results

The search outlined above identified 969 potentially relevant studies, abstracts of which were assessed for inclusion. Two further articles were identified through supplementary search strategies. Twenty two articles were retrieved in full text form

for evaluation. Study flow and reasons for exclusion are summarised in Figure 4.3. Four studies were identified which fulfilled the inclusion criteria; all were cross-sectional, and are summarised in Table 4.5. Examples of reasons for studies being excluded were: not structural imaging data, individuals had experienced cerebrovascular events, and abuse/dependence on substances other than tobacco. Structural data were extracted from all included studies and recorded along with a description of the image analysis technique employed, the type and unit of measurement (area or volume) and characteristics of the subject and control groups which possibly confounded any observed differences (including age, gender ratio and use of other substances). Before considering the findings of the systematic review, a small number of studies not meeting criteria for inclusion in the review but nonetheless of relevance to subsequent discussion must be considered.

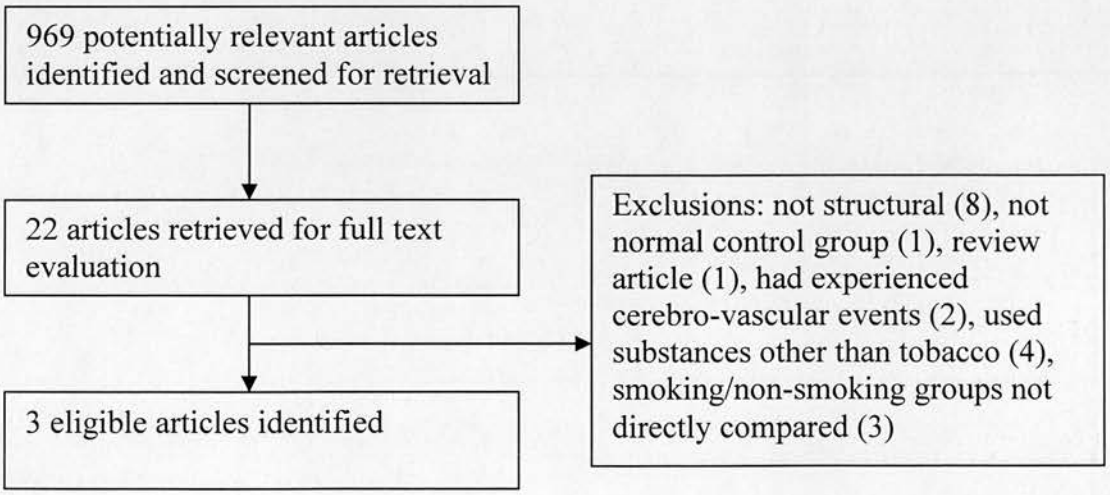

Figure 4.3
Study flow and reasons for exclusion in systematic review of structural imaging finding in adult smokers.

Structural data were extracted from all included studies and recorded along with a description of the image analysis technique employed, the type and unit of measurement (area or volume) and characteristics of the subject and control groups which possibly confounded any observed differences (including age, gender ratio and use of other substances). Eligible studies are summarised in Table 4.5.

Study	Imaging modality	Image analysis	Gender S/C (M:F)	Mean age (SD) S/C (M/F)*	Inclusion criteria	Smoking status	Use of other subs	Structures compared	Findings
Brody *et al.* 2004[432]	MRI	Semi-automated ROI and VBM	11:8/ 10:7	39.5 (10.3)/ 37.9 (12.9)	Recruited through newspaper advertisements Excluded if any lifetime history of Axis I psychiatric disorder or medical condition or medication use thought to affect brain structure or function	Smoked at least 20 cigarettes a day and met DSM-IV criteria for nicotine dependence. Controls never smokers.	Excluded if any lifetime history of illicit drug use other than cannabis, use of cannabis greater than once a month, or alcohol use more than one drink a day.	VBM: whole brain comparison of grey matter density ROI: Dorsolateral and ventrolateral prefrontal cortices, dorsal and ventral ACC, ventral striatum, and thalamus.	Smokers reduced grey matter volumes and lower grey matter densities than nonsmokers in the PFC bilaterally (DLPFC and VLPFC). Smaller volumes in the left dorsal ACC Lower grey matter densities in the right cerebellum
Gallinat *et al.* 2006[433]	MRI	VBM	12:10/ 12:11	30.8(7.5)/ 30.3(7.9)	Recruited through newspaper advertisements Excluded if Axis I or II disorder	Mean (SD) 13.5(13.0) pack years and 14.5(9.2) cigarettes a day. Controls never smokers.	Excluded if more than a single use of illicit drugs, consumed alcohol more than three times a week with more than two drinks a day, or consumed cannabis more than three two joints a month	Whole brain analysis	No difference in whole-brain volume. Reduced grey matter volume and density were observed in the frontal regions (anterior cingulate, prefrontal and orbitofrontal cortex), the occipital lobe and the temporal lobe including parahippocampal gyrus. Reductions of either grey matter volume or grey matter density were also found in the thalamus, cerebellum and substantia nigra. No higher grey matter volumes or density were found in smokers relative to never smokers. No significant group differences in white matter volume or white matter density were found.
Paul *et al.* 2008[396]	MRI DTI	VBM Voxel-based ROI	6:4/ 4:6	38.5 (14.2)/ 38.6 (12.5)	Individuals archived in a database. Excluded if they reported any history of a medical or neurological disorder, or psychiatric illness	Current smokers with any level of nicotine dependence compared to non-smokers	Excluded if they reported any history of drug abuse or dependence (aside from nicotine abuse or dependence)	Corpus callosum	No volumetric differences between groups. Smokers exhibited significantly higher FA in the body and whole corpus callosum and a strong trend for higher FA in the splenium.

Table 4.5
Studies investigating structural imaging abnormalities in adult smokers.
ACC: anterior cingulated cortex

Three studies did not meet inclusion criteria as they focused on individuals whoab used substances other than tobacco; in all cases these compared alcohol

abusing populations who did and did not smoke. Though not meeting inclusion criteria for the review, they do however warrant discussion. Gazdzinski *et al.*, in their 2006 report, compared regional grey and white matter volumes in 24 alcohol dependent subjects who smoked to 13 who did not.[434] They reported that cigarette smoking was associated with less parietal and temporal grey matter and with more temporal white matter. A subsequent report from the group utilised a non-treatment-seeking heavy drinking sample, again comparing regional brain volumes in those who smoked (17) to those who did not (16).[435] Smoking heavy drinkers demonstrated smaller temporal and total grey matter volumes than non-smoking heavy drinkers. These findings led the authors to suggest that the combination of chronic heavy alcohol consumption and cigarette smoking has particularly deleterious effects on cortical grey matter.

Gazdzinski *et al.* recently published longitudinal data and further baseline analyses from their study investigating the co-morbid effects of alcohol dependence and chronic cigarette smoking.[436] The focus of this study was the comprehensive investigation of white matter injury and recovery by simultaneously employing diffusion tensor imaging, magnetic resonance imaging and spectroscopy in the same cohort. Baseline findings (one week post abstinence) were generally in keeping with earlier data; for example, non-smoking alcohol dependent individuals demonstrated *higher* MD than non-smoking light drinkers in the temporal and parietal lobes, this indicating greater impairment of white matter structural integrity in the former group. Differences in patterns of white matter recovery on comparing longitudinal data from non-smoking and smoking alcohol-dependent individuals were rather unexpected however. In non-smoking alcohol-dependent individuals, the increase in fractional anisotropy of temporal white matter was accompanied by a pattern of decreased mean

diffusivity in all regions over 1 month of abstinence; no corresponding changes were observed in smoking alcohol-dependent individuals. As discussed above, this indicates that repair of white matter structural integrity is occurring in the non-smoking subjects. In contrast, a pattern of white matter volume increase in frontal and temporal lobes was apparent in smoking alcohol-dependent individuals but not in non-smoking alcohol-dependent individuals. The authors conclude that these data demonstrate that the pattern of white matter recovery following abstinence from alcohol is greatly dependent on an individual's smoking status; microstructural recovery predominates in non-smokers, whereas volumetric increases occur in smokers.

4.1.3.3.2 Summary of structural brain imaging abnormalities in smokers

As was the case in young drinkers, data investigating the non-cerebrovascular brain imaging associations of tobacco use are few. On reviewing the few studies identified however, two do report reduced grey matter density in the prefrontal cortex;[432, 433] indeed, in one of these studies this was detected on employing both VBM and ROI-based methodologies. There is even less evidence for white matter abnormalities in non-atherosclerotic smokers. Indeed, *increased* white matter volume has been reported in alcohol dependent subjects who smoke (compared to alcohol-dependent non-smokers),[434] and *increased* white matter integrity has been reported both when smokers are and are not alcohol dependent.[396, 436]

4.1.3.4 Structural imaging abnormalities associated with the use of other drugs

The other drugs which have been the subject of previous reviews are amphetamines, cocaine and ecstasy. I will also consider, in turn, data ascertaining structural imaging abnormalities associated with the use of these substances. As ever, but even more so when investigating these substances, the problem of multiple substance use is a major issue. The extent to which included studies accounted for substance misuse comorbidity will be a major consideration of this review.

4.1.3.4.1 Amphetamine

A number of reviews have been undertaken characterising the structural imaging abnormalities associated with amphetamine use. The majority of these have focused on methamphetamine, reflecting the explosion in use of this drug that has occurred in the East Coast of America in recent years. Case-control studies, in which structural imaging findings in methamphetamine users are compared to those in normal controls, are detailed in Table 4.6. In addition, the few studies focusing on amphetamine use (the stimulant more commonly used in the UK) are also included in this table. Studies included in the aforementioned reviews have been supplemented by a literature search using the search terms amphetamine OR methamphetamine AND magnetic resonance imaging. Combined review of amphetamine and methamphetamine studies is justified by the fact that though methamphetamine may

be more potent, the actions of these two drugs are generally regarded as remarkably similar.

Study	Imaging modality	Image analysis	Gender S/C (M:F)	Mean age (SD) S/C (M/F)	Inclusion criteria	Amphetamine/methamphetamine using status	Use of other subs	Structures compared	Findings
Bartzokis *et al.* 2000[437]	MRI	Semi-automated ROI	9:0/ 16:0	27.8 (4.3)/ 28.6 (ND)	Recruited from treatment programmes and research clinics. Met DSM-IV diagnosis of amphetamine dependence Excluded if comorbid psychiatric condition of sufficient severity to require treatment or clinically significant medical condition	Currently using amphetamine Mean(SD) duration of amphetamine use 6.7(3.9)	Excluded if met DSM-IV criteria for dependence on opiates, benzodiazepines, or other sedative-hypnotics. One currently alcohol dependent, five past history of same. Five past history of cocaine dependence	Grey and white matter of whole brain, temporal lobes and frontal lobes	Reduced total volume of temporal lobe. Trend towards reduced grey matter volume of temporal lobe
Thomson *et al.* 2004[438]	MRI	Autom ated segmen tation with cortical and hippoc ampal pattern matchi ng	10:11/ 15:7	31.9 (1.47)/ 35.3 (1.66)	Methamphetamine users. No DSM-IV diagnosis other than MA or nicotine dependence. Excluded if on psychtropic medication or had relevant medical illness	Mean(SD) duration of methampheta mine use 10.5(1.09)	No substance dependence other than MAor nicotine	Whole brain	Grey-matter deficits in the cingulate, limbic, and paralimbic cortices. Reduced hippocampal volumes.
Oh *et al.* 2005[439]	MRI	Semi-automated ROI	23:4/ 14:4	36.7 (5.6)/ 33.6 (6.7)	Recruited through advertisements in papers. Lifetime diagnosis of DSM-IV MA dependence. Excluded if DSM-IV axis I disorder, antisocial or borderline PD, significant medical illness	All users abstinent for at least 4 weeks. Total IV MA use at least 50.0g	Excluded if lifetime history of exposure to any dependence forming drugs except nicotine, caffeine, alcohol and prescribed medication.	Corpus Callosum. Measured as a single structure and divided in to seven regions: rostrum, genu, rostral body, anterior midbody, posterior midbody, isthmus and splenium	NSD in either total or subregional areas
Kim *et al.* 2005[440]	MRI	VBM	16:2 (LTA) 11:0 (STA)/ 15:5	LTA: 35.6 (5.2) STA: 37.9 (6.0) CTR: 33.2 (6.5)	Recruited through advertisements in papers. Lifetime diagnosis of DSM-IV MA dependence. Excluded if DSM-IV axis I disorder, antisocial or borderline PD, significant medical illness	All users abstinent for at least 4 weeks. Divided in to two groups, (LTA and STA), based on whether had/had not used methampheta mine in the last 6 months. Total IV MA use at least 50.0g	Excluded if alcohol dependence or lifetime history of exposure to any dependence forming drugs except nicotine, caffeine, alcohol and prescribed medication. NSD between prevalence of drinking and smoking between groups	Whole brain	After correction for multiple comparison, there was a grey-matter density decrease in the right middle frontal gyrus. This was most pronounced in the STA group, with the magnitude of reduction in the LTA group being intermediate between the STA group and controls.
Jernigan *et al.* 2005[441]	MRI	Semi-automated ROI	17:4/ 17:13	38.2 (7.7)/ 38.1 (10.5)	DSM-IV diagnosis of MA dependence. Excluded if schizophrenia, neurological conditions, head injury		Excluded if met criteria for dependence on any substance of abuse other than MA in the previous 5 years	Whole brain, lenticular nucleus, nucleus accumnbens, thalamus, amygdala, frontal lobe, temporal lobe, parietal lobe, occipital lobe	Larger caudate, lenticulate, accumbens and parietal cortices
Chang *et al.* 2005[442]	MRI	Semi-automated ROI	24:26/ 24:26	M: 33.1 (7.8)/ 30.9 (7.8)	History of DSM-IV diagnosis of MA dependence. No history of medical or psychiatric illness	Abstinent for at least one week. Average MA use .25 g	No current or past history of other drug dependence	Whole brain, cerebellum (and subregions), corpus callosum (and subregions),	Enlarged putamen and globus pallidus. Female MA users only also had

				F: 30.9 (6.4)/ 30.0 (9.2)	which may confound findings	/day, at least 4 days per week, for at least 2 years.		thalamus, midbrain, globus pallidus, putamen, caudate	enlarged mid-posterior corpus callosum
Chang *et al.* 2007[443]	DTI	Voxel-based ROI	23:9/ 20:10	M: 36.0 (6.7)/ 33.3 (6.6) F: 29.0 (7.2)/ 28.7 (6.0)	Recruited through newspaper advertisements. Lifetime DSM-IV diagnosis of MA dependence. Excluded if DSM-IV axis I disorder, antisocial or borderline PD, significant medical illness	Abstinent for greater than 4 weeks. Total MA abuse over 50.0g	Excluded if alcohol dependence or lifetime history of exposure to any dependence forming drugs except nicotine, caffeine, alcohol and prescribed medication. No significant difference between prevalence of drinking and smoking between groups	Frontal lobe white matter	Significantly reduced FA in regions in right and left frontal lobes in combined gender analysis. On examination of genders separately, this was only significant in males.
Salo *et al.* 2009[444]	DTI	Voxel-based ROI	13:24/ 9:8	36.29 (8.7)/ 32.18 (7.5)	Lifetime diagnosis of DSM-IV MA dependence. No other DSM-IV Axis I disorder. No relevant medical condition	Mean duration of use (SD) 11.61 (7.1), and abstinence (SD) 20.98 (31.9)	Excluded if other substance dependence (aside from nicotine) in last year, alcohol abuse in last 5 years	Corpus callosum genu and splenium	No significant difference between groups

Table 4.6
Studies investigating structural imaging abnormalities in amphetamine users
Abbreviations: ND: no data; NSD: no significant difference; MA: methamphetamine
LTA: long term abstinent; STA: short term abstinent

Results

Despite only eight studies being identifiable which have compared structural imaging findings in amphetamine/methamphetamine dependent subjects to controls, this topic has been subject to two reviews.[443, 445] In both reports reviewers discuss the findings of lower cortical grey matter volume and higher striatal volume in amphetamine/methamphetamine abusers. These findings are also apparent in this review, the latter finding seeming particularly robust. Hippocampal volume reduction may also occur, though rather surprisingly this has only been specifically investigated in a single study.[438] On considering which particular regions of the cerebral cortex are affected, the data are rather inconsistent. Whereas Kim *et al.* did report reduced frontal lobe grey matter,[440] this was not observed in two other studies.[437, 441] Similarly, while a single study has reported reduced temporal lobe grey matter volume,[437] this has not been replicated in other studies (e.g. [441]). Corpus callosum deficits seem

particularly unlikely, these being detected by neither volumetric nor DTI methodologies.[439, 444]

Both the reviews of Chang *et al.* and Bergman *et al.* discuss the possibility that the robust finding of enlargement of the striatum could reflect either a compensatory response to methamphetamine toxicity, or be a trait characteristic associated with risk of amphetamine use. Given that the data reviewed are cross-sectional unfortunately neither possibility can be refuted or confirmed. The study of Kim *et al.* investigated regions of density reduction rather than increase, and no abnormalities were localised to the stratum.[440] In this study individuals who have been abstinent for longer and shorter than six months were compared to each other and to normal controls. Abnormalities in the prolonged abstinent group were intermediate between those in the other two, suggesting that some recovery of structural abnormalities can occur.[440] This does suggest that at least some of the brain structural abnormalities observed in amphetamine users are the result of a dynamic process, and do not simply reflect trait characteristics associated with a propensity to use the drug. Nevertheless, longitudinal data investigating the dynamic effects of amphetamine use on brain structure would clearly add substantially to the existing knowledge base.

4.1.3.4.2 Cocaine

A small number of studies have specifically compared MRI brain scans in individuals with a history of cocaine dependence to those in normal controls. These are detailed in Table 4.7. In addition to these case control comparisons a small number of other studies are also of relevance to this discussion.

Study	Imaging modality	Image analysis	Gender S/C (M:F)	Mean age (SD) S/C (M/F)	Inclusion criteria	Cocaine using status	Use of other subs	Structures compared	Findings
Bartzokis *et al.* 2000[437]	MRI	Semi-automated ROI	10:0/ 16:0	31.4(3.8)/ 28.6(ND)	Recruited from treatment programmes and research clinics. Met DSM-IV diagnosis of COC dependence Excluded if comorbid psychiatric condition of sufficient severity to require treatment or clinically significant medical condition	Currently using COC. Mean(SD) duration of cocaine use 7.9(4.3) years	Excluded if met DSM-IV criteria for dependence on opiates, benzodiazepines, or other sedative-hypnotics. One currently alcohol dependent, five past history of same	Grey and white matter of whole brain, temporal lobes and frontal lobes	Reduced total and grey matter volume of temporal lobe
Jacobsen *et al.* 2001[446]	MRI	Semi-automated ROI	15: 10/ 11:9	35.6(5.7)/ 35.0(6.8)	Chronic COC dependence without comorbid Axis I disorder.	Smoked mean(SD) 4.5 g (5.9) of crack COC per week for 15.3 years (SD=6.5)	Had consumed mean(SD)3.7 (5.2) alcoholic drinks per day, one had history of alcohol dependence, no abuse/ dependence any other drug	Whole brain, caudate, putamen	Enlarged caudate and putamen. Caudate finding was not significant on controlling for alcohol consumption or race, though putamen result did remain significant
Fein *et al.* 2002[447]	MRI	Voxel-based ROI	29:0/ 20:0	39(7)/ 39(6)	Inpaients/ residents in substance misuse treatment centre. Met DSM-IV criteria for COC (crack) dependence.	Currently abstinent. Mean duration of crack COC use (in months, SD) 174(75)	Did not meet criteria for dependence on any other substance	Total and regional white and grey matter in four cortical regions of interest: prefrontal, parietal, occipital, temporal	Reduction in prefrontal cortical grey matter
Lim *et al.* 2002[448]	DTI	Voxel-based ROI	12:0/ 10:3	44.17 (5.09)/ 40.36 (6.80)	Participants in COC treatment studies and programmes. Met DSM-IV criteria for COC dependence in last 6 months. Excluded if significant psychiatric or medical comorbidity	Abstinent. Average duration of COC use 17.3 years	No lifetime history of alcohol dependence or dependent on any other illicit substance	Various frontal regions Genu and splenium of corpus callosum. Temporal white matter	Significant reduction in WM FA in the inferior frontal region
Franklin *et al.* 2002[449]	MRI	VBM	13:0/ 16:0	42(6)/ 32(7)	DSM-IV diagnosis of COC dependence. No clinically significant medical or psychiatric illness.	Mean years (SD) of COC use was 13 (6.5). Average number of days (SD) of COC used during the last 30 (7.3)	No current alcohol or other drug (except nicotine) dependence. No past or current use of opiates	Whole brain	Grey matter concentration decreases in the ventromedial orbitofrontal, anterior cingulate, anteroventral insular and superior temporal cortices
Matochik *et al.* 2003[450]	MRI	VBM	11:3/ 7:4	36.3(4.7)/ 33.8(4.5)	DSM-IV COC dependence. Excluded if past or current psychiatric disorder (except cocaine dependence) or neurological disorder	Abstinent minimum 20 days. Used for minimum of two years and at least 4 times a month. Use verified by duplicate urine drug screens.	Consumed fewer than 10 alcoholic drinks a week. Excluded if abuse/dependence on any other drugs (except nicotine)	Cingulate gyrus, perigenual and dorsal midcingulate. Prefrontal cortex (whole), orbitofrontal cortex, lateral prefrontal cortex.	Reduced grey matter density in the infragenual and perigenual regions of the anterior cingulate gyrus, lateral prefrontal and medial orbitofrontal cortices, and the whole lobar region of the prefrontal cortex.
Makris *et al.* 2004[451]	MRI	Semi-automated ROI	23:4/ 21:6	33.9/ 35.6	DSM-IV diagnosis of cocaine dependence	At time of scanning received COC infusion.	Not detailed	Hippocampal and amygdala volumes	Reduced amygdala volume, greater on right than left.

						Years (SD) of drug intake 9.5(8.4) Number of days(SD) in the past month they had used COC 16.3 (8.5)			
Sim *et al.* 2007[452]	MRI	VBM	27:13/ 26:15	41.4(6.9)/ 38.7(8.8)	Enrolled in treatment studies. DSM-IV diagnosis of COC dependence. Excluded if schizophrenia, BPAD or relevant medical illness.	Used on at least 6 occasions in 28 days preceding scan.	Excluded if current dependence on any psychoactive drug other than cocaine, alcohol, or nicotine.	Whole brain	Reduced grey matter volumes in bilateral premotor cortex, right orbitofrontal cortex, bilateral temporal cortex, left thalamus, and bilateral cerebellum. Reduced white matter volume in right cerebellum.
Lim *et al.* 2008[453]	MRI DTI	Voxel-based ROI	11:10/ 11:10	42.5(6.1)/ 40.9(7.4)	Recruited via newspaper advertisements. Met DSM-IV criteria for COC dependence in last month and use confirmed on drug screen. No history of other DSM-IV Axis I condition (aside depression), or relevant medical condition	Abstinent for at least 4 days.	Excluded if current dependence on any psychoactive substance other than COC, caffeine or nicotine	Grey and white matter volumes in superior and inferior frontal regions. Frontal white matter FA	Reduced FA in inferior frontal white matter. Reduced inferior frontal grey matter volume..
Ma *et al.* 2009[454]	DTI	Voxel-based ROI	13:6/ 9:9	39.1(7.8)/ 35.1(10.7)	Lifetime DSM-IV COC dependence. Excluded if lifetime history of non-substance related DSM-IV psychiatric diagnosis or relevant medical history	Years (SD) of lifetime COC use 10.7(6.7). Used COC on a mean(SD) of 7(7.8) of the last 30 days	Some current alcohol abuse/dependence (3) or cannabis abuse/dependence (1). Greater numbers past use of these and some other illicit drugs	Corpus callosum divided in to seven regions: rostrum, genu, rostral body, anterior midbody, posterior midbody, isthmus and splenium	After controlling for lifetime alcohol use, higher radial diffusivity (indicating reduced myelin integrity) in the rostral body and isthmus

Table 4.7
Studies investigating structural imaging abnormalities in cocaine users
Abbreviations: COC: cocaine

On reviewing the above studies, it is clear that the most consistent brain imaging abnormality observed in cocaine dependent individuals is grey matter reduction in the prefrontal lobe. Indeed, this was detected in the prefrontal cortex (either in the structure as a whole or in subregions of it) in five of the six studies which specifically investigated it.[447, 449, 450, 452, 453] Additionally, (though they are not the subject of this review), this is also consistent with functional neuroimaging

findings, which have demonstrated decreased prefrontal cerebral blood flow in cocaine-dependent subjects.[455, 456]

Several of the studies enable more accurate identification of which specific subregions of the prefrontal cortex exhibit structural abnormalities in the context of cocaine dependence. The three VBM studies are particularly interesting in this regard, these consistently identifying reduced grey matter volume/density in the orbitofrontal cortex. Localisation of abnormalities to the orbitofrontal cortex is notable, as deficits of this brain region have been particularly associated with compulsive drug intake behaviours,[457] and functional dysregulation of the orbitofrontal cortex has long been recognised in cocaine dependence.[458, 459]

Reduced grey matter volume in the temporal cortex of cocaine dependent subjects has also been reported by several independent groups. Specifically, it was identified in two out of the three VBM studies and one of the two ROI studies which specifically investigated it.[437, 449, 452] These findings are also consistent with prior functional brain imaging studies which have reported decreased temporal cortex blood flow in cocaine abusers.[460, 461]Additionally, a single study reported reduced amygdala volumes in cocaine-dependent subjects.[451]

Studies of white matter are much more inconsistent. One study did report that white matter volume was reduced in the frontal cortex,[448] but others have not. [447] DTI studies have been similarly inconsistent; again, one study reported deficits in the corpus callosum, while another did not.

All the data outlined above are cross-sectional, meaning it can not inform us of direction of causality. It is the case however that a number of studies have examined if correlations exists between structural findings and age. This was done, for example, in the amygdala study.[451] Notably, in this report no correlation was found between years

of drug use and volume. Similarly, Lim *et al.* reported that the inferior frontal FA decrease they detected in cocaine users was also unrelated to age and duration of use. These findings have led authors suggest that these abnormalities may represents early neural marker of cocaine use or possibly even constitute developmental traits that increase the risk of drug dependence.[451] In contrast however, other researchers have reported that abnormalities do relate to duration of cocaine use.[450] Cleary, and as has previously been discussed, longitudinal studies in this area are greatly needed.

4.1.3.4.3 MDMA

Though the chemical structure of ecstasy is very similar to that of amphetamine, the psychological effects produced differ substantially. Thus, it is important to establish, (distinct from the review of amphetamine), if use of this drug is associated with brain structural abnormalities. Case-control studies which have addressed this question are detailed in Table 4.8.

A further cross-sectional study of the effects of ecstasy use also warrants mention. This was undertaken by deWin *et al.*, with recruitment being designed to identify both heavy ecstasy users and 'polydrug-but-no-ecstasy' users.[462] Rather than directly comparing ecstasy using subjects to never exposed controls, this group then used a regression model in which ecstasy history (as well as use of other drugs) was dichotomised as either never exposed or consumed on greater than 10 occasions in lifetime. By employing this model they hoped to better control for the polydrug use which so frequently confounds these studies. Using this model the researchers then investigated the association between ecstasy use and DTI measures (as well as a

number of functional imaging variables). DeWin *et al.* reported that ecstasy use was associated with decreased FA in the thalamus, suggesting axonal damage in this structure. Interestingly, the other imaging tools employed (such as SPECT) also localised abnormalities to this region.

Study	Imaging modality	Image analysis	Gender S/C (M:F)	Mean age (SD) S/C (M/F)*	Inclusion criteria	MDMA using status	Use of other subs	Structures compared	Findings
Cowan *et al.* 2003[463]	MRI	VBM	17:14/ 18:11	24.3 (3.5)/ 21.7 (3.3)	Recruited via newspaper advertisements. At least 5 lifetime episodes of MDMA use. Excluded if history of substance dependence or Axis I substance dependence	Used on at Least 5 occasions	Vast majority (unlike controls) had used other drugs including cannabis, cocaine and hallucinogens	Whole brain	Reduced grey matter concentration in left temporal lobe, left frontal lobe, bilateral occipital lobes, bilateral cerebellum, and midline brainstem. These findings were generally robust to controlling for the effects of other drugs of abuse
Moeller *et al.* 2007[464]	DTI	Voxel-based ROI	10:2/ 13:7	27.3 (5.4)/ 25.5 (4.5)	Did not meet DSM-IV criteria for any Axis I disorder aside from substance abuse	Used on at least 15 occasions. Mean duration since last use 39.8 days.	Had consumed significantly more alcohol than controls. Majority had used cannabis, benzodiazepines and hallucinogens	Corpus callosum divided in to seven regions: genu, rostral body, anterior midbody, posterior midbody, isthmus and splenium, (rostrum excluded)	Decreased longitudinal eigenvalues in corpus callosum rostral body, indicating axonal damage

Table 4.8
Studies investigating structural imaging abnormalities in ecstasy (MDMA) users

In contrast to the paucity of prospective studies investigating amphetamine and cocaine use, the research group of DeWin *et al.* has also successfully undertaken a prospective imaging study investigating the effects of ecstasy on brain structure.[465] This was achieved by identifying and assessing (including scanning) a group of ecstasy-naïve individuals, at high risk of using the drug, before their first episode of use. They were then followed up after they had used the drug with further assessments at this point. Various imaging technologies were employed, including DTI. The initial report from this group was an uncontrolled cohort study, comparing individuals before and after ecstasy exposure. The mean inter-scan interval was 8.1+/-6.5 months,

and the mean number of ecstasy tablets consumed was 1.8+/-1.3 tablets. The researchers report that as well as changes in cerebral blood volume (a measure indicating brain perfusion), ecstasy use was followed by an increase in FA in frontoparietal white matter, and a decrease in apparent diffusion (ADC) in the thalamus. As discussed above, axonal cell membranes are known to be responsible for most of the restriction of water diffusion, with axonal damage leading to decreased FA and increased ADC. These findings would thus not suggest that ecstasy exposure was associated with reduced axonal integrity. After correction for multiple comparisons however none of these structural differences remained significant anyway, only the cerebral blood flow decrease in the dorsolateral frontal cortex differentiating subjects before and after ecstasy use.

The above study was followed by a larger report, which did include a control group.[466] This was achieved utilising the whole cohort of 188 young people who were ecstasy naïve but at high risk of using the drug. They were assessed as described above, but follow up was after a longer time period, approximately 17 months after their initial assessment. Successful follow up was achieved for 158 subjects of whom 64 had used ecstasy in the interim period and 94 had not. Of the 64 users, 59 agreed to a follow-up assessment and these individuals were matched to 59 individuals from the persistent ecstasy-naïve group. This report again demonstrated changes in cerebral blood flow in the ecstasy-exposed group (in this case in the globus pallidus and putamen), but also demonstrated DTI changes. Specifically, they demonstrated decreased FA in thalamus and frontoparietal white matter, but *increased* FA in globus pallidus. They also demonstrated an *increased* apparent diffusion coefficient in the thalamus, in direct contrast to the earlier analysis of a subset of the cohort. It is thus the case that these findings do suggest detrimental brain structural consequences of

ecstasy use, possibly reflecting ecstasy-induced axonal damage in the thalamus and frontoparietal white matter. It is of course the case that these findings also parallel the cross-sectional study of heavy ecstasy users reported above.[462]

On considering the studies of De Win *et al.* together with the two cross-sectional studies in Table 4.8, it does seem likely that, in the case of heavy ecstasy use at least, use of the drug is associated with subtle brain imaging abnormalities. These seem to be most likely to occur in the thalamus, given that it is here that abnormalities are observed in those studies with the most robust methodology.

4.2 Structural imaging findings in schizophrenia and those at high risk of schizophrenia

The central aim of this study is to investigate if substance misuse by those at high risk of schizophrenia promotes the development of the imaging abnormalities associated with schizophrenia. Given this, it is clearly essential that the nature of these imaging abnormalities is fully characterised. Additionally however, given that the population being investigated is one at elevated genetic risk of schizophrenia, it is also important to clarify if any brain structural abnormalities are already evident at people at high risk of the condition. Given these requirements this chapter will have two aims. Firstly to characterise the structural imaging findings in those with established schizophrenia, and secondly to do the same for those people currently well but at elevated risk of developing the condition.

4.2.1 Structural imaging findings in people with established schizophrenia

On attempting to synthesise the imaging data relating to people with established schizophrenia, one is immediately confronted with this issue of what constitutes established illness. While this could potentially be defined as anything from the point at which people have developed clear psychotic symptoms, this does raise the concern that not all first episodes of psychoses are actually schizophrenic. Thus, studies which include all people who present with a first episode of psychosis may ultimately transpire to have contained substantial numbers of people with, for example, an affective illness. Imaging findings in these individuals may differ

substantially from those in schizophrenia.[467] Equally, though evidence of actual longitudinal decline in cognitive function in chronic schizophrenia is curiously weak,[468, 469] it is also recognised that even in those people with an established diagnosis of schizophrenia, symptom development is not static and the impairment associated with the condition does tend to accrue over time.[470] If this impairment were to be reflected in imaging abnormalities, then it would be expected that those individuals with the more chronic condition would display more pronounced brain imaging abnormalities than those in whom schizophrenia has only recently been diagnosed.[471] It thus follows that the data on structural brain imaging abnormalities in people with schizophrenia are best considered as relating to two overlapping populations: those with the chronic condition, and those who have only recently developed the condition (i.e. first episode patients). If neuroanatomical abnormalities are indeed associated with schizophrenia, they would be expected to be most marked in those with chronic condition. Thus, I will first consider the data relating to them.

4.2.1.1 Imaging findings in chronic schizophrenia

As was the case in alcohol dependence, the first studies to apply *in vivo* imaging technologies to the study of schizophrenia utilised pneumencephalography. Though a primitive technique, and associated with considerable morbidity, these early investigations did suggest that the lateral and third ventricles were enlarged in at least some cases.[472]

In the 1970s the less invasive technique of computerised tomography was developed. This was utilised by Johnstone *et al.* to compare lateral ventricular

volumes in a group of chronically institutionalised schizophrenic patients to that in normal controls.[473] They were significantly enlarged, a finding which was replicated in the majority of subsequent studies. Indeed, not only was this finding demonstrated in patients with less extreme illness severity,[474] but it was also demonstrated that treatment effects could not account for the increased ventricular size shown.[475]In the 1980s MRI became available. With its superior resolution it enabled better delineation of tissue types, and permitted the measurement of cortical and subcortical structures. It was first applied to the study of schizophrenic patients in 1984,[476] and has since been applied in numerous studies. These studies have compared schizophrenics to normal controls, but also looked at diagnostic, aetiological and prognostic associations of various brain structural parameters. The two primary image analysis techniques that have been utilised to enable comparison of structural imaging characteristics in schizophrenics and controls are, as was the case in imaging studies of substance misuse, region of interest approaches and voxel based morphometry. Data obtained from both of these image analysis techniques have now been extensively reviewed, and the subject of often multiple meta-analyses. These meta-analyses will be referred to in the following passage, with single studies being emphasised if they are of particular importance.

Several meta-analyses of region of interest MRI studies in schizophrenia have now robustly demonstrated a reduction in the volume of the whole brain, this being particularly marked in grey matter and being associated with an increase in ventricular volume.[477, 478] Meta-analytic methodology has also been applied to the synthesis of studies comparing the volumes of specific cortical and subcortical structures. These report that volume reductions are particularly prominent in temporal lobe structures, with volumes of the hippocampus, amygdala, and the superior temporal gyri being

reduced by a greater amount than the whole brain.[479, 480] This is also the case in the prefrontal cortex,[478] and the thalamus,[481] while the corpus callosum is reduced to a roughly similar extent as the whole brain.[482]

VBM data was itself synthesised by Honea *et al.*, who undertook a meta-analysis of the 15 VBM studies comparing people with schizophrenia to controls which had been published up until May 2004.[483] They reported that the left superior temporal gyrus and the left medial temporal lobe were reported as decreased in more than 50% of studies, with fifty percent of the studies also reporting volumetric decreases in the left parahippocampal gyrus, right superior temporal gyrus, left inferior frontal gyrus, and left medial frontal gyrus. Moreover, superior temporal gyrul volumes and reductions in medial temporal volumes correlated significantly with positive symptoms and memory impairment respectively.[472, 484] It is notable that only a minority of VBM studies reported volume reduction in the thalamus, a structure demonstrated as reduced in volume by ROI methodology. Honea *et al.* suggest that this may reflect the smoothing approaches employed, the studies with positive findings in this (and a number of other regions) tending to have employed smaller smoothing kernels.[483] While such factors must be taken in to account when reviewing the VBM data, it does nonetheless seem reasonable to conclude, as did Lawrie *et al.*, that VBM studies have generally supported the findings of ROI approaches.[485]

While the reports outlined above do robustly demonstrate that structural imaging abnormalities are present in the brains of people with schizophrenia, they do not inform us if these abnormalities are static or dynamic. To answer this question a number of longitudinal studies have be undertaken in this population, ascertaining if brain changes do occur over time. These were recently reviewed in a report published

by Hulshoff Pol and Kahn, whose systematic review identified 11 MRI or CT studies comparing longitudinal brain changes in chronically ill patients to those in healthy controls.[471] In this population, which had been ill for on average 10 years before the initial scan, excessive brain tissue decreases and lateral ventricle volume increases were observed over an interscan interval of one to five years. The tissue loss was most pronounced in the frontal and temporal grey matter areas, with more pronounced changes being associated with more negative symptoms, and a decline in neuropsychological performance in some of the studies, but not consistently so. Of particular interest, higher daily cumulative dose of antipsychotic medication intake was either not associated with brain volume changes or with less prominent brain volume changes. The authors conclude that the available data suggest that progressive brain changes are indeed continuing to occur in chronically ill patients.

4.2.1.2 Imaging findings in first episode schizophrenia

The meta-analytic studies outlined above consisted of studies undertaken in patients who had had schizophrenia for variable periods of time, but in whom the condition was generally well established. In the meta-analysis of thalamic size undertaken by Konick *et al.* for example, the mean age of the 485 included subjects was 34.8 meaning they had generally been ill for at least a decade.[481] The meta-analysis of Wright *et al.* was similarly weighted towards people with the chronic condition, with only 7 of the 58 studies included focussing exclusively on first episode subjects.[478] This emphasis on studies investigating well-established illness, while it does facilitate diagnostic certainty, does have less desirable consequences.

Importantly, it means that any structural imaging abnormalities observed in meta-analyses could potentially be attributable to the effects of chronic illness, medication and other variables as well as to schizophrenia itself. For this reason it is important to establish what the imaging findings are in people experiencing their first episode of schizophrenia. While such studies do of course have their own potential pitfalls (e.g. the inherent risk of including subjects who do not ultimately transpire to have schizophrenia), clearly the potential for any abnormalities observed to be a consequence of variables such as the non-specific effects of chronic illness or antipsychotic exposure will be substantially reduced.

Two recent meta-analyses of studies which employed region of interest techniques to compare brain regional volume in subjects with their first episode of schizophrenia to normal controls were published in 2006 by Steen *et al.* and Vita *et al.*[486, 487] In both reviews the studies included focussed predominantly on first episode of schizophrenia, but subjects with other related conditions (e.g. schizoaffective disorder) were also included. The study of Vita *et al.* was a little more restrictive, and consequently included fewer studies (21 versus the 52 included in the review of Steen *et al.*). Despite these differences however, the conclusion of these two reports are strikingly similar. Both report that whole brain volume is reduced and lateral and third ventricular volumes increased in the first episode subjects. The only regional brain structure which both studies report as significantly reduced in the first episode subjects is the hippocampus, volume of this structure being evaluated in six of the studies identified by Vita *et al.*, and eleven of the studies identified by Steen *et al.*. Both groups discuss the fact that a paucity of data on other structures hinders meta-analytic comparison of their volumes. Utilising four studies in each case, Vita *et al.* do attempt this comparison for the temporal lobes and amygdala. Though volumetric

differences are not significant on comparison to controls, this could clearly simply reflect the limited data available.

More recently, Ellison-Wright *et al.* have applied meta-analytic techniques to synthesise data from studies using VBM to compare MRI scans from people with first episode schizophrenia to those from controls.[488] Though the primary goal of this interesting study was in fact to compare the pattern of brain deficits associated with first-episode psychosis to that in chronic schizophrenia, comparisons between first episode schizophrenics and normal controls were also undertaken. This component of the report was based on seven VBM studies, these comparing 224 patients with first episode of schizophrenia to 248 controls. Grey matter decreases were reported in the anterior cingulate gyrus and the bilateral insulae, inferior frontal gyri, unci/amygdalae, caudate heads and thalami. On comparing first episode individuals to those with chronic schizophrenia, grey matter decreases were more pronounced in the latter group in the cortex, this deficit being particularly marked in the medial frontal gyrus and the dorsolateral prefrontal cortex. The authors note that localisation of deficits in first episode subjects to the medial temporal lobe does demonstrate overlap with the meta-analyses of ROI studies, but that the VBM meta-analysis does detect more extensive abnormalities; this is of course not entirely surprising given that detection of abnormality in a given region with this technique is not, as is the case with ROI-based techniques, constrained by prior hypotheses. The authors also discuss that their findings suggest that as an individual moves from the early to the chronic condition, so brain abnormalities accrue in cortical regions. In keeping with this, and as was discussed above, longitudinal studies have reported that frontal and temporal cortical loss does continue to occur even after a schizophrenic illness is well established.[471, 471]

4.2.2 Structural imaging findings in high risk populations

The current study utilises data obtained from a population identified as being at high risk of schizophrenia. This risk is conferred by virtue of having two relatives affected by the condition and having not yet passed through the age range when schizophrenia most commonly develops. Given that the aim of the study is to ascertain what the impact of substance use by this population is on brain structure, it is clearly of great importance to clarify what brain imaging abnormalities are already present in populations at elevated risk of the condition. Of most direct relevance in this regard are of course previous reports from the EHRS, and a summary of previous imaging findings from this study will be presented first. Data from other studies of high risk populations are of course also of potential relevance however. These potentially relevant study populations are as follows, and data arising from each will also be discussed in turn:

1. Other studies which have characterised structural brain imaging findings in the relatives of people with schizophrenia.
2. Studies in people with schizotypy, a condition believed to be closely related to schizophrenia
3. Studies in people who are at elevated risk of schizophrenia for clinical reasons.

One of the great benefits of clarifying structural imaging characteristics in populations such as those listed above is that people in these groups have not actually developed a schizophrenic illness. Thus, these groups offer an opportunity to investigate any structural brain abnormalities which are associated with schizophrenia free of

potential illness-associated confounders such as antipsychotic medication treatment and lifestyle factors associated with severe mental illness.

4.2.2.1 Previous findings from the Edinburgh High Risk Study

As is apparent from the discussion above, it is now beyond doubt that patients with schizophrenia have demonstrable abnormalities on structural brain imaging. It is also clear that at least some of these abnormalities are present at the time of the first episode of schizophrenia. If the development of the condition is to be more fully understood however, then it is clearly crucial to ascertain from what point in an individual's trajectory towards schizophrenia these abnormalities are apparent. Are they present from birth, for example, or do they develop during the prodromal period that frequently precedes psychosis?

Clearly, the ideal study design to address this question would be a prospective study, with people being regularly scanned and clinically assessed from shortly after birth to the onset of schizophrenia and beyond. Unfortunately however, such a study is impractical for a variety of technological, clinical, and epidemiological reasons (for further discussion of these issues see Chapter 5). An alternative method is to study at-risk populations leading up to and during the time period of maximum risk of onset of the disorder. Indeed, this was exactly the model which was adopted in the Edinburgh High Risk Study, from which considerable data are already available.

The methodology of the EHRS will be discussed in more detail in Chapter 5, but to appreciate the significance of findings from it, it is important to outline the basic structure of the study now. The study essentially centred on the identification of

a cohort of individuals aged 16-24 who were currently well, but at high risk of developing schizophrenia. This risk was a consequence of having two close relatives with the condition, one of these relatives generally being either a parent or sibling. A total of 229 suitable HR subjects were identified. One hundred and sixty two provided some clinical, neuropsychological, and/or imaging data, and 150 had one or more sMRI scans between 1994 and 1999. A group of similarly aged healthy controls and first episode subjects was also examined. Most of the HR subjects and controls returned for at least one further sMRI and an fMRI scan between 2000 and 2005, when the study ended.

Both baseline and longitudinal imaging findings from the EHRS have now been widely published, and were recently summarised by Lawrie *et al.*[485] Analyses of baseline scans reported that the amygdalo-hippocampal complex was significantly smaller (about 4% than controls), but about 4% larger than first episode subjects.[489] The volume of the thalamus was also reduced in the high risk subjects compared to the controls, but interestingly this was not the case in the first episode subjects. These findings were largely replicated on VBM analysis, though deficits in the region of the hippocampus were localised to the left parahippocampal gyrus with this methodology.[490] Additionally, the VBM analysis also showed anterior cingulate and medial prefrontal reductions in grey matter density with a first episode schizophrenia < high risk < controls pattern.

Psychotic symptoms were elicited with the Present State Examination, and isolated or transient symptoms were found in more than half of the sample (many more than who actually developed schizophrenia). In those with psychotic symptoms at any point in the first 5 years of the study, the whole-brain volume at study entry was reduced compared with those who did not have psychotic symptoms over this

time.[491] No other brain volumes at intake were related to a liability to psychotic symptoms. As many of the participants had multiple scans, longitudinal analyses of brain structural changes over time were possible. Those with psychotic symptoms, and two or more sMRI scans over approximately 18 months, had significant reductions in the (right) temporal lobes and nonsignificant reductions in whole-brain and (left) AHC volume.[492] Job *et al.* extended these findings of changes over time by using VBM.[493] While all high risk subjects had distributed changes in grey matter concentration in the prefrontal and temporal lobes as compared with healthy controls, these were more marked in those who had psychotic symptoms at one or both assessments. Moreover, there were additional grey matter density reductions in left (para) hippocampal uncus, fusiform gyrus, and right cerebellar cortex in the 8 individuals at high risk who subsequently developed schizophrenia when compared with 10 individuals who also had psychotic symptoms but did not make the transition to illness.

By the close of the study 21 subjects (13%) had developed schizophrenia, on 20 of whom there was clinical data.[187] As one of the aims of the study was to establish if structural imaging measures could be used to predict who would ultimately develop schizophrenia, this was specifically investigated. While analyses did find that reduced AHC or thalamic volume did have some predictive effect, unfortunately the predictive effect of any baseline regional brain volumes (or indeed grey matter densities) for the subsequent development of schizophrenia was weak.[187, 493] While longitudinal grey matter loss, (particularly that in the inferior temporal gyrus), may constitute a more accurate predictor, even this does not at present have clinical utility.[494] What this study does make absolutely clear however, is that brain structural abnormalities are present in those at high risk of developing schizophrenia, and that (in those destined

to develop the condition), brain changes do occur years prior to diagnosis. As the individuals in this study were all well at the time of recruitment, these abnormalities and changes are clearly due neither to the effects of medication, nor are they the consequence of chronic illness. Clearly this raises the question of whether substance misuse may be one of the factors which results in these changes arising. This possibility is the focus of the current study.

4.2.2.2 Brain imaging abnormalities in the relatives of people with schizophrenia

The EHRS investigated the brain imaging characteristics of a clearly defined group of individuals at greatly elevated genetic risk of schizophrenia. While the design of this study was unique, other studies have also characterised the brain imaging characteristics of relatives of people with schizophrenia. The rationale behind this stems from the fact that the first-degree relatives of people with schizophrenia share 50% of a patient's genome. Given that schizophrenia has a 70-80% heritability,[495] any differences that exist between these relatives and normal controls would therefore be expected to reflect the genetic risk factors they posses for the condition. As was the case with the EHRS cohort, studying these unaffected relatives has important advantages to investigating people who actually have schizophrenia. Of particular importance, given that these individuals are well, any abnormalities which are detected cannot be attributed to secondary effects of the illness or its treatment. Boos *et al.* undertook a meta-analysis exploring exactly this question, synthesising data from studies comparing both global and regional brain volumes in the non-psychotic first degree relatives of patients with schizophrenia to those in healthy

control subjects.[496] They identified 25 studies eligible for inclusion, with volumes of the following structures being ascertained in at least 3 studies: whole brain volume, grey matter volume, amygdala-hippocampal complex, hippocampus, lateral ventricles and third ventricle. This study found that the largest effect was observed for hippocampal volume, with unaffected relatives exhibiting significantly smaller volumes than controls, but larger volumes than their relatives with schizophrenia. Smaller effects were also observed for cerebral grey matter (reduced in relatives), and third ventricular volume (increased in relatives). Interestingly, analysis of lateral ventricular volume, a robust finding in people with schizophrenia, did not show significant effects. The authors conclude that brain volumes in relatives of patients with schizophrenia do differ from those of healthy control subjects, and that this reflects the increased vulnerability of these individuals to the development of schizophrenia.

Out-with the EHRS, automated techniques of image analysis have not been widely applied to the comparison of brain imaging characteristics in the relatives of people with schizophrenia to normal controls. An independent group has however also reported reduced grey matter density in the prefrontal cortex of relatives at high risk for schizophrenia.[497]

4.2.2.3 Brain imaging studies in people with schizotypal personality disorder

A further group of interest when investigating biological markers associated with schizophrenia are those individuals with 'schizophrenia spectrum disorders', foremost among which is schizotypal personality disorder, (SPD), DSM-IV diagnostic

criteria for which are detailed in Table 4.9. This condition, similarly to schizophrenia, is characterized by positive or psychotic-like symptoms and negative or deficit-like symptoms.[498] The positive-like symptoms include ideas of reference, cognitive or perceptual distortions, and magical thinking. Negative symptoms encompass social deficit and interpersonal difficulties. Cognitive disorganisation is also seen. As well as symptom similarity there is also evidence of a genetic association between the two disorders, with a greater prevalence of schizotypal disorder being found in the relatives of those with schizophrenia,[499] and also psychophysiological correlates between the two conditions.[500] Similarly to what was discussed in relation to studies in the relatives of people with schizophrenia, there are clear advantages to studying people with schizotypal personality disorder rather than frank schizophrenia; importantly, this group is freer from the multiple artefacts that potentially confound schizophrenia research, including the effects of long-term medication treatment, multiple hospitalizations or institutionalization, and prolonged functional impairment secondary to chronic psychosis and social deterioration. Additionally, there must be an underlying reason (or reasons) why these individuals do *not* express the more severe phenotype of schizophrenia. Thus, the investigation of brain structural abnormalities in schizotypy may lead to a greater understanding of why some individuals progress to schizophrenia while others do not.

A)	A pervasive pattern of social and interpersonal deficits marked by acute discomfort with, and reduced capacity for, close relationships as well as by cognitive or perceptual distortions and eccentricities of behaviour, beginning by early adulthood and present in a variety of contexts, as indicated by five (or more) of the following: 1) ideas of reference (excluding delusions of reference) 2) odd beliefs or magical thinking that influences behaviour and is inconsistent with subcultural norms 3) unusual perceptual experiences, including bodily illusions 4) odd thinking and speech (eg. vague, circumstantial, metaphorical, overelaborate or stereotyped) 5) suspiciousness or paranoid ideation 6) inappropriate or constricted affect 7) behaviour or appearance that is odd, eccentric, or peculiar 8) lack of close friends or confidants other than first-degree relatives 9) excessive social anxiety that does not diminish with familiarity and tends to be associated with paranoid fears rather than negative judgements about self
B)	Does not occur exclusively during the course of schizophrenia, a mood disorder with psychotic features, another psychotic disorder, or a pervasive developmental disorder and is not due to the direct physiological effects of a general medical condition

Table 4.9
DSM-IV diagnostic criteria for schizotypal personality disorder

Structural imaging findings in schizotypal personality disorder (SPD) were reviewed by Dickey *et al.* in 2002,[501] and Andrew Stanfield in 2007.[502] The latter review benefits from the inclusion of several important studies undertaken by Suzuki, Takahashi and others in Tokyo; these involved a cohort of up to 39 people with SPD, the largest such cohort ever studied.[503, 504] Given this, greater emphasis will be placed on the Stanfield review. No relevant studies comparing structural brain imaging findings in people with SPD and normal controls and published after the review of Stanfield were identified.

The available data find little evidence of prefrontal lobe reduction in SPD, with the Japanese group finding that it may even show subregional enlargements relative to normal controls.[503] By contrast, there is evidence for regional volume reduction in the temporal lobes. The most consistent deficit observed in this structure is in the superior temporal gyrus; three out of four studies which compared volume of this region to that in controls identified reductions in the SPD subjects,[504-506] whereas

only one did not.[507] By contrast, findings in the amygdala, hippocampus, Heschl's gyrus and planum polare are more equivocal; in each case one positive and one negative finding is reported for each structure. Temporal lobe abnormalities, localised particularly to the superior and medial temporal gyri, were also reported in a VBM study from the Tokyo group.[508] Other brain regions specifically investigated include the cingulate gyrus, basal ganglia, thalamus, corpus callosum and lateral ventricles. Three studies report that there is no evidence of volume differences between SPD individuals and controls in the cingulate gyrus.[509-511] Conflicting findings are reported for the basal ganglia. Hazlett *et al.* found that there were no differences in the total thalamic volume between individuals with SPD and normal controls;[512] in a subset of this group however, Byne *et al.* found the size of the pulvinar was reduced in both schizophrenia and SPD relative to controls, whereas the mediodorsal nucleus did not differ between the groups.[513] The single study investigating corpus callosum volume reported that the genu of the corpus was larger in patients with SPD than in control subjects, whereas the posterior corpus was larger in controls.[514] Two studies report that lateral ventricular volume is not increased in SPD.[515, 516]

On the basis of these finings Stanfield concludes that SPD is associated with some temporal lobe structural abnormalities, in a similar but less severe pattern than those seen in schizophrenia. By contrast the prefrontal region seems to be relatively spared. Furthermore, he also argues that the findings of sparing of the mediodorsal thalamic nucleus and the enlargement of the callosal genu add further weigh to this explanation. Given that both of these regions are closely associated with the prefrontal lobe, it would be expected that abnormalities of that structure would be reflected as abnormalities of these regions.[500, 502] That such abnormalities are not detectable suggests that communication from or through these structures with prefrontal regions

powerful scanner (3T rather than 1.5T) once again reports that (left) hippocampal volume is reduced in UHR individuals.[522] As they had previously reported however, this measure did not differentiate those who made the transition to psychosis from those who did not. In interpreting these results however it is important to acknowledge that only 7 of the 66 ultra high risk subjects did actually develop psychosis, which clearly raises the possibility of a Type II error. On considering these most recent findings Wood *et al.* conclude that though hippocampal volume may be reduced in UHR individuals, it seems unlikely that this measure will prove to be a useful predictive marker for later psychosis

The Melbourne group have also recently specifically investigated if volume of the superior temporal gyri predicts who will go on to develop psychosis.[523] Using semi-automated tracing methodology they reported that that though the structure was significantly reduced bilaterally in UHR individuals, volume did not differ in those who did and did not subsequently develop psychosis.

Other groups employing the same criteria for the identification of UHR subjects have now also published baseline data from their studies. Of note, Borgwardt *et al.* utilised VBM to compare 22 controls and 35 UHR patients (12 of whom developed psychosis over the following 2 years).[524] Significantly reduced grey matter volume was found in a number of regions, including the left hippocampus, but there were no hippocampal differences between those who did and those who did not later develop psychosis. By contrast, individuals who became unwell did have less grey matter in the right insula, inferior frontal and superior temporal gyrus than those who remained well. Similar findings were also reported by a German group (Witthaus *et al.*), who reported that when compared to controls their UHR sample demonstrated lower grey matter volume bilaterally in the cingulate gyrus and hippocampi and also

in the right inferior frontal and right superior temporal gyri.[525] Additionally, a Korean group which investigated cortical thickness reported thinning of the prefrontal cortex, anterior cingulate cortex, inferior parietal cortex, parahippocampal cortex, and superior temporal gyrus in the UHR group compared with healthy controls.[526] By contrast however, Ziermans *et al.* in their admittedly very young Dutch sample (mean age 15.8) do not detect *any* differences between UHR individuals and controls.[527]

In a follow-up paper to their 2008 report Witthaus *et al.* acknowledge the existence of conflicting data in this area, and that this is particularly problematic in relation to whether or not hippocampal volume is reduced in UHR individuals destined to become psychotic. They suggest one potential explanation for this inconsistency is that it may be abnormalities of regional rather than total hippocampal volume which are associated with risk of transition to psychosis.[528] Specifically, they suggest that it may be particularly reduction of hippocampus corpus and tail volumes which are indicative of the prodromal phase of schizophrenia and represent risk factors for transition into psychosis. As they acknowledge, whether such refinement resolves the current confusion can only be answered by further studies.

Studies undertaken by the Melbourne and Swiss groups have incorporated sequential scans, enabling exploration of the relationship between structural brain changes over time and clinical outcomes. In their first longitudinal report, utilising VBM methodology and based on 21 participants, Pantelis *et al.* demonstrated reductions in grey matter in the left parahippocampal and fusiform gyri, left orbitofrontal cortex and cerebellar cortex and bilateral cingulate gyri. These changes occurred over approximately a year in 11 people as they developed a diagnosis of psychosis, usually schizophrenia.[520] In their review Wood *et al.* discuss the limitations of applying VBM to these analyses, and specifically the fact that poor registration

limited the sensitivity of the technique. Consequently they also applied a tensor based technique, based on that described by Thomson *et al.*[438] but augmented by their own processing, to a slightly extended sample of 35 UHR individuals. On applying these more sensitive analyses they reported significantly greater brain contraction in the right prefrontal region in those 12 UHR individuals who went on to develop psychosis (five being diagnosed with schizophrenia spectrum disorders), indicative of an accelerated rate of grey matter loss in these people.[529] The authors do acknowledge the limitations of this study, not least among these the fact that follow-up scans were not acquired during or immediately after the psychosis onset, raising the possibility that factors secondary to psychosis or its treatment contributed to the greater brain change in the converters. Nonetheless, these findings do fit with a number of other studies in unmedicated individuals (discussed above) implicating changes in the prefrontal lobe as associated with transition to psychosis. Interestingly, and as Wood *et al.* note in their review, the pattern of longitudinal change seen in the UHR group who made the transition to psychosis was similar to that observed in healthy controls, albeit exaggerated in magnitude; on this basis Wood *et al.* suggest that the transition to psychosis is associated with an exacerbation of normal neurodevelopmental processes.[530] Once again, only further studies can ascertain what the factors influencing these exaggerated changes are, but Sun *et al.* do cite previous findings demonstrating larger pituitary volumes in UHR individuals who make the transition to psychosis to suggest that activation of the hormonal stress response may play a role.[531]

The Melbourne group have also investigated the possibility that white matter changes differentiate those individuals who make the transition from UHR to psychosis from those who do not.[532] On comparing baseline MRI data from 75 UHR

individuals, 23 of whom subsequently developed psychosis, individuals who later developed psychosis had larger volumes of white matter in the frontal lobe, particularly in the left hemisphere. Twenty one participants had a second scan, ten of whom developed psychosis. Longitudinal comparison of these groups revealed a reduction in white matter volume in the region of the left fronto-occipital fasciculus in those who develop psychosis. Participants who had not developed psychosis showed no reductions in white matter volume but increases in a region subjacent to the right inferior parietal lobule. Two important limitations of this study are that the interscan interval for the 'remained well' group was almost double that in the 'developed psychosis' group and that many more of the psychosis group were treated with antipsychotic medication; thought the former potential confounder was controlled for in the analysis, the latter was not.

The Swiss group successfully followed up (and rescanned) 20 UHR individuals, 10 of whom had developed psychosis.[533] Their VBM analysis reported that in subjects who developed psychosis there were longitudinal volume reductions in the orbitofrontal cortex and the right superior frontal, inferior temporal and superior parietal cortices, as well as the right hemisphere of the cerebellum and the left precuneus. Conversely, there were no longitudinal changes in subjects who did not develop psychosis. Though they clearly remain relevant factors in interpreting these results, the difference in interscan interval between groups was less in this study than in that of Waterfang *et al.*, and fewer of the converters (approximately half) had been treated with antipsychotics at the time of the second scan.

Given the superficial similarities between the methodology employed in the EHRS and in 'ultra-high risk' studies some discussion of how comparable these approaches are is essential. This is particularly important as many findings from the

EHRS and ultra-high risk appear to be divergent. For example, hippocampal volumes are reduced in those in the EHRS who go on to develop psychosis whereas those who go on to develop psychosis in the Melbourne study have *normal* hippocampal volume.

The truth is that despite the superficial similarities between these two methodologies they actually have the potential to recruit very different individuals. Methodologies between different UHR groups does differ, so in this section I will focus on those differences between the EHRS and the UHR studies undertaken by the Melbourne group (whose UHR studies have been most influential).

Firstly, in contrast to the EHRS study (in which participants were identified by virtue of a/more than one close relation/s with schizophrenia, the Melbourne group used a 'close-in' strategy, recruiting symptomatic help-seeking individuals. It is clearly possible that individuals who actively seek help for partial psychotic symptoms/functional decline are quite different from those who are well but have a family history of schizophrenia. This leads to a further potentially important implication of this difference between the groups, namely the stage in development of illness at which individuals are identified. As UHR subjects are, by definition, help seeking, those who go on to develop schizophrenia may have already experienced some structural brain changes in associateion with this (which could potentially preceeed the development of psychosis by some time); consequently even longitudinal studies in these subjects will not observe these already apparent changes arise. The final important issue I will highlight relates to the illness that recruited individuals actually develop. In the case of the EHRS all individuals who became psychotic were diagnosed with schizophrenia. In the publications from the Melbourne group the reported outcome is generally *psychosis*, which in only approximately 50% of cases is a schizophreniform disorder. It is now established that imaging findings do

differ in schizophrenia and bipolar affective disorder,[467] meaning that utilization of the concept of a unitary psychosis may cloud imaging findings somewhat.

4.2.3 Summary of structural imaging findings in people with schizophrenia or at high risk of the condition

On considering the imaging data deriving from both people with schizophrenia and those at risk of the condition, a number of firm conclusions can be drawn. Firstly, the evidence that structural imaging abnormalities exist in established schizophrenia is now overwhelming. These are present at the whole brain level, manifesting both as a reduction in total grey matter and increased ventricular volume, but reductions in a number of brain regional volumes have also been repeatedly demonstrated. The brain regions particularly affected are the medial temporal lobes (particularly the amygdala and hippocampus), the superior temporal gyrus, the frontal lobes and the thalami. Comparable abnormalities are also present in first episode subjects, but they are less pronounced; indeed, deficits in the frontal lobe in particular may be very subtle in these individuals.

It has now also been established that structural abnormalities are also detectable in people who are at high risk of schizophrenia for either genetic or clinical reasons. These deficits are detectable in the medial temporal lobe/hippocampus, regions of the prefrontal cortex and possibly also the thalamus. The magnitude of these deficits is considerably less marked than in first episode subjects, a fact which likely contributes to it not being detected in several studies of these high risk groups. It is the case however that even the most consistently reported deficits in high risk

groups (medial temporal lobe/hippocampal deficits) can not reliably predict who from a high risk cohort will go on to develop psychosis. Several groups have now also reported on longitudinal changes that occur in high risk subjects who do make the transition to psychosis. These reports suggest that in these individuals further temporal lobe volume loss occurs, seemingly some time before transition to psychosis, while additional prefrontal lobe volume loss likely occurs in closer proximity to the point of actual transition.

4.2.4 Influence of genotype on susceptibility to brain structural changes

As discussed above, many of the studies investigating brain structural abnormalities in schizophrenia have identified their study group on the basis of a genetic propensity for schizophrenia. In the EHRS the association between genetic propensity and these abnormalities was also explored *within the high risk group*, the relationship between volumes of various structures being related to the strength of genetic liability to schizophrenia. Volumes of the PFC, thalamus, and whole-brain, but not those of the AHC, were reported to be negatively associated with this variable.[491] Additionally, the influence of specific genes on structural imaging findings has also been explored in the EHRS. Specifically, it was found that high risk individuals with the COMT Val allele had reduced grey matter density in anterior cingulate cortex, and that possession of this allele increased the risk of developing schizophrenia in a dose dependent manner.[534]

Importantly, there is also evidence of a significant interaction between genotype and cannabis use in determining an individual's risk of developing

schizophrenia. Intriguingly, and as was discussed in Section 3.2.2.1.1, it is again the COMT gene which has been reported as mediating such an interaction.[121] Given such data, it would obviously be desirable to explore specific gene/substance misuse interactions further in this study. Specifically, it would be of great interest to explore if there was an interaction between particular genotypes and vulnerability to the brain structural and illness potentiation effects of cannabis within the high risk cohort. For reasons of power however, (only 75 number of the high risk subjects were both genotyped and had usable scans), this will not be possible. Thus, though brain structural abnormalities potentially arising as a consequence of gene/substance misuse interactions are clearly an important issue to explore, unfortunately I will not be address them in this report. Consequently, given that it is beyond the scope of the current report, I will not discuss the important issue of the contribution of specific genes to an individual's risk of developing schizophrenia on exposure to cannabis any further.

4.3 Studies in dual diagnosis

As has been repeatedly stated, the central aim of this study is to investigate if substance misuse by those at high risk of schizophrenia promotes the development of the imaging abnormalities associated with schizophrenia. Essential in interpreting any data investigating this possibility is, as has already been discussed, data establishing: 1) the structural abnormalities associated with use of drugs of abuse; 2) structural abnormalities associated with the condition of being at high risk of schizophrenia; and 3) the structural abnormalities associated with schizophrenia itself. Of arguably most direct relevance to addressing this question however are studies which directly investigate the brain imaging consequences of substance misuse by people who have schizophrenia. What follows is a review of studies characterising brain structural imaging findings in people with both these conditions. In this section I will also include a summary of studies investigating structural imaging findings in alcoholic hallucinosis. As this condition represents a phenomenon by which psychotic symptoms are directly attributable to use of a substance (alcohol), it is clearly of particular interest to this report.

4.3.1 Studies addressing the brain imaging findings associated with substance misuse by people with schizophrenia

4.3.1.1 Cannabis

Only a handful of cross-sectional studies comparing structural imaging findings in cannabis using schizophrenic individuals to those in subjects with

schizophrenia but with minimal exposure to cannabis have been undertaken. The first such study was undertaken by Cahn *et al.* and published in 2004.[535] It compared volumes of the whole brain, total grey and white matter, cerebellum, caudate nucleus and third and lateral ventricles in 27 patients with recent-onset schizophrenia and a lifetime diagnosis of cannabis abuse or dependence to that in 20 patients with recent-onset schizophrenia who were cannabis-naive. Patients with a lifetime diagnosis of abuse of or dependence on a substance other than cannabis were excluded. The cannabis exposed group was significantly younger than the comparator subjects, but age was included as a covariate in the analysis. A significant difference in volume was found in none of the brain regions investigated.

Two more recent MRI studies have again compared structural brain imaging findings in people who do and do not smoke cannabis. The first, undertaken by Szeszko *et al.*, compared structural characteristics of the prefrontal lobes in 20 first episode subjects who had an additional diagnosis of cannabis abuse, 31 first episode subjects with no such history and 56 healthy controls without a history of any substance misuse diagnosis.[536] Six of the cannabis using first episode subjects had a history of an alcohol use disorder, whereas this was the case for only two of the non-cannabis using patients; use of other drugs was minimal. Antipsychotic exposure was also minimal, the median duration of use of these drugs being quoted as 0 weeks. Volumes of the superior frontal gyrus, anterior cingulate gyrus and orbital frontal lobe were outlined manually using a semi-automated ROI approach and then automatically segmented in to grey and white matter. These volumes were then compared between the groups with age and intracranial volume included as covariates. Szezko *et al.* report that first episode of schizophrenia patients who use cannabis had less anterior cingulate grey matter than both patients who did not use the drug and healthy

volunteers. Significant differences were not observed in any other of the regions studied. This result remained significant even when any patients with substance misuse diagnoses other than cannabis were excluded from the analysis. In their discussion Szezsko *et al.* speculate that the reason these findings contrast with those of Cahn *et al.* likely derives from the fact that the latter study did not examine discrete frontal cortical regions.

More recently Bangalore *et al.* (2008) analysed data from subjects similar to those included in the Sezezsko *et al.* report.[537] In this study MRI scans from 15 people experiencing a first episode of schizophrenia who had a lifetime history of cannabis use on more than 10 occasions were compared with 24 similar patients who were cannabis naive and 42 healthy volunteers who did not use cannabis. Eight of the cannabis using patients were polysubstance abusers, though specific substances are not detailed. Bangalore *et al.* conducted a voxel based morphometric analysis of a priori determined regions of interest, focusing on CB1 receptor rich brain regions. These regions included the DLPFC, hippocampus, posterior cingulate and cerebellum. Though they report that there were no statistically significant differences in age and gender between the groups, these factors were included as covariates in the analyses. Bangalore *et al.* report that in the main analysis comparing the cannabis exposed and unexposed first-episode subjects, a trend towards reduced grey matter density in the right posterior cingulate was observed in the former group, this being significant in post-hoc analyses conducted separately for the individual a priori hypothesized regions. Comparisons were also made between the groups using modulated normalized images, these being believed to better identify volume (rather than density) differences between subjects. This noted a decrease in grey matter volumes of the right posterior cingulate and left hippocampus in the cannabis exposed

(compared to unexposed groups); no differences were noted between cannabis unexposed subjects and controls.

A further cross-sectional volumetric MRI study, this undertaken by Wobrock *et al.* is also relevant to this discussion.[538] In this study 20 patients with recent onset schizophrenia or schizoaffective disorder (onset within the last three years) and a history of alcohol or drug abuse were compared to 21 schizophrenia/schizoaffective disorder patients without such comorbidity. Substances used by the comorbid group were heterogeneous, but all had used cannabis; additionally abused substances were cocaine (8), amphetamines/ecstasy (7), opiates (2), alcohol (2), and hallucinogens (1). Volumes of the amygdala-hippocampal complex, superior temporal gyrus/Heschl's gyrus and cingulate gyrus were ascertained using semi-automated ROI methodology, and compared between the two groups while covarying for age and height. No significant difference was observed between the groups in volumes of any of the structures investigated.

In addition to the cross-sectional data, a single longitudinal study investigating the impact of cannabis on brain volume loss over time has now been published.[539] This study was based on the same cohort of patients previously reported on by Cahn *et al.* In this follow up study brain volume loss over a five year period was compared between 19 with patients with a diagnosis of a schizophrenia spectrum psychosis who used cannabis and no other illicit drugs in the inter-scan period, 32 patients with a schizophrenia spectrum disorder who used no illicit drugs in the inter-scan period, and 31 healthy controls who did not use any illicit drugs. An automated brain segmentation tool was applied to both baseline and follow up scans to obtain volumes of the total brain, total grey and white matter of the cerebrum, and lateral and third ventricles. At baseline there was no difference on any of these measures between the

groups aside from for third ventricular volume, which was significantly larger in the non-cannabis-using schizophrenic group in relation to healthy comparison subjects. Over the five years of the study however cannabis using patients did show significantly greater loss of grey matter and lateral and third ventricular enlargement. The authors stress that this excessive brain volume loss could not be attributed to differences in baseline characteristics, such as brain volume or clinical measures; indeed, they stress that the cannabis-using group did not display more pronounced brain volume abnormalities at onset compared to the nonusing group, and there was no significant difference in scores on symptom rating scales. Similarly, the effects of medication cannot explain the difference as medication use over the follow-up period was qualitatively and quantitatively similar in both groups. No significant correlations were present between brain volume change and measures of symptomatic and functional outcome in either of the patient groups. Patients who continued to use cannabis did show a less pronounced improvement in positive and negative symptoms compared to nonusing patients however.

It is thus the case that two of the four available cross-sectional studies report brain structural abnormalities on comparing people experiencing their first episode of schizophrenia who use cannabis to those who do not use the drug.[536, 537] In each of these studies abnormalities were detected in the cingulate, though in each case different subregions (either the anterior or posterior cingulate) were investigated. Additionally, one of these studies detected abnormalities in the hippocampus,[537] whereas the only other study investigating this region (it actually looked at the amygdalahippocampal complex as a single entity) did not.[538] A single longitudinal study has further strengthened the case that cannabis use by people with schizophrenia is associated with magnified brain structural changes.[539] Given that structural imaging

studies have not found any consistent alterations in brain structures in cannabis using adolescents, the finding of structural brain changes in schizophrenia patients with comorbid cannabis use are of particular interest. They may suggest that it is an interaction between schizophrenia pathophysiology and cannabis use which is resulting in the structural brain alterations observed in these studies.

4.3.1.2 Alcohol

As is apparent from Sections 4.1.3.2 and 4.2.1, the nature of the brain structural deficits in schizophrenia and chronic alcohol dependence overlap. This observation prompted the first study directly comparing brain structural brain imaging findings in the two conditions, this being undertaken by Sullivan *et al.* and published in 1998.[540] In this American study brain structural volumes were compared between 62 detoxified alcohol dependent subjects (abstinent for approximately one month and without significant psychiatric or medical comorbidity), 71 schizophrenics (without a history of alcohol abuse or dependence) and 73 healthy controls. The ages of participants included in each group spanned many decades, the youngest being 21 and the oldest 70; though the alcoholic group was significantly older than the participitants with schizophrenia (mean age 44.6 vs. 44.0), age was included as a covariate in the analyses. Semi-automated methodology was used to segment grey and white matter and divide the cortex into six regions on the basis of anatomical landmarks; lateral ventricular volume and third ventricular area (the latter being determined on the slice on which it appeared largest) were also ascertained. On comparing these measures across the groups, Sullivan *et al.* report that whereas both groups had grey matter volume deficits and CSF volume enlargement, only the

alcoholics had white matter volume deficits. Furthermore, whereas the schizophrenics had significantly greater volume deficits in the prefrontal and anterior superior temporal grey matter than in the more posterior cortical regions, the deficits in the alcoholics were relatively homogeneous across the cortex. On statistical comparison only the superior temporal lobe deficits were significantly greater in the schizophrenic patients than the patients with alcohol dependence.

The same group built on this early work, scanning a fourth group of men, these people being comorbid for schizophrenia (or schizoaffective disorder) and a lifetime history of alcohol abuse or dependence.[541] This enabled a four way comparison of structural imaging findings between 35 comorbid men, 64 people with schizophrenia/schizoaffective disorder alone, 62 men with alcohol dependence alone, and 62 healthy men. The comorbid group matched the schizophrenia group on age and illness severity, but was younger and had consumed five times less alcohol in their lifetimes than the alcohol dependence alone group. The same image analysis methodology was utilized as in the previous study of Sullivan *et al.*, though obviously in this case comparisons were made across four groups rather than three. On undertaking these comparisons, Mathalon *et al.* report that grey matter volume deficits relative to controls were present in all three patient groups. These were particularly pronounced in the comorbid group, these people having greater prefrontal cortex and anterior parietal grey matter volume loss than either the alcoholic or schizophrenic controls. By contrast however deficits in the temporal lobe, a region known to exhibit abnormalities in schizophrenia, were equivalent. Mathalon *et al.* conclude that, as they had hypothesised, it does appear that people comorbid for schizophrenia exhibit compounded grey matter volume deficits. As would be expected given evidence that it is affected in both disorders, these compounded

deficits are particularly pronounced in the prefrontal cortex. Additionally however, it seems that the anterior parietal region is also susceptible to these compounding effects; by contrast, the temporal lobe is not.

Mathalon *et al.* go on to compare the magnitude of the prefrontal deficit in the comorbid patients to that in the two patient comparator groups. They note that the prefrontal grey matter volume deficit of the comorbid patients (mean $z=-1.70$) was equivalent to the additive effects of alcoholism (mean $z=-0.71$) and schizophrenia (mean $z=-1.00$). As they discuss however, given that the lifetime alcohol consumption of the comorbid patients was nearly five times less than that in the alcoholic patients, the compounded effect of schizophrenia and alcoholism may best be characterized as interactive rather than additive. In other words, comorbid patients exhibited the full detrimental effect of alcoholism on prefrontal (and anterior parietal) grey matter volume, despite significantly less lifetime alcohol intake than the alcoholic patients. The authors conclude that their data provide biological evidence consistent with clinical observations of heightened vulnerability to adverse outcomes among patients with schizophrenia who also have alcohol problems.

The same group has also published three other studies investigated the effects of comorbidity on the volumes of various subcortical structures. These studies utilise the same subjects and focussed on the cerebellum, the pons, the thalamus and the stiatum (caudate, putamen and nucleus accumbens). In each case volumetry was undertaken using semi-automated ROI methodology, with adequate inter-rater reliability.[542-544] The only other group to investigate the effect of alcohol consumption on brain structure in schizophrenia was a Scandinavian group, who investigated a group of drinkers with schizophrenia who were not receiving treatment for an alcohol problem.[545] Their findings will be considered after those published by the group

comprising Sullivan, Pfefferbaum, Mathalon and others.

Sullivan *et al.* found regional volume deficits in the cerebellum and pons in the comorbid compared to schizophrenia alone group, but this was not the case for the thalamus or striatum.[542-544] The two studies with positive findings will be discussed further. Firstly, in the study focussing on the cerebellum, the vermis was divided in to four regions and each cerebellar hemisphere was outlined separately.[542] Volume of the fourth ventricle was also ascertained. On comparing these volumes between the groups, the comorbid group had significantly greater cerebellar hemisphere and vermian grey matter volume deficits and fourth ventricular enlargement than in either single diagnosis group, despite significantly lower levels of alcohol consumption compared with the alcoholic group. The authors propose a model of cerebellar supersensitivity to alcohol-related tissue volume deficits in schizophrenia to explain these findings. As further support for this they report that among the combined group of patients with schizophrenia, (both with and without a diagnosis of alcoholism), lifetime alcohol consumption was significantly related to grey matter volume in the anterior superior vermis, with amount of alcohol consumption accounting for the observed group differences in vermian volumes. As they stress, this relationship also argues against the contention that these vermian volume deficits predated the onset of alcoholism, increasing confidence that the have arisen secondary to alcohol use. They conclude by cautioning that by virtue of a supersensitivity of the cerebellum in schizophrenia to the detrimental effects of alcohol, patients with schizophrenia and alcohol problem comorbidity are exposed to risks for motor and cognitive dysfunction common to both diseases.

The study focusing on the thalamus and pons also employed semi-automated ROI methodology to enable comparison of structure volumes across the four

participant groups.[543] Interestingly, in this study volume deficits in either structure were not identified on comparing the (non-alcoholic) schizophrenia group to the controls. Additionally, thalamic volume did not differ between alcoholic patients with schizophrenia and controls or between patients with schizophrenia with and without comorbid alcohol dependence. It was however reduced in those people with alcohol dependence alone. By contrast however deficits in the pons were apparent in both the subjects with alcohol dependence alone and those subjects comorbid for alcohol dependence and schizophrenia when compared to the healthy controls. The authors conclude that patients with schizophrenia and comorbid alcohol dependence are at risk for alcohol-related reduction of pontine structures that are not necessarily affected by schizophrenia *per se*. They suggest the absence of volume loss in the thalamus in comorbid subjects may be explained by the effects of alcohol dependence on the thalamus in schizophrenic patients being mitigated by the antipsychotic medication they receive. Again they conclude by emphasizing that the comorbid group had consumed a fifth of the alcohol of the schizophrenia alone group, indicating a particular vulnerability in schizophrenia. This puts patients with schizophrenia and comorbid alcoholism at particular risk for impairment in cognitive and motor functions involving fronto-ponto-cerebellar circuitry.

As mentioned above, the study of Nesveg *et al.* investigated the effects of alcohol on brain structure in a population of 69 patients with schizophrenia who were non-clinical alcohol consumers; i.e. they were not recruited on the basis of having an alcohol use disorder.[545] They were compared to 97 healthy control subjects. Both subjects with schizophrenia and control subjects demonstrated a negative association between historic levels of alcohol consumption and white matter volumes. A significant association between alcohol consumption and grey matter volume is not

reported. Unfortunately, in this study a direct comparison was not made between people with schizophrenia who did and did not use alcohol; given that the methodology of this study is so different from that of the American group, the results of the two studies are not directly comparable.

The report of Nesveg *et al.* aside, it is thus the case that the body of work outlined above suggests that people with schizophrenia are particularly at risk of brain structural abnormalities in association with heavy alcohol use. It seems to be particular brain regions which are susceptible to this, the prefrontal cortex, anterior parietal grey matter, cerebellum and pons being identified as such regions to date. It is of course the case that these data arise from a single cohort of patients. The study of Nesveg *et al.* employed a design which was not comparable, meaning that repetition of the study of Sullivan *et al.* remains urgently needed. Nevertheless, it is the case that the studies of Sullivan *et al.* are well conducted and are consistent with the observation of particularly poor outcomes arising in cases of schizophrenia and alcohol dependence comorbidty. They make a strong case for people with schizophrenia being particularly vulnerable to brain structural sequelae secondary to heavy alcohol use, providing a biological underpinning to the devastating consequences of comorbidity which are observed clinically.

4.3.1.3 Tobacco

A single cross-sectional study has been undertaken comparing brain structure in 14 schizophrenic patients who do and 18 who do not smoke tobacco. The study also included 32 healthy comparison controls of whom only 2 smoked. Remarkably,

no information is given about use of any other substances in any of the included groups. Whole brain, voxel-wise analysis of regional grey matter was conducted using VBM. As has been demonstrated in other studies, the schizophrenic group as a whole had reduced grey matter in regions such as the orbitofrontal cortex, dorsolateral prefrontal cortex and superior temporal gyri. Somewhat surprisingly however, compared to those who did not smoke, schizophrenic smokers had relative *preservation* of lateral prefrontal and superior temporal grey matter.[546] The authors suggest that it is possible that these unexpected findings are explained by nicotine, in the context of schizophrenia at least, having a neuroprotective effect.

The report of Tregallas *et al.* has been followed by a longitudinal study which compared brain structural changes over five years in smokers to those in non-smokers.[547] This study included both 96 patients with schizophrenia (54 smokers/42 non-smokers) and 113 healthy control subjects (35 smokers/78 non-smokers). This was undertaken by the same group which published on the effects of cannabis over the same time period,[539] and may have utilsed some of the same patients. The authors do state however that none of the patients had a diagnosis of drug abuse or dependence at baseline, though three did have such a diagnosis at follow up. Van Haren *et al.* report that cigarette smoking does not explain the excessive brain tissue loss over time that they observed in patients relative to the healthy controls. They do report however that extremely heavy smoking (>25 cigarettes per day, a level of consumption present only in the patient group) was associated with excessive brain loss over time, although this did not explain the excessive tissue loss in the schizophrenia patients as a whole.

4.3.1.4 Other drugs

Despite the extensive literature searches undertaken, no structural imaging studies could be identified which specifically elucidated the structural brain imaging consequences of the use of the other substances of interest to this report by people with schizophrenia.

4.4 Alcoholic hallucinosis

The psychiatric enigma that is alcoholic hallucinosis (AH) was introduced in Section 3.2.2.1.2. In ICD-10 it is classified as Alcohol Induced Psychotic Disorder and is described as being characterised by hallucinations, delusions, psychomotor disturbance and abnormal affect; these symptoms occur in the context of a clear or only marginally clouded sensorium. AH occupies an interesting position in psychiatry, being a psychotic condition for which causation is essentially entirely attributed to an environmental exposure, heavy alcohol consumption. Though generally transient, it does have the potential for chronicity; in possibly 10% of patients the condition will run such a course.[548] The psychosis experienced closely resembles schizophrenia, though a recent report does outline a number of features in which the two conditions differ. Specifically, patients with AH are reported to exhibit greater levels of depressive and anxiety symptoms, fewer negative and disorganized symptoms, better insight and judgment, and less functional impairment compared to patients with schizophrenia.[549] Previous researchers have also reported that schizophrenic symptoms such as thought disorder and passivity are generally absent in this condition.

Given the clinical overlap between the two conditions, early researchers were optimistic that study of AH may have the potential to shed light on the pathophysiology of schizophrenia.[550] Family and genetic studies failed to demonstrate a greater prevalence of schizophrenia in relatives of patients with AH however, which led to genetic predisposition for the two conditions being regarded as independent.[271] This limited interest in AH, which combined with its rarity has meant that little research has been undertaken into the condition in recent years. Reflecting this lack of

research, I could find no structural imaging studies investigating the structural imaging characteristics of people with this condition. There have however been a small number of functional imaging studies. Given the relevance of AH to the current study I will outline the findings from these, despite the fact that findings from functional imaging studies are in general outwith the scope of the current report. Up until this year even the totality of functional imaging studies consisted solely of PET and single photon emission computed tomography (SPECT) reports undertaken as single case studies in individuals with unusually chronic AH. These have consistently reported a significant absolute or relative decrease in metabolism in either one or both thalami.[551-553] Interestingly in those subjects in whom the hallucinations resolved these abnormalities normalised.[551, 553]

Earlier this year the first case controlled imaging study investigating AH was published.[554] This utilised SPECT to compare regional cerebral blood flow (rCBF) in 19 patients with AH, 16 with schizophrenia, 20 with uncomplicated alcohol dependence and 19 healthy volunteers. Increased rCBF was demonstrated in the right calcarine area (medial occipital lobe) in patients with AH compared to healthy volunteers, with a trend towards increased rCBF to the frontal and temporal lobes and the right pallidum. Decreased left sided rCBF to the putamen, parietal, mid-frontal and mid-temporal lobes and heterogenous flow to the cerebellum were demonstrated in patients with AH when compared to patients with uncomplicated alcohol dependence. The left posterior cingulate and right cerebellum showed higher and lower rCBF respectively in patients with AH compared to patients with schizophrenia. The authors conclude that their findings implicate the right occipital lobe and possibly the cerebellum in the pathogenesis of AH. They state that they could not confirm the reduced rCBF to the thalamus suggested in previous case reports. Overall, on

integrating these data with previous reports, it is clear that the presence or absence of any functional imaging abnormalities in alcoholic hallucinosis remains at this point unclear.

Chapter 5

Questions raised by the systematic review and hypotheses to be tested in this study

In the preceding chapters a body of work is reviewed which has greatly increased our understanding of substance misuse, schizophrenia and the condition of 'dual diagnosis'. It is the case however that many questions about the inter-relationship of these conditions continue to be unanswered. It is my hope that answers to some of these remaining questions may be provided by this study, and later in this chapter I will outline the specific questions which this study aims to address. Firstly however I will summarise those themes from the preceding chapters which are of particular importance in guiding the focus of the data analysis sections. This will be followed by a consideration of findings from (nonhuman) experimental studies which may also have relevance in informing our understanding of the relationship between substance misuse and psychosis. This will lead on to a discussion of the specific hypotheses that this study is designed to address.

5.1. The importance of dopamine in addiction and psychosis

The importance of dopaminergic mechanisms in the development of substance dependence was discussed in Section 1.2. As part of this discussion the centrality of the mesolimbic dopaminergic pathway both in the experience of pleasure (reward) and imbuing the prevailing circumstances with salience (and so promoting memory) was reviewed. This pathway, centred on the nucleus accumbens, is believed to be crucial when drug use is initiated. As use continues however, it is argued that projections to the dorsal striatum become more important.[30] In this way behaviour

evolves from being a declarative process involving prefrontal executive functions into a habitual behaviour utilizing working memory circuitry.[31]

As discussed in Section 2.2 however, dopamine is also regarded as central in the development of psychosis. This was suggested by the key observations that stimulation of dopamine release by amphetamines resulted in psychotic symptoms, whereas dopamine blockade by antipsychotics attenuated them. In recent years one of the most influential hypotheses proposed to explain the *mechanism* by which dopamine overactivity in mesolimbic circuits could give rise to psychotic symptoms has (similarly to the addiction literature) focused on the importance of dopamine in imbuing salience to phenomena. Kapur proposed that psychosis is predominantly a condition of increased salience, which arises as a consequence of dopamine release and through which neutral experiences are mistakenly endowed with personal meaning.[110]

The overlap between the models proposed to explain the development of addiction and psychosis do not end there however. Further overlap is also apparent when the importance given to frontal lobe abnormalities is considered. In our understanding of schizophrenia, the idea that there is simultaneously overactivity of dopamine in the striatum and underactivity in the prefrontal cortex remains influential.[106] Complementing this in the addictions field, there is burgeoning neuroimaging and neuropsychological evidence suggesting that chronic drug abusers show deficits in tests of inhibitory control and decision making.[555-557]

It should thus be clear from the above that there is much overlap in the roles dopamine is purported to have in the models formulated to explain both the development of addiction and psychosis. As reviewed in Section 3.2.3, this is in-

keeping with the suggestion (first coherently formulated by Chambers *et al.*[317]), that the excess of substance misuse observed in schizophrenia may actually arise as a consequence of neuropathology associated with schizophrenia itself. By this argument this neuropathology impacts on the neural circuitry mediating drug reward and reinforcement, meaning that people with schizophrenia are biologically more vulnerable to the rewarding effects of drug abuse and thus at increased risk of addictive behaviour. Utilising findings from human and animal studies, Chambers *et al.* hypothesized that abnormalities in hippocampal-cortical function in schizophrenia impair the inhibitory hippocampal projections to the nucleus accumbens, resulting in reduced inhibitory control over dopamine-mediated functional hyperresponsivity to dopamine release. In this model, dysregulated neural integration of dopamine and glutamate in the nucleus accumbens resulting from frontal and hippocampal dysfunction could lead, in subjects without prior drug exposure, to neural and motivational changes similar to those in long-term substance use.

It is conceivable however that the overlap between the neural circuitry important for the development of schizophrenia and addiction has implications beyond simply explaining why people with schizophrenia are at increased risk of developing substance misuse problems. Though not proposed by Chambers et al, it also seems conceivable that in an adolescent *at risk* of schizophrenia (by, for example, abnormalities of hippocampal-cortical function), dysregulated neural and motivational changes arising as a consequence of substance use could further increase their risk of actually developing the condition. In short, by stimulating dopaminergic circuits directly (amphetamine) or indirectly (cannabis), (and possibly by resulting in other changes as well), certain illicit drugs could interact with other risk factors for psychosis, influencing an individual's risk of making the transition to illness. Thus, as

well as explaining why people with schizophrenia are more likely to develop substance misuse problems, the overlap between the neural circuitry implicated in addiction and psychosis could provide a possible explanation as to why certain drugs (see Section 3.2.2.1) appear to increase an individual's risk of developing schizophrenia. Data supporting the possibility that certain drugs of abuse can indeed have this effect will be discussed below.

5.2 Data from the review section supporting the possibility that substance use can increase the risk of an individual developing schizophrenia

5.2.1 Use of some drugs in adolescence is associated with an increased risk of developing schizophrenia

The evidence that use of various substances may increase an individual's risk of developing schizophrenia is reviewed in Section 3.2.2.1. The strongest evidence comes from cohort studies, which suggests that, even when confounders are controlled for, cannabis use is associated with an increased risk of subsequently developing schizophrenia. Two further important findings have arisen from this research however. Firstly, it was reported that early use of cannabis is associated with a greater risk of developing psychosis than later use, with several studies reporting that use before age 16 was associated with particular risk.[238, 255, 248] Secondly, it was shown that an allelic variant moderated the influence of adolescent cannabis use, with

cannabis users homozygous for the high activity Val allele having an at least fivefold increased risk of developing a schizophreniform disorder, whereas homozygosity for the M*et* allele offered relative protection.[121] These findings are important as they suggest that particular individuals, by virtue of factors such as youth or genetic variants, are particularly vulnerable to the risk modifying effects of cannabis use. Consequently, it is conceivable that structural brain abnormalities arising as a consequence of cannabis use may be detectable in specific, vulnerable populations which would not be observed in the general population.

In contrast to the considerable research examining if cannabis is a tenable risk factor for schizophrenia, comparable studies for other drugs of abuse are few. It is notable however that chronic heavy alcohol use has long been associated with induction of a hallucinatory state (alcoholic hallucinosis). Additionally, and as discussed in Section 3.2.2.1.2, in their analysis of data from the Swedish conscript study Lewis *et al.* report that there was an increased risk of schizophrenia in those with an ICD-8 diagnosis of alcohol abuse at age 18.[237] In a Japanese study 5% of methamphetamine psychosis cases were shown to have residual symptomatology many years later.[281]

The reports outlined above suggest that cannabis use, and possibly also use of alcohol or methamphetamine, may indeed contribute to an individual's risk of developing schizophrenia or other protracted psychoses. Furthermore there is a suggestion (arising from the cannabis and alcohol data at least), that certain individuals, by virtue of youth or genetic variability, may have a particular vulnerability to these effects. This is particularly interesting when considered with the possibility (discussed above) that it could be the dysregulated neural and motivational

changes arising as a consequence of substance use which increase an individual's risk of developing the condition. It seems understandable that use of such substances at a time of rapid biological change and particularly by a person already at elevated genetic risk of psychosis would be more likely to lead to alterations in neurobiology that increase psychosis risk.[256, 257]

5.2.2 Brain imaging abnormalities in dual diagnosis individuals are more pronounced than those observed in people with either schizophrenia or substance misuse alone.

Data reviewed in Sections 4.3.1.1 and 4.3.1.2 reported that individuals with established schizophrenia with a history of heavy use of either cannabis or alcohol exhibit more pronounced brain volume loss than those who do not use these substances. Though cannabis using control groups were not included in the former studies, in the case of the alcohol study an alcohol dependent control group was. In this study it was reported that the deficits observed in the alcohol-using schizophrenic group were considerably greater than the additive effects of alcohol and schizophrenia alone. Indeed, the combined effect of schizophrenia and alcoholism were reported as being best characterized as interactive rather than additive. Mathalon *et al* refer to this group as exhibiting compounded effects, and suggest that individuals with schizophrenia have a particular sensitivity to brain volume loss on exposure to alcohol.[541] The available data suggest that this may also be the case for cannabis given that (in contrast to studies in the normal population) substantial grey matter loss is reported in people with schizophrenia who use the drug.[539]

If it is true that people with schizophrenia have a particular susceptibility to the brain structural consequences of alcohol and cannabis use, then it is conceivable that this susceptibility is present prior to illness onset. If this is the case, it may characterise a group in whom alcohol or cannabis use is a particularly important risk factor for the development of psychosis. In the section above it is discussed that the risk modifying effects of cannabis appear to be particularly pronounced in adolescents, particularly those possessing genetic variants associated with risk of schizophrenia. Thus, it may be expected that adolescents at elevated risk of schizophrenia for genetic reasons may be a group in whom the structural brain consequences of cannabis use (as well as alcohol and possibly other drugs) may be particularly pronounced.

5.3 Findings from animal studies of relevance to understanding the relationship between substance misuse and psychosis

The data reviewed above suggest that cannabis use is a risk factor for schizophrenia, and that if the drug is used in adolescence the individual may be particularly vulnerable to this risk modifying effect. Additionally, as reviewed in Section 3.2.1.1, experimental studies have also demonstrated that acute administration of THC produces transient psychotic symptoms. Obviously however, true experimental studies examining whether repeated administration of cannabis/THC to an adolescent population results in an increased risk of subsequent *schizophrenia*

(rather than simply acute and transient psychotic symptoms) are not feasible. This however can be explored through animal models, which also offer the opportunity to directly examine the neurobiological consequences of adolescent cannabis/THC exposure. Important studies in this research area will be outlined below. In addition to studies examining the consequences of cannabis/THC exposure, relevant animal studies examining the consequences of alcohol use will also be included.

Animal data examining this possibility that cannabis can affect the central nervous system during a critical period and result in irreversible changes were recently reviewed by Bassong and Niesink.[558] They note early research demonstrating that not only can administration of cannabis extracts to rodents cause long-lasting effects at the behavioural level,[559-561] but that exposure of immature rats to THC induces more irreversible residual effects on behaviour than in mature rats.[562] Clearly this suggests that age during exposure may be a critical determinant of neurotoxicity outcome.[563] They go on to note that this possibility is further supported by later animal studies demonstrating that chronic peri-pubertal, but not adult, cannabinoid exposure causes long-lasting alterations in memory and behaviour, with functions mediated by the PFC such as working memory and prepulse inhibition (abnormalities also observed in schizophrenia) being particularly effected.[564-566]

Other studies provide insights into what may be occurring at a cellular level. As discussed by Demerika et al in their 2011 paper,[567] molecular and cell biology research has demonstrated that endocannabinoids promote oligodendrocyte progenitor survival,[568] and control axonal growth by inducing chemorepulsion or collapse of axonal growth cones.[569] Cannabionoid-1 (CB- 1) receptor activation increases astroglial progenitor proliferation and differentiation in vitro, and neural progenitor

proliferation and astrogliogenesis are decreased in adult CB1-deficient mice.[570] In keeping with these findings studies in rats have demonstrated that when exogenous THC is added, CB1 receptors in the hippocampus are down-regulated,[571] and endocannabinoid-mediated synaptic plasticity as well as the formation of new synapses is blocked.[572, 573] Other studies reported that the number of hippocampal neurons and synapses were decreased after THC administration and hippocampal neurons were smaller.[574, 575] Together these preclinical studies support the view of THC inhibiting neurogenesis. In adult rat studies these effects are observed predominantly in the hippocampus. Given data that human adolescence is a time of dramatic change in brain structure and connectivity, with widespread synaptic refinement and myelination occurring in cortical and subcortical in addition to hippocampal changes,[576] the effects of human adolescent cannabis exposure could potentially be even more pronounced. These effects could be detectable as change in volume of specific structures in human imaging studies.

In summary therefore the above suggests that cannabis (or THC) exposure at a vulnerable period in neurodevelopment could conceivably adversely influence brain maturation, increasing an individual's risk of developing schizophrenia. This most likely occurs through THC interfering with the interaction of endogenous cannabinoids with CB1 receptors which are critically involved in brain maturation. Through this means it is likely that THC disturb this normal physiological process, potentially resulting in disturbed neurotransmitter release, subtle neurotoxic effects, structural defects and potentially psychosis.

In keeping with the paucity of data from other fields of research, the potential contribution of alcohol exposure to the neurodevelopmental abnormalities believed to be important in the development of schizophrenia has received little attention in animal work. Indeed, no studies could be identified which had investigated the effect of adolescent alcohol exposure utilising animal models of psychosis. That the adolescent brain is particularly vulnerable to the effects of alcohol does seem to be the case however, being suggested by animal experiments undertaken by several groups.

Firstly, studies of the effects of binge drinking on rat brain structure have found that the effects are age-dependent, forebrain damage being more pronounced in the adolescent than adult rats.[577] Furthermore it has also been reported that ethanol more potently inhibits NMDA-mediated excitation and stimulus-induced long-term potentiation in hippocampal slices from early adolescent rats versus slices from adult hippocampus.[578, 579] In addition, adolescent rat brain has been found to be particularly sensitive to ethanol induced inhibition of neurogenesis.[580] Finally, a recent study found that a binge-drinking model of consumption increases cell death in the neocortex, hippocampus and cerebellum, and produced adverse neurobehavioural consequences detectable adults. Behavioural deficits on motor coordination and conditional discrimination learning tasks in alcohol-exposed animals persisted into adulthood.[581]

Consideration of the above data does suggest that alcohol-related toxicity could potentially result in deranged dynamic synaptic remodeling of the maturing adolescent brain. As is discussed by Crews *et al.*, this may have the effect of enhancing the strong learning components of heavy drinking behaviours while simultaneously resulting in the loss of important self-control and goal setting

components of the maturing brains executive centres.[582] One manifestation of this is a persisting propensity to alcohol problems, likely reflected in the fact that individuals who start drinking before the age of 15 are four times more likely to become alcohol dependent at some time in their life.[583] It is also conceivable however that these same effects (a primed 'reward system', impaired executive function and other, potentially even more dramatic consequences of aberrant synaptic remodelling) may also increase an individual's risk of developing psychosis. This is an area which (at the very least) would seem to warrant further research.

5.4 Hypotheses which will be tested in this study

The findings outlined above are central to the design of the current study. This will utilise data from the Edinburgh High Risk Study to examine whether people who are at familial risk of schizophrenia (and who are consequently assumed to be at elevated genetic risk of the condition) have a particular vulnerability to the brain structural consequences of substance use. This will be examined in a number of stages:

1. The brain structural associations of historic substance use (up to point of entry in to the study) will be contrasted between those at elevated risk of schizophrenia for familial reasons and matched controls. The null hypothesis is: 'the brain structural abnormalities associated with historic substance use

are no different in those at high risk of schizophrenia for familial reasons compared to controls'.

2. Within the high risk group the relationship between different levels of use of specific substances and volume of specific brain structures will be examined. The null hypothesis for this part of the study is: 'within the high risk group there is no relationship between historic levels of use of specific substances and volume of specific brain structures'.
3. The brain structural consequences of substance use between scan points will then be compared between high risk individuals who do and do not use specific substances between the scan points. The null hypothesis in this part of the study is: 'longitudinal structural brain changes in individuals at high risk of schizophrenia are not influenced by use of substances of abuse.'

Other secondary considerations will include examination of the influence of substance use before study entry on the rate of subsequent development of schizophrenia.

Chapter 6

Recruitment and ascertainment of clinical measures

6.1 Recruitment

The objective of the Edinburgh High Risk study was to identify people at high genetic risk of schizophrenia when well, and follow them up during the period during which they would be expected to develop schizophrenia. High risk individuals were defined as young people aged between 16-25, who did not have a history of serious psychiatric problems and had never been considered as psychotic, but who had at least two first or second degree relatives affected with schizophrenia.

Identification of high risk subjects was a labour intensive process. It was done by examining casenotes of all patients with schizophrenia known to individual hospitals where there appeared to be two related cases. Consent was sought from one of the affected subjects to speak to a healthy relative, from whom a full family history was obtained. In taking this history particular emphasis was given to the possibility of there being family members, aged 16-25, who were first or second degree relatives of the affected subjects. This methodology has been fully described in a number of published works.[584]

When planning the investigation it was calculated that the likelihood of genetically high risk individuals such as those recruited developing schizophrenia by the age of 30 was between 10 and 15%. It was therefore determined that 200 high risk subjects should be sought, with a view to 20-30 of them developing schizophrenia. Two control groups, each planned to be of about 30 subjects to enable comparison with those who went on to develop schizophrenia, were also recruited. One group was of age-matched individuals with no family history of psychotic disorder, while the other was age-matched cases of first episode schizophrenia who were not known to have a family history of the condition. In this study I will make no reference to the

first episode schizophrenia control group; this is because the issue of primary interest is the impact of substance misuse on brain structure in people who are at high risk of schizophrenia, but currently well. As discussed, studies have already been undertaken investigating the structural associations of substance misuse in people with established schizophrenia (Section 4.3.1).

Two hundred and twenty nine suitable high risk individuals who were prepared to consider inclusion in the study were identified and, in the first five years of the study, 162 of these provided useful data. In order to achieve these numbers participants were recruited from mental health services over much of Scotland. Thus, in addition to having substantial numbers of subjects from the city of Edinburgh, people were also included from families living in the rural areas of Argyll, Clyde, Borders, Forth Valley, Lothian, Highlands & Islands and the towns and cities of Inverness, Dumfries, Perth and Greenock. These are areas of stable population where traditional patterns of family life largely persist, providing the extended family networks necessary for the study. As subjects were from such diverse environments, the well controls were recruited from the social networks of the subjects themselves.

6.2 Plan of study, assessments used, and characteristics of participants

The study was conducted in two phases. The first took place from 1994 to 1999 and the second from 1999 until 2004. In the first phase all of the high risk subjects and both groups of controls were assessed at the point of entry in to the study in terms of psychopathology, neuropsychology and brain structure as determined by structural magnetic resonance imaging (MRI). As part of this battery of assessments, lifetime highest level of use of drugs of abuse was ascertained by self report in face to face interviews. Of the high risk subjects successfully recruited to the study 147 had usable scans, and so could be included in the following analyses.

Following entry in to the study, assessment of the high risk subjects and well controls were then repeated approximately every two years. These repeat assessments included a repeat MRI scan, assessment of psychiatric symptomatology, and ascertainment of both current levels of substance use and levels of exposure to tobacco, alcohol and illicit drugs in the intervening period. Both at initial assessment, and at follow up the main psychopathological instrument used was the Present State Examination, ninth edition (PSE-9).[585] Present State Examination (PSE) ratings were also obtained after diagnosis in subjects who became ill. After confirmation of a diagnosis of schizophrenia people left the study, and thus no further assessments were obtained. Unfortunately, the MRI scanner used in the study changed in 1998, meaning that only the second round of scans were all conducted with the same scanner as those at baseline. Quantitative comparison across a change in scanner is of uncertain feasibility while utilising standard image analysis methods.[586] For this reason the longitudinal analyses only utilise scans taken at timepoints 1 and 2.

A control group of 36 healthy individuals without any family history of schizophrenia was recruited from the same areas of Scotland as the high-risk participants. These people were of similar age to the high-risk group and their numbers were comparable to the number of people expected to develop psychosis. They underwent the same assessments, including scanning, as the high risk subjects.

6.2.1 Collection and analysis of baseline demographic and drug use data

6.2.1.1 Quantification of levels of drug use up to point of entry into study

As discussed above, on entry into the study lifetime use of alcohol, cigarettes and illicit drugs was ascertained by self report in face to face interviews. Current levels of substance use were also recorded, in a similar manner. At follow up assessments, the researchers recorded level of use since last seen, and again recorded current substance use.

Information on use of all substances was comprehensively recorded for the vast majority of participants at the vast majority of assessments. The manner in which it was recorded did exhibit some variability however, and to some extent was substance dependent. In the case of cannabis for example, consumption was generally recorded as frequency of consumption (e.g. has smoked cannabis twice a week for approximately six months) rather than absolute quantities consumed (e.g. ¼ ounce a week). In relation to cannabis use it is important at this point to comment on the *type*

of cannabis that the subjects included in this study would have consumed. At the time this study was conducted use of 'skunk' cannabis was not common, and the form of cannabis which would have been consumed would most commonly have been cannabis resin, which has a considerably lower THC:cannabidiol ratio. As discussed in Section 3.2.1.1 preparations with a relatively high THC:cannabidiol ratio may be particularly psychotomimetc.

Alcohol consumption was often recorded as number of units consumed per week, both currently and historically. This was however far from universal, and in a substantial number of cases consumption was not documented in a manner that could feasibly be quantified on a simple ratio scale; this would include, for example, '>21 units/week but no symptoms of dependence' or 'regular but less than 14 units a week'. Alcohol consumption was actually recorded in such a manner for a considerable number of subjects; it was the case for 51 of the 140 subjects with both baseline scans and data on past alcohol consumption.

Given the manner in which data were collected, for substances such as cannabis, cigarettes and ecstasy a categorical scale to quantify substance use was unavoidable. Ideally in the case of alcohol I would have employed a ratio scale, using number of units per week to quantify historic highest level of use of alcohol. This would however have meant excluding the 51 subjects on whom useable data on alcohol use had been collected, but not as a specific number of units per week. Given my wish to include as many subjects with usable data in my analyses as possible, this did not seem the optimal approach. Instead, it seemed most appropriate to employ a categorical system for quantification of levels of use of alcohol as well as the other substances under investigation. With this approach, all 140 subjects with usable data on highest levels of alcohol use could be included in the analysis.

The volume, pattern and frequency in which different drugs of abuse are consumed varies with the substance under consideration. This necessitates derivation of a categorical scale appropriate for each substance. Optimally, the cut-off points in such a scale would fulfil two criteria: (1) They would reflect potentially biologically significant points of transition in level of drug use; (2) They would reflect the manner in which drug use data were collected, enabling inclusion of the maximum number of subjects. The categorical scale chosen for each of the substances under discussion is discussed further below:

Alcohol

The categorical scale to quantify highest level of use of alcohol at baseline is detailed in Table 5.1. As can be seen, we included an 'occasional use' category, for people who had a history of alcohol consumption, but this has never exceeded 3 units/week. The inclusion of this minimal use category was felt to be necessary for two principle reasons. Firstly, and particularly given the prevalence and levels of alcohol consumption in Scotland (91.5% of Scottish adults drink alcohol, and 34% of men usually exceed government recommendations),[5] it was conceivable that teetotal subjects may have quite different characteristics to those who did consume alcohol, but at a low level. There is certainly considerable evidence to support this possibility. Abstention from alcohol use has been reported to be associated with social isolation, social anxiety, hostility and depression.[587] Additionally, though these data do admittedly arise from older populations, there are also reports of lower scores on cognitive testing[588, 589]and structural imaging abnormalities[398] in lifetime abstainers compared to low/moderate drinkers.

Category	Level of use
Teetotal	Lifetime abstinence
Occasional	Maximum level of use never exceeded approximately 3 units/week
Regular	Consumption has been >3 units/week, but never exceeded government recommended safe limits (14 units/week for women and 21 units/week for men)
Excessive use	Consumption has exceeded government recommended safe limits, but no history of dependence
Dependence	History of alcohol dependence syndrome

Table 6.1.
Categories employed in ordinal scale quantifying historic levels of alcohol consumption

In addition to there being a rationale for separation of low users of alcohol from lifetime abstainers, it also seemed important to distinguish between low and moderate users of the drug. I thus devised two categories for people who did consume alcohol, but not exceeding government recommendations; the occasional use category of people who did not consume more than 3 units/week, and the category of regular use but never having exceeded government recommendations (21 units/week for men and 14 units/week for women). This was important because grouping all non-teetotal subjects who reported using alcohol within government recommendations together (particularly given the accepted belief that people may under-report their consumption), would group a wide range of alcohol consumption levels together. At the lower end would be those reporting 'a glass of wine once a week' or 'occasionally a pint of lager once a week' (genuine very low users), while at the other would be some, (e.g. '10 pints of lager a week'), who may even just exceed government recommendations. Specifically choosing 3 units a week as the cut off enabled our inclusion of subjects reporting 'a pint a week' (pints being the most common quantity in which alcohol is consumed in Scotland), within this group. A similar approach has been used in other studies to identify comparable groups of occasional drinkers.[321, 393]

Given the considerations discussed above, I believed the separation of lifetime abstainers, occasional users and regular users to be clearly justified. As well as being

theoretically robust however, it also provided other advantages. Specifically, it enabled the identification of a group of very low users of alcohol unlikely to posses the atypical characteristics that could potentially be associated with being teetotal. As will become apparent in the subsequent section discussing the statistical methods used, the use of logistic regression is largely dependent on the presence of such a group.

The fourth and fifth alcohol consumption categories were, respectively, alcohol consumption exceeding government recommendations (but no history of dependence) and a history of alcohol dependence syndrome. In this instance I believed that a history of the dependence syndrome was a more appropriate cut-off point rather than an arbitrarily chosen consumption level.

Other substances

Categories comparable to those employed in the quantification of historic levels of alcohol consumption were used for the other substances. These are detailed in Tables 6.2-6.5. The categories used in cannabis consumption are similar to those used by previous researchers (see Table 6.2).[189] Similarly, the method for quantification of historic levels of tobacco consumption was fairly standard (see Table 6.3).[590, 591] A relatively small number of studies have been conducted quantifying levels of ecstasy and amphetamine use, but these have generally employed approaches to quantification similar to that in Table 6.4.[592, 593] This scale was also used to quantify exposure to LSD.

Category	Level of use
Never	Report that they have never consumed cannabis
Isolated	Have consumed cannabis on a maximum of three occasions
Occasional	Have consumed cannabis on more than three occasions, but frequency of use has never reached monthly
Frequent	Have used cannabis regularly. This has been on at least two occasions in a single month, but never exceeded three days in a single week
Most days/daily	Level of use has exceeded three days in a single week

Table 6.2
Categories employed in ordinal scale quantifying historic levels of cannabis consumption

Category	Level of use
Never	Non-smoker
0-10	Smoker, but not more than 10 cigarettes (or equivalent) a day
11-20	Smoker, consuming 11-20 cigarettes (or equivalent) a day
>20	Smoker, consuming >20 cigarettes (or equivalent) a day

Table 6.3
Categories employed in ordinal scale quantifying historic levels of tobacco consumption

Category	Level of use
Never	Report that they have never consumed the drug
Isolated	Have consumed the drug on a maximum of three occasions
Repeated	Have consumed the drug on more than three occasions, but frequency of use has never reached monthly
Frequent	Have used the drug regularly, this being on at least two occasions in a single month.

Table 6.4
Categories employed in ordinal scale quantifying historic levels of ecstasy, amphetamine, cocaine and LSD consumption

Participants were also questioned about use of other illicit drugs of interest, such as solvents, opiates, benzodiazepines and cocaine. Exposure to any of these substances was however relatively rare. For use of opiates and benzodiazepines, a scale incorporating a history of dependence was employed (see Table 6.5). For solvents the scale used was identical to that for ecstasy and amphetamines.

Category	Level of use
Never	Report that they have never consumed the drug
Isolated	Have consumed the drug on a maximum of three occasions
Repeated	Have consumed the drug on more than three occasions, but frequency of use has never reached monthly
Frequent	Have used the drug regularly, this being on at least two occasions in a single month.
Dependence	History opiate/benzodiazepine dependence syndrome

Table 6.5
Categories employed in ordinal scale quantifying historic levels of opiate and benzodiazepine consumption

6.2.1.2 Other relevant data collected from subjects

In addition to ascertainment of level of drug use and brain imaging, participants in the study were given detailed clinical and neropsychiatric assessments. These included ascertainment of IQ using the Wechsler Adult Intelligence Scale Revised,[594,594] and quantification of the experience of schizotypal symptoms using the Rust Inventory of Schizotypal Cognitions (RISC).[595]

The experience of schizotypal cognitions, as measured by the RISC, has been shown to be one of the strongest predictors of the subsequent development of schizophrenia.[187] Given the significance of this measure in this population, it will be informative to establish if scores on it vary with level of drug exposure. As a measure of psychotic-type symptomatology, it will also useful in suggesting whether or not self-medication of subclinical psychotic symptoms may be playing some role in substance use.

Low IQ is itself associated with risk of development of schizophrenia, and is also associated with use of some substances (see Section 3.2.3.1). It will thus be important to compare it across levels of substance use, and it will also be included as a covariate in analyses exploring the relationship between substance use and structural measures when relevant.

6.2.1.3 Statistical analyses

Statistical testing was conducted with the Statistical Program for the Social Sciences 14. Data were checked for normalcy and outliers by plotting graphically and conducting the Kolmogorov-Smirnov test. Demographic and clinical variables were compared between high risk (and control) subgroups with different levels of exposure to the various substances with analysis of variance and the χ^2 test.

6.2.1.4 Comparison of demographic and clinical characteristics between subjects with different level of exposure to the substances of interest

High risk subjects

One hundred and 47 high risk and 36 healthy control subjects had usable scans. For the vast majority of individuals full information on past and present use of each of the three substances of primary interest was available. However, information on alcohol use was not available for 7 high risk subjects, cannabis use for 5, and tobacco use for 10.

Demographics and clinical details, organised by level of use of each of the three substances, are shown for high risk subjects in Table 6.6. There is a (non-significant) trend for more males in the highest exposure groups to both cannabis and alcohol. There is also a significant difference in age across the alcohol exposure groups, occasional users tending to be younger. Additionally, there is a significant difference in IQ across the tobacco exposure groups, the heaviest smokers having the lowest IQ. There was no significant difference in RISC scores between individuals

with different levels of exposure to alcohol or tobacco. A difference did however exist between those with different levels of exposure to cannabis, those with greater levels of exposure tending to have higher scores.

Some high risk subjects had a history of use of illicit drugs other than cannabis. In only three subjects, with a history of opiate addiction, did level of past use constitute dependence. Data on levels of use of cocaine, ecstasy, amphetamines and LSD are given in Table 6.7.

Alcohol						
Level of exposure[a]	Teetotal	Occasional	Regular	Exceed safe limits	Dependence	*P*
Number of subjects (%)	10 (7.1)	28 (20)	56 (40)	37 (26)	9 (6.4)	
Mean age in years (SD)	20.9 (2.3)	19.8 (3.2)	21.4 (2.8)	21.9 (2.8)	21.2 (2.9)	**.05**
Gender (M:F)	4:6	14:14	29:27	18:19	7:2	.53
Handedness (R:L:both)	10:0:0	23:4:1	51:2:3	32:2:1	7:0:2	.16
Mean IQ (SD)	100.9 (16.9)	104.0 (16.0)	98.4 (12.2)	94.9 (9.4)	94.3 (8.9)	.06
RISC (SD)	25.7 (12.7)	26.6 (11.3)	30.7 (9.4)	29.2 (11.5)	34.9 (12.5)	.19
Cannabis						
Level of exposure[b]	Nil	Isolated	Occasional	Frequent	Most days	*P*
Number of subjects (%)	50 (35.2)	23 (16.2)	26 (18.3)	15 (10.6)	28 (19.7)	
Mean age in years (SD)	21.1 (3.0)	21.5 (3.3)	21.7 (2.5)	20.6 (2.5)	21.0 (2.9)	.77
Gender (M:F)	20:30	11:12	15:11	8:7	20:8	.11
Handedness (R:L:both)	42:5:2	22:0:1	24:1:1	13:1:0	23:2:3	.65
Mean IQ (SD)	98.4 (14.3)	101.8 (13.9)	100.1 (12.8)	96.6 (7.0)	95.3 (11.1)	.41
RISC (SD)	28.4 (10.6)	29.4 (9.2)	23.6 (10.5)	34.5 (11.7)	33.4 (11.0)	**.005**
Tobacco						
Level of exposure[c]	Nil	0-10	11-20	21+		*P*
Number of subjects (%)	62 (45.3)	42 (30.7)	20 (14.6)	13 (9.5)		
Mean age in years (SD)	21.4 (3.1)	20.6 (2.5)	21.0 (3.0)	22.5 (2.7)		.20
Gender (M:F)	32:30	24:18	10:10	7:6		.94
Handedness (R:L:both)	55:5:2	37:3:1	16:1:2	12:0:1		.72
Mean IQ (SD)	102.1 (13.6)	95.3 (9.3)	101.6 (12.6)	89.8 (8.4)		**<.01**
RISC (SD)	28.0 (10.5)	32.1 (10.5)	31.5 (12.8)	27.1 (12.6)		.24

Table 6.6
Demographic details of high risk subjects in each of the substance exposure categories.

[a] Exposure categories, based on highest level of consumption of alcohol during period of maximal use, are as follows: teetotal = no history of alcohol use; occasional = use never exceeded approx 3 units/week; regular = regular use but not exceeding 14U/week for women or 21U/week for men; exceed safe limits = exceeding safe recommendations; dependence = history alcohol dependence.

[b] Exposure categories, based on highest level of exposure to cannabis during period of maximal use, are as follows: nil = never used cannabis; isolated = used on maximum of 3 occasions; occasional = regular use, but less than monthly; frequently = use of cannabis monthly or greater; most days = use of cannabis more than three days a week.

[c] Highest level of tobacco use: number of cigarettes (or equivalent) per day.

Drug	Level of use: N (% of all high risk subjects with that level of use)			
	Never	Isolated	Repeated	Frequent
Cocaine	140 (94.6)	0	2 (1.4)	0
Ecstasy	111 (75.0)	9 (6.1)	9 (6.1)	13 (8.8)
Amphetamine	96 (64.9)	21 (14.2)	13 (8.8)	12 (8.1)
LSD	103 (69.6)	14 (9.5)	14 (9.5)	11 (7.4)

Table 6.7
Highest levels of use of cocaine, ecstasy, amphetamines and LSD by the high risk subjects.

Control subjects

Demographic details and data on use of alcohol, cannabis and tobacco for the control subjects are detailed in Table 6.8. Information on tobacco use was available for all of the control subjects, and that on both alcohol and cannabis use unavailable in only one case. Data on use of cocaine, ecstasy, amphetamines and LSD are given in Table 6.9. No control subjects had a history of misuse of opiates or benzodiazepines.

Level of exposure[a]	Teetotal	Occasional	Regular	Exceed safe limits	Dependence	*P*
Number of subjects (%)	2 (5.7)	4 (11.4)	18 (51.4)	10 (28.6)	1 (2.9)	
Mean age in years (SD)	20.4 (2.7)	21.1 (2.8)	22.9 (1.5)	21.1 (1.4)	22.6[d]	.77
Gender (M:F)	1:1	2:2	8:10	4:6	1:0	.11
Handedness (R:L:both)	1:0:1	4:0:0	17:1:1	8:1:1	0:1:0	.65
Mean IQ (SD)	118.5 (13.4)	114.5 (18.7)	106.4 (12.9)	98.9 (10.4)	79.0[d]	.41
Cannabis						
Level of exposure[a]	Nil	Isolated	Occasional	Frequent	Most days	*P*
Number of subjects (%)	12 (34.3)	8 (22.9)	7 (20.0)	7 (20.0)	1 (2.9)	
Mean age in years (SD)	20.3 (2.7)	21.1 (2.8)	22.9 (1.5)	21.1 (1.4)	22.6[d]	.25
Gender (M:F)	7:5	1:7	4:3	4:3	0:1	.15
Handedness (R:L:both)	9:2:1	7:1:0	7:0:0	6:0:1	7:0:0	.61
Mean IQ (SD)	114.8 (12.7)	95.4 (13.0)	107.0 (14.4)	104.0 (9.1)	85.0[d]	.02
Tobacco						
Level of exposure[c]	Nil	0-10	11-20	21+		*P*
Number of subjects (%)	18 (50.0)	10 (27.8)	6 (5.6)	2 (5.6)		
Mean age in years (SD)	21.4 (2.5)	20.6 (2.1)	21.6 (1.7)	20.9 (5.0)		.26
Gender (M:F)	10:8	3:7	2:4	2:0		.04
Handedness (R:L:both)	15:2:1	9:1:0	5:0:1	2:0:0		.81
Mean IQ (SD)	108.8 (13.6)	97.9 (13.1)	106.8 (12.6)	101.5 (27.6)		.03

Table 6.8

Demographic details of control subjects in each of the substance exposure categories.

[a] Exposure categories, based on highest level of consumption of alcohol during period of maximal use, are as follows: teetotal = no history of alcohol use; occasional = use never exceeded approx 3 units/week; regular = regular use but not exceeding 14U/week for women or 21U/week for men; exceed safe limits = exceeding safe recommendations; dependence = history alcohol dependence.

[b] Exposure categories, based on highest level of exposure to cannabis during period of maximal use, are as follows: nil = never used cannabis; isolated = used on maximum of 3 occasions; occasional = regular use, but less than monthly; frequently = use of cannabis monthly or greater; most days = use of cannabis more than three days a week.

[c] Highest level of tobacco use: number of cigarettes (or equivalent) per day.

[d] Standard deviation not calculable as only single subject

Drug	**Level of use: N (% of all high risk subjects with that level of use)**			
	Never	**Isolated**	**Repeated**	**Frequent**
Cocaine	33 (94.3)	0	0	2 (5.7)
Ecstasy	28 (80.0)	2 (5.7)	3 (8.6)	2 (5.7)
Amphetamine	25 (71.4)	1 (2.9)	5 (14.3)	4 (11.4)
LSD	28 (80.0)	1 (2.9)	6 (17.1)	0

Table 6.9

Highest levels of use of cocaine, ecstasy, amphetamines and LSD by the control subjects.

6.2.2 Collection and analysis of longitudinal drug use data

As discussed above, participants were followed up for up to ten years following entry in to the study. Outcome, in terms of whether or not they developed schizophrenia, is thus known for all subjects who had a baseline scan. In addition however, substantial numbers have had subsequent detailed assessments, including ascertainment of drug use in the intervening period and further MRI scanning.

The EHRS study was initially funded for five years. Recruitment of subjects took approximately three years, and rescanning was planned (in those who had not become unwell), after approximately eighteen months. This meant that only half of the subjects recruited were in the study long enough to be able to have a second scan during the initial funding period. Thus, in the initial part of the study, only 66 of the subjects recruited had a second scan. Subsequently additional funding was obtained, and those subjects who were not rescanned in the initial period did receive a second scan. Unfortunately however, and as discussed in the opening to Section 5.2, by this time the scanner available for use by the study had changed. Thus, these scan were undertaken with a different scanner. It is widely acknowledged that scanner change during the course of a longitudinal study introduces substantial problems, impacting on the consistency of data.[472, 586] When investigating what are likely to be subtle effects, (such as we would expect to observe in association with generally non-dependent levels of substance use), such factors can potentially have an important impact on the chances of observing positive findings, increasing the likelihood of a type II error. The fact that 66 subjects had two scans undertaken using the same scanner thus makes these subjects a particularly important group. Given this consistency of scanner use, (combined with our knowledge of drug use in the interim

period), we have an opportunity to investigate, in a longitudinal manner, the impact of substance use on brain structure in this unique group of people at genetically high risk of schizophrenia. Such data are sorely lacking in schizophrenia research. These analyses will thus have particular value, and constitute an important compliment to the baseline data.

It is thus the case that the substantial drop in participant numbers between scanning times 1 and 2 (147 vs. 66), is largely explicable by the manner in which the study was conducted. Additionally, a small number of subjects were not rescanned because they had developed schizophrenia between recruitment and the second scan point. As it had been planned from the outset that individuals would leave the study once they became unwell, it is thus the case that the vast majority of eligible subjects were rescanned; consequently, though the numbers with a scan at timepoint 2 was substantially lower than the total number recruited, this does not represent subjects being 'lost' from the study. There is thus no reason to believe that those subjects in whom follow up was achieved are not representative of the group as a whole.

Only 20 control subjects had baseline and follow-up scans with the same scanner. Clearly this is too small a number to enable meaningful exploration of the effects of substance use with in this group. Consequently longitudinal data from these subjects will not be discussed any further. I do not believe that the lack of a normal control group detracts substantially from the longitudinal analyses; clearly the comparison of primary interest is between high risk subjects who do and do not use the various substances of interest.

6.2.2.1 Quantification of levels of drug use between scans

Complete drug use data were available for the majority of those subjects who were scanned at both timepoints 1 and 2. As at baseline, this was ascertained by face to face interview, participants being asked about levels of use of the various substances of interest since the first assessment.

Ideally, similarly to analyses of baseline data, I would have wished to investigate if a dose response relationship existed between substance use and structure volume change. The desirability of establishing the presence or absence of such a biological gradient arises from the fact that this has long been regarded as an aid in inferring if causation is the link between an exposure and an outcome.[596] By these criteria, the establishment of such an association bolsters the argument that it is genuinely the drug exposure which is resulting in structure volume change. Unfortunately however, given the much reduced subject numbers available for the longitudinal analysis, this was not feasible. Instead, in these analyses data on use of each drug of interest were dichotomised, with the following cut-offs being employed: cannabis use during this period or not; alcohol use exceeding government recommendations during this period (greater than 14 units/ week for women and 21 units/week for men) or not; ecstasy use during this period or not; amphetamine use during this period or not; tobacco smoker during this period or not. This approach of dichotomisation of users and non-users does in fact have an established history of use in longitudinal studies for the investigation of brain structural changes consequent to drug use.[539] The choice of some of the specific cut off points does however require some further explanation.

Firstly, the choice of cut off points for cannabis consumption (and other illicit drugs) must be explained. It is the case that including subjects reporting *any* use of cannabis in the inter-scan period will result in inclusion of people with only isolated exposures to the drug in the interim period in the exposure group. It may be expected that, even if cannabis does have effects on brain structure, the effects associated with such low levels of cannabis exposure will be minimal. This raises concerns, as the inclusion of these people in the exposure group will potentially dilute any effects that are present and result in a Type II error. Unfortunately however, though this concern is genuine, choosing an alternative cut off to this does not seem feasible. If only subjects with greater than isolated exposures to cannabis in the interim period were included in the analysis, then there would be only 20 subjects in the exposure group. The analysis would consequently be relatively low powered. On balance it thus seems that, though the effects occurring may be less in these subjects (and thus it may jeopardise the chances of positive findings), including those people with low levels of consumption of cannabis in the interim period in the exposure group is the best decision overall. This has the clear benefits of yielding reasonably balanced groups to contrast (see below), has literature precedent,[539] and enables the inclusion of all available subjects.

As in the cannabis analyses, the relatively low numbers of subjects available for longitudinal analyses meant investigation of a dose response relationship between alcohol consumption and brain structural abnormalities was also not possible. Given the concerns discussed about the potential non-representativeness of the teetotal subjects, an important decision was whether or not to exclude these subjects from the longitudinal analyses. For two distinct reasons however I did not believe that this was appropriate. Firstly, due to the smaller numbers of subjects with scans at timepoints 1

and 2, the priority was to maximise inclusion of subjects; excluding the teetotal subjects would have resulted in a substantial loss of data. Secondly, as this was a longitudinal analysis, I believed that the arguments for exclusion of teetotal subjects on grounds of unrepresentativeness were less robust. In undertaking the baseline analysis, the concern was that these subjects may posses confounding factors, resulting in their characteristics being out of keeping with the general trend of associations with alcohol consumption. Any such factors would however be expected to be trait characteristics, and as such would not be subject to change over time; they thus would not confound the longitudinal analysis. As such, there was little rationale for excluding them from this analysis.

Concerns could also potentially be raised about inclusion of subjects in the 'regular use' category in these analyses. As will be discussed in more detail on considering subjects included in the baseline VBM analyses (see Section 7.2.6.2), there are sound arguments for exclusion of these subjects from the analysis, on the basis that the intermediate nature of their alcohol consumption levels will obscure differences that may well be present. Though ideally this would be done, again because of the relatively low number of subjects available for the longitudinal analyses, in practice this was not feasible. Thus, the 63% of subjects who used alcohol regularly but reported this as being within government recommendations were also included in the analysis, being placed in the 'not exceeding government recommendations category'.

Consumption of other drugs in the interscan period (namely tobacco, ecstasy, amphetamine, cocaine, opioids and benzodiazepines) was binarised as either did or did not use during the interscan period. Thus, use of the drugs to any extent conferred membership of the 'used' category, and complete abstinence membership of the 'not

used category'.

6.2.2.2 Other relevant variables from subjects with longitudinal data

Demographic and clinical details of subjects who did and did not use cannabis, alcohol and tobacco in the interscan period are detailed in Tables 5.10, 5.11 and 5.12. As for subjects included in the baseline analysis, RISC score at the time of the initial scan has been included in this table; as before I believed these data to be potentially informative in indicating if subclinical psychotic-type symptoms could be contributing to drug use. Other data included are that which may potentially be important to include as a covariate in the subsequent longitudinal analyses. Though IQ was included as a covariate in the baseline analyses, given the within-subject nature of any longitudinal analyses, I did not believe it appropriate to include this as a covariate in these. This is in common with the models employed by previous investigators.[539] Thus it is included in the tables for descriptive purposes only.

6.2.2.3 Statistical analyses

Statistical testing was conducted with the Statistical Program for the Social Sciences 14. Data were checked for normalcy and outliers by plotting graphically and conducting the Kolmogorov-Smirnov test. Demographic and clinical variables were compared between high risk subjects with and without each drug exposure with the independent t test and the χ^2 test. Rate of change in whole brain volume between the

scan periods was calculated by subtracting whole brain volume at the time of the second scan from that at the first and dividing the difference by the time that elapsed between the scans (i.e. [WBV2-WBV1]/time between assessments).

6.2.2.4 Comparison of demographic and clinical characteristics between subjects in different between scan substance misuse categories

Scans were obtained at both time points in 66 high risk individuals of whom data on use of all the substances of interest in the inter-scan period were available for 57. Longitudinal analyses will thus focus on these subjects.

Of the 57 subjects eligible for inclusion in longitudinal analyses, 25 consumed cannabis between the two assessments while 32 did not. Demographic and clinical characteristics of these two groups are compared in Table 6.10. In addition to the substances detailed in Table 6.10, two subjects used opiates, two cocaine and three LSD in the period between scans; in all cases these people were in the cannabis exposure group. As can be seen from Table 6.10, though demographic variables and score on the RISC were reasonably well balanced between the two cannabis exposure groups, the prevalence of use of tobacco, ecstasy and amphetamines were all greater amongst the cannabis consuming subjects.

	No cannabis use N = 32	Cannabis use N = 25	*P*
Mean age at first assessment (SD)	21.11 (2.87)	21.76 (2.52)	.38
Gender (male/female)	15:17	15:10	.33 (a)
Handedness (R:L:both)	28:4:0	21:2:2	.24 (a)
IQ (SD)	100.59 (14.73)	100.12 (11.69)	.90
Exceed recommended max alcohol consumption	4	7	.18 (b)
Smoke tobacco	8	18	<.001 (a)
Use ecstasy	0	9	<.001 (b)
Use amphetamines	0	10	<.001 (b)
Days between assessments (SD)	648.38 (128.06)	679.12 (206.85)	.49
Rust Inventory of Schizotypal Cognitions baseline score	25.44 (9.94)	27.92 (11.70)	.39

Table 6.10.
Demographic and clinical characteristics of high risk subjects who do and do not consume cannabis between timepoints 1 and 2.
P = independent t test, except (a) = chi squared and (b) = Fisher's exact test

Table 6.11 compares demographic and clinical variables in subjects whose alcohol consumption between the two scan periods did and did not exceed government recommendations. In addition to the substances detailed in Table 6.11, two subjects used opiates, two cocaine and six LSD in the period between scans. All the LSD and cocaine using subjects were in the 'within recommended limits' group, while the two subjects who used opiates were in the hazardous/harmful drinking group.

In contrast to the cannabis exposure contrasts, in the case of alcohol the exposure group did score significantly higher on the RISC. In terms of use of other substances however this group only exceeded the comparator group on the measure of tobacco consumption.

	Within recommended limits N = 46	Exceed recommended limits N = 11	*P*
Mean age at first assessment (SD)	21.31	21.73	.650
Gender (male/female)	5:6	25:21	.596 (a)
Handedness (R:L:both)	39:5:2	10:1:0	.762 (a)
IQ (SD)	101.93 (13.52)	93.91 (11.04)	.073
Use cannabis Y:N	18:28	7:4	.184 (a)
Smoke tobacco Y:N	17:29	9:2	.007 (a)
Use ecstasy Y:N	6:40	3:8	.354 (b)
Use amphetamines Y:N	7:39	3:8	.387 (b)
Days between assessments (SD)	656.09 (144.05)	686.00 (246.35)	.597
Rust Inventory of Schizotypal Cognitions baseline score	24.80 (10.12)	33.73 (10.60)	.012

Table 6.11
Demographic and clinical characteristics of high risk subjects who do and do not report hazardous or harmful levels alcohol consumption between timepoints 1 and 2.
P = independent t test, except (a) = chi squared and (b) = Fisher's exact test

Table 6.12 compares demographic and clinical variables in subjects who did and did not smoke tobacco between the two scan periods. Of those subjects who used other substances between scan points, both opiate users, one of the two cocaine users and four of the six LSD users were smokers between the scan ponts.

Again in contrast to the comparisons made on the basis of cannabis consumption, people who use tobacco in the interscan period had higher ratings on the RISC at the point of entry in to the study.

	Non-smoker during inter-scan period N = 31	**Smoker during inter-scan period N = 26**	***P***
Mean age at first assessment (SD)	21.50 (2.90)	21.27 (2.53)	.755
Gender (male:female)	16:15	14:12	.866 (a)
Handedness (R:L:both)	28:3:0	21:3:2	.275 (a)
IQ (SD)	101.39 (15.11)	99.19 (11.13)	.54
Use cannabis Y:N	24:7	8:18	<.001 (a)
Alcohol consumption exceeds government recommendations Y:N	2:29	9:17	.016 (b)
Use ecstasy Y:N	1:30	8:18	.008 (b)
Use amphetamines Y:N	2:29	8:18	
Days between assessments (SD)	662.84 (137.09)	660.69 (198.46)	.962
Rust Inventory of Schizotypal Cognitions baseline score	22.97	30.78	.005

Table 6.12
Demographic and clinical characteristics of high risk subjects who did and did not smoke tobacco between timepoints 1 and 2.
P = independent t test, except (a) = chi squared and (b) = Fisher's exact test

6.2.3 Does RISC rating predict alcohol or tobacco use independent of its association with the use of other substances?

As discussed above, in those subjects for whom longitudinal data were available, there was a strong associations between RISC rating at point of entry in to the study and subsequent levels of both alcohol and tobacco consumption. This is despite RISC ratings not being significantly different between the alcohol and tobacco exposure groups at point of entry in to the study (see Table 6.6). This difference is not simply due to exposure groups to these substances being determined in a different manner in the baseline and longitudinal analyses. Specifically, when baseline RISC ratings are compared between those with a history of being smokers (with any level of consumption) and those with no such history there is no significant difference seen. The same is also true when people with a history of exceeding government alcohol

intake recommendations are compared to those who have no such history. This raises the intriguing possibility that those with a higher RISC rating at entry in to the study may have an increased risk of subsequent use of these substances.

Teasing apart these effects is made more difficult by the substantial cross-correlation between use of these two substances; smokers are more likely to be heavy drinkers, making it difficult to ascertain if these behaviours are independently predicted by RISC rating. To ascertain if this was the case, I employed logistic regression in an attempt to clarify if either alcohol or tobacco use were predicted by RISC rating at point of entry in to the study. This analysis was undertaken while controlling for use of other substances, age and gender. This analysis will be detailed in Section 9.4.

6.3 History of substance use at entry into study and subsequent development of schizophrenia

Nineteen of the subjects with baseline data on drug use went on to develop schizophrenia. The χ^2 test or likelihood ratios (where sample size was small) were used to compare numbers developing schizophrenia in high and low exposure groups. Rate of development of the illness was compared between: 1) Those with a history of alcohol dependence and those without, 2) Those who had used cannabis at a rate greater than isolated and those who had not and 3) Smokers and non-smokers. Where significant differences were found, odds ratios (OR) were calculated. As relatively few people had used the other substances under investigation, comparable analyses were not undertaken for them.

In undertaking these analyses it was decided not to control for numerous factors which could potentially confound such an association (e.g. other substance use, IQ etc). This was decided as the primary aim of this study was to ascertain the consequences of substance misuse by this at risk population on brain structure. Subject numbers in the current study are obviously substantially smaller than studies designed to investigate specifically if substance use is associated with an increased risk of subsequent schizophrenia (e.g. Zamit *et al.*[237]); consequently the current study is inadequately powered to enable inclusion of all the covariates that would ideally have been incorporated in to such an analysis. Nonetheless, this basic calculation will clearly give an indication of whether or not use of a specific substance is associated with an increased risk of subsequent schizophrenia in the population under study.

6.4 Other considerations

6.4.1 History of substance use between timepoints and subsequent development of schizophrenia

For the reasons discussed in Section 6.2.2, the number of subjects with scans at timepoint 2 was substantially lower then at timepoint 1. Again as discussed above, one of these reasons was that subjects who developed schizophrenia between the timepoints did not have a second scan. As a consequence, of those subjects who did go on to develop schizophrenia, longitudinal data were available for many fewer than baseline data. In fact, in contrast to the 19 subjects with baseline data who went on to develop the illness, only 8 of those with longitudinal data went on to develop the illness. Given this marked loss of power it did not seem feasible to investigate if use of a specific substance between the timepoints was associated with subsequent risk of schizophrenia.

6.4.2 Reliability of self report as a measure of substance misuse

A criticism which could potentially be levelled is the reliance on self report as a means of determining substance misuse history. It could be postulated that people may not give a truthful account of their substance use, thus meaning that this approach is unreliable. While an objective method to determine history of drug use would have been desirable, unfortunately no appropriate tool exists. Given that we

were interested in lifetime history of drug use, drug testing would have added little to the study. Furthermore, self-report of drug use is in fact a reliable tool in the research context.[597]

Chapter 7

Scanning methods and overview of approach to image analysis

7.1 Scanning methods

Each participant underwent MRI scanning on a 1 T Siemens (Erlangen, Germany) Magnetom scanner. Midline sagittal localization was followed by two sequences to image the whole brain. The first scan was a double-spin echo sequence that gave simultaneous proton density and T2-weighted images (repetition time [TR] = 3565 msec, echo time [TE] = 20 and 90 msec, 31 contiguous 5 mm slices acquired in the Talairach plane, field of view 250 mm), which were used to exclude any gross brain lesions. The second scan, for the volumetric analysis of whole brain and regions, was a fast gradient echo sequence consisting of a 180° inversion pulse followed by a Fast Low Angle Shot collection (flip angle 12°, TR = 10 msec, TE = 4 msec, TI = 200 msec, relaxation delay time 500 msec, field of view 250 mm), giving 128 contiguous 1.88-mm-thick slices in the coronal plane orthogonal to the Talairach plane. Any inhomogeneity in the head coil was corrected for after scanning a flood phantom (see Whalley *et al.* for further details).[598] Regular phantom scanning was employed to ensure the reliability of the scan sequences over the course of the study.

7.2 Plan for image analyses

This study will have two fundamental features guiding its structure. The first will be that for analysis of baseline and longitudinal data, both semi-automated hand tracing and an automated technique will be employed in image analysis. The second is that findings from baseline analyses will be used, as much as appropriate, to plan longitudinal comparisons. The importance of these two approaches will be discussed below.

7.2.1 Importance of including automated processes as well as ROI

The remarkable sophistication of MRI allows neuroanatomical structures to be visualised in vivo with exquisite detail. Thus, cerebral structures preferentially affected in a given disorder can be identified, together with the manner in which they are altered. Quantitative tools such as volumetric measures based on manually traced regions of interest have been extensively used to assess the overall size of brain structures in individuals with schizophrenia, though data from people who are destined to develop the condition but still well at the time of assessment are few (see Section 4.2). This methodology has revealed important insights into brain abnormalities associated with schizophrenia, many of which have already been discussed.

Despite the insights gained from manually traced region of interest techniques however, sole reliance on such approaches has considerable limitations. Firstly, by their very nature these approaches rely on changes in the total volume of a structure to

be able to detect any abnormalities. This can be a particular weakness when applied to a population such as that in the EHRS, in which all are well at the time of assessment and not all of whom will ever develop schizophrenia; in such a population it is likely that any abnormalities present are fairly subtle, and changes in overall *size* of structures fairly minimal. This is likely the case even in the context of additional insults, such as exposure to drugs of abuse. Automated voxel based techniques, such as VBM, have the potential to detect very localised, substructural changes.[599] They may thus be able to detect abnormalities missed by ROI techniques as the magnitude of these substructural abnormalities falls within the range of measurement error of the structure as a whole.

A further advantage of techniques such as VBM arises from the fact that in a given condition brain structure may differ in multiple brain areas, and without assessment of other areas of the brain, a particular regional loss may lack specificity. As it relies on preselected regions of interest, which are then hand traced, analysis at the whole brain level is not feasible with semi-automated ROI techniques. VBM, by contrast, is not limited by these constraints.[599] Furthermore, automated procedures are not as labour intensive as manual measurements, and as they are almost completely user-independent, inter- and intra-observer variations are avoided.[599, 600]

Despite the accepted potential advantages of automated techniques such as VBM however, it is also generally acknowledged that they do have their own limitations. These arise primarily as a consequence of the fact that inferences drawn in VBM are dependent upon local coregistration, and due to the limitations of the currently available algorithms the degree of registration varies across the brain. This, when combined with the fact that significance corrections are made for multiple comparisons over the whole brain, can mean that SPM-based automated techniques

can potentially be overly conservative, and not identify findings which are genuinely present. Indeed, it is recognised that this can be a particular issue when applied to structural imaging data (the techniques originally having been developed for the analysis of functional imaging data).[601]

The limitation of VBM outlined above means that ROI techniques remain the gold standard method for image analysis. Nevertheless however, techniques can be applied to focus the VBM analysis and limit the volume over which correction for multiple comparisons is applied. Most commonly this is achieved by restricting the analysis to regions which have been determined *a priori* to be of particular interest.[601-603] This is achieved by using the small volume correction (SVC) function in SPM, significance being correcting for the voxels included in this restricted analysis rather than the whole brain. An important consideration in undertaking these analyses is thus which regions to target with these small volume corrections. This will be discussed when planning of the specific VBM analyses are discussed later. An obvious problem with this approach is that these SVCs can only be employed in regions for which there are pre-existing reasons to believe that structural abnormalities will be found. Similarly to ROI techniques, the identification of abnormalities is dependent on expecting them to be present in a particular region, meaning once again that unexpected but potentially important abnormalities will only be picked up if they are detectable at whole brain level.

There is also a further limitation of VBM which must also be acknowledged, and which is not easily correctable. It essentially arises as the flip side of the ability of VBM to detect very localised abnormalities. Given this sensitivity for the detection of localised changes, VBM is unfortunately less sensitive to the diffuse, distributed effects which may well be identified by hand tracing techniques.[604] This is a limitation

which reflects the fundamental methodology underpinning the technique and is largely insurmountable.

Given the considerations outlined above, it would be expected that VBM and manual tracing techniques have differing sensitivities. Indeed, this is exactly what has been observed in studies applying the two techniques to the same dataset.[604, 605] It follows from this that for optimal image analysis both methods should ideally be employed. This enables the advantages of each technique to be exploited and the sensitivity of image analysis maximised. It also follows however, (as has again been noted by previous researchers),[606] that the abnormalities detected by VBM and hand-tracing may not always exactly tally. Thus, though analysis of the current dataset is undoubtedly best served by the combined use of complementary methodologies, it would not be entirely unexpected if the regions identified as exhibiting abnormalities in association with substance misuse by the two approaches did differ. In this study VBM will be used to compliment ROI based analysis of baseline data, and Tensor-Based Morphometry (TBM, a complementary technique better suited to the analysis of longitudinal data) used to compliment longitudinal ROI-based analyses.

7.2.2 Determining regions in which small volume corrections will be employed

As discussed above, the use of SVCs is an established practice in VBM analysis. This is also the case in TBM analyses. The use of an SVC in a particular circumstance must however have clear justification, this arising from an *a priori* expectation that abnormalities would be expected to be found in the particular region to which an SVC is being applied. On the basis of the literature review, the

amygdala/hippocampus would qualify as such a region, being consistently demonstrated to exhibit abnormalities which may predate development of illness, (Section 4.2) and also possibly exhibiting abnormalities in association with abuse of several of the substances of interest (Section 4.3). Other candidate regions would be the thalamus and frontal lobes, and if there is evidence from the ROI analyses to suggest that abnormalities of these regions would be expected in association with use of a substance in this population, then thalamic and frontal lobe SVCs should certainly be considered. The initial plan for investigation will thus be that these regions, similar to the regions chosen for investigation with manual tracing, will be specifically investigated for abnormalities in association with a history of substance use with specific SVCs.

7.2.3 Using baseline findings to guide longitudinal analyses

As is apparent from the literature review in Section 4.2.2, longitudinal studies examining the impact of substance misuse on brain structure in people at genetic risk of schizophrenia are particularly lacking in the imaging literature. Given the rarity of these data, it is thus particularly important that optimal use is made of the dataset available to me. This necessity is compounded by the fact that, for the reasons discussed in Section 6.2, the number of subjects on whom longitudinal imaging data are available is substantially fewer than those with baseline data. This reduces statistical power to detect structural imaging consequences of drug use by this population, and makes it unlikely that any effects that are present will be robust to correction for multiple comparisons.

Given the above considerations, it is particularly important that the analyses that are undertaken on the longitudinal data are carefully planned. If this is the case, then the number of comparisons undertaken can be kept to a minimum, and the need for correction for multiple comparisons obviated.

Abnormalities of a number of brain regions (in particular the AHC and frontal lobes) have been so repeatedly reported in schizophrenia, that it would be difficult to exclude exploration of structural changes in them from any longitudinal analysis of imaging findings in a high risk population. For other regions, though there may be replicated findings of abnormalities in established schizophrenia, the case for their inclusion in the current longitudinal analysis is less irrefutable. The most appropriate regions to include in the analysis need to be chosen. As there is no study directly comparable to the present, pre-published data can only be used to guide the selection of these regions to a limited extent. What will be available however will be results from the (greater powered) baseline analysis. It would be expected, (if they did indeed arise as a consequence of substance exposure), that the brain structural abnormalities observed in association with substance misuse in the baseline analyses will overlap with those occurring between timepoints 1 and 2. It thus seems reasonable that findings from the baseline analysis are used to guide the selection of brain regions for longitudinal study. In this way the number of analyses can hopefully be minimised, statistical power maximised, and any brain structural abnormalities arising in association with substance use accurately identified. Baseline findings will thus be given appropriate importance in planning longitudinal analyses, how these findings have influenced the planning of longitudinal contrasts being explicitly discussed in the relevant section.

Chapter 8

Analysis of baseline data

8.1 Volumetric analysis

8.1.1 Semi-automated volumetric methodology

Image processing used the software package Analyze (Mayo Foundation, Rochester, MN, USA) to outline neuroanatomical structures and ascertain their volumes, with a semi-automated combination of automated edge detection and manual editing.[598] Structures were identified with the assistance of a MRI brain atlas,[607] and volumes were calculated by summing voxels on all brain slices included. The volumes of structures with the strongest pre-existing evidence of structural abnormalities secondary to substance use were included in this analysis. Structures selected were the: lateral ventricles, third ventricle, fourth ventricle, right and left prefrontal lobes, amygdala-hippocampal complex (AHC) and thalamic nuclei. Strict anatomical definition criteria incorporating anatomical landmarks were used to define these structures. These, together with relevant references justifying the use of particularly contentious boundaries, are outlined in Table 8.1. Further explanatory information is detailed below.

Region	Boundaries		
	Anterior	**Posterior**	**Medial**
Pre-frontal lobes	Frontal pole, when distinct from meninges	Slice anterior to corpus callosum [608]	Inter-hemispheric fissure
Thalamic nuclei	When clearly distinct from surrounding white matter and walls of third ventricle	Slice anterior to crus fornicis	Walls of third ventricle or the cistern beyond
Amygdalo-hippocampal complex	When clearly distinguishable from surrounding white matter. Not before temporal stem	Last slice of temporal lobe [609]	Naturalistic
Lateral ventricles	As defined by the autotrace, frontal horns included	As defined by the autotrace, occipital horns included	As defined by the autotrace
Third ventricle	When chamber is enclosed by the optic chiasm, as defined by the autotrace	Last slice of conical shape as distinct from transverse cerebral fissure beyond	As defined by the autotrace
Fourth ventricle	First slice of rhomboid shape as distinct from the cerebral aqueduct, as defined by the autotrace	As defined by the autotrace	As defined by the autotrace, lateral recesses included

Table 8.1
Summary of definition criteria of regions of interest.

For the purposes of this study, all thalamic nuclei were grouped together, as MRI resolution makes distinction between the individual thalamic nuclei difficult. Lateral and posterior boundaries are often indistinct. Given this, the posterior boundary of the thalamic nuclei was defined as the last slice in which the grey matter of the pulvinar nucleus was distinct from the emerging gyrus fasciolaris of the posterior hippocampus with the crus fornicis obscured behind it. Laterally, the posterior limb of the internal capsule was used as a boundary.

The distinction between the amygdala and hippocampus is also difficult due to resolution. For the purposes of study definition, both nuclei were defined together as a complex. Anterior, as the temporal stem appears, the gyri that enclose the amygdaloid nucleus become more evident. The lateral boundary was defined by the medial aspect of the temporal horn of the lateral ventricle, and the white matter of the temporal stem. The medial boundary was defined by the edges of the subiculum bordering the

subarachnoid space. Moving posterior, as the pulvinar nucleus disappears, the full extent of the crus fornicis is revealed and the fibre bundles gyrus fasciolaris and fasciolaris cinerea of the posterior hippocampus emerge. The posterior hippocampus was defined as distinct from the pulvinar nucleus when the full extent of the crus fornicis was present. Manual outlining of the thalami and AHC are illustrated for anterior and posterior regions of the brain in Figure 8.1. For clarity selected other brain regions have also been identified.

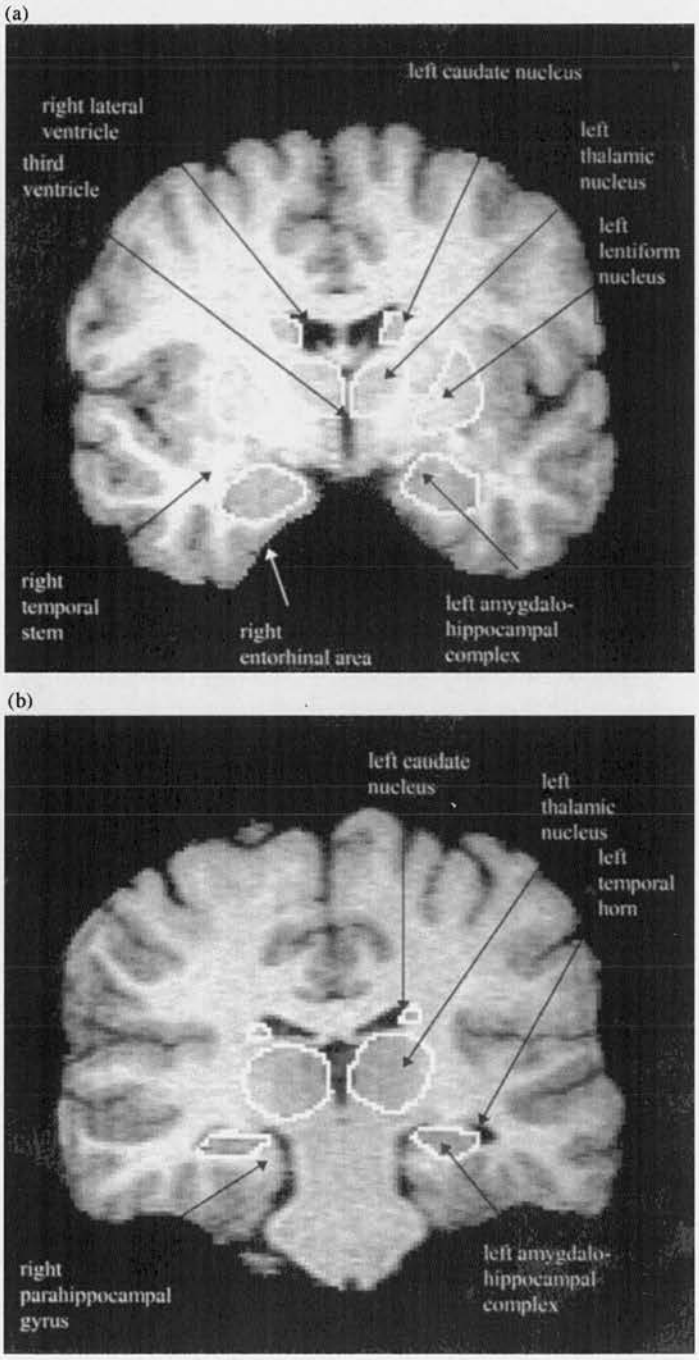

Figure 8.1
Sample of manual tracing.
a. Illustration of manual outlining of thalamus and AHC on an anterior slice of an edited whole brain.
b. Illustration of regional outlining of a more posterior slice.
In both examples, for reasons of clarity, other brain regions have also been identified.

Volumetric image processing was done by three investigators, who examined all the brain regions above on five brains to ensure reliability between raters (mean correlation coefficient 0·94 [range 0·78–0·99]). Intra-class correlation co-efficients (ICCs) for each of the structures of interest were as follows: 0.99 for whole brain and lateral ventricles, 0.98 for prefrontal lobes, 0.93 for third ventricle, 0.92 for fourth ventricle, 0.84 for thalamus, 0.82 for the amygdalo-hippocampus. All the semi-automated ROI data were available to me, thus meaning I did not need to undertake any of the hand tracing myself.

The ICCs quoted above are generally regarded as acceptable in image analysis.[610] To maximise the rigour with which repeatability of volumetric measurement was ascertained however, an additional method based on proposals made by Bland and Altman was also included.[611] By this methodology, the mean difference observed between a pair of raters (equivalent to bias between raters) was compared to the mean volume of the raters. This yielded an overall mean difference between ratings by different raters as a percentage of mean volume. For each of the regions of interest this was as follows: 0.7% for the whole brain, 0.8% for prefrontal lobes, 2.2% for the lateral ventricles, 4.4% for the third ventricle, 5.3% for the thalamic nuclei, 9.4% for the amygdalo-hippocampus.

In addition the main rater also measured her intra-rater reliability every six months on the same five brains. Given the longitudinal nature of the EHRS, this was of course fundamental to the validity of the study. Intra-class correlation co-efficients for each of the structures of interest were as follows: 0.99 for whole brain and lateral ventricles, 0.83 for prefrontal lobes, 0.97 for third ventricle, 0.98 for fourth ventricle, 0.88 for thalamus, 0.81 for the amygdalo-hippocampus. Similarly to the additional methodology employed in examining mean differences between raters, mean error

over time was calculated by dividing the mean difference between a pair of ratings by the mean volume of the two. Expressed as a percentage, the mean error over time was less than 1% for the whole brain, 4.1% for the prefrontal lobes, 5.5% for the lateral ventricles, 4.5% for the thalamic nuclei and 3.2% for the amygdala–hippocampus. It was however 23.3% for the third ventricle, indicating that despite the satisfactory ICC for this structure, there is potential for substantial measurement error in manually delineating this structure.

8.1.2 Statistical analysis of volumetric data

As before, statistical testing was conducted with the SPSS 14. Analyses were conducted for both high risk and control subjects. Wherever possible, due to their greater power,[612] parametric methods of analysis were employed. Data were checked for normalcy and outliers by plotting graphically and conducting the Kolmogorov-Smirnov test. In common with other studies, the distribution of ventricular volumes showed a right sided skew.[516] These data were therefore log transformed prior to the regression analysis. Subsequent standardised residuals were checked for normality.

The relationship between substance use and structure volumes was explored in two steps: firstly, investigation of a dose-dependent relationship and subsequently by multiple regression analysis. This two-step analysis was required because of the potential collinearity between both the levels of different substances used by individuals and the effects of the various substances on structure volumes. Multiple

regression has the potential to clarify which relationships do exist, while accounting for potential confounders.

For the reasons discussed in Section 6.2.1, level of substance use was ascertained using an ordinal, rather than ratio measure. This precluded the use of a parametric technique, such as Pearson's correlation, for exploration of the existence of a dose response relationship between the level of each substance used and structure volume. Instead I used Spearman's correlation co-efficient, a non-parametric test, with separate analyses being performed for each substance and each region of interest. I also wished to establish if the relationship between substance use and structure volume was significantly different on comparing the high risk and control groups. To enable this, the significance of differences between correlation coefficients in the high risk and control groups was tested using Fischer's Z test.

Multiple regression analysis employed a backward elimination model. Separate analyses were undertaken for each of the structures which showed a significant dose-response relationship between levels of use and structure volume. These structures were the third and lateral ventricles and the prefrontal lobes. For each analysis the dependent variable was volume of the structure of interest (e.g. third ventricle) with the predictor varibles entered simultaneously into the full model. These blocks were: gender; age; whole brain volume; alcohol use; cannabis use; and tobacco use. Gender was entered as a binary variable, age and whole brain volume as continuous variables. In the blocks representing exposure to each of the substances, levels of use were represented as dummy variables in relation to a reference group. In the case of cannabis and cigarettes the reference group was no use ever. As there were only 10 teetotal subjects (and there is reason to believe that the characteristics of teetotal individuals are not in-keeping with the general trends seen with increasing

levels of alcohol use, see section 6.2.1), for alcohol the reference group was occasional use.

In undertaking this backwards elimination analysis SPSS begins with all of the predictors included in the model. The programme then tests if any of the predictors can be removed without having a substantial effect on how well the model fits the observed data. The first predictor to be removed will be the one that has the least impact on how the model fits the data. This then proceeds in successive steps until the only predictors left are those which have a substantial influence on the dependent variable. As discussed above, these analyses complement the initial dose-respone analyses by clarifying which factors are indeed significant precitors of structure volumes while accounting for potential confounders.

Analysis was also repeated with current IQ (measured using the Wechsler Adult Intelligence Scale Revised),[594] as an additional block. Due to low subject numbers, the multiple regression analyses were not undertaken in the control group.

As was expected, a number of the high risk subjects had used illicit drugs other than cannabis. Regression analysis was therefore repeated excluding any individuals with a history of dependence on any substance other than alcohol, cannabis or tobacco. As excluding subjects with *any level* of exposure to illicit drugs other than cannabis would have resulted in too great a loss of statistical power, it was not feasible to repeat regression analysis without these subjects. A supplementary regression analysis was therefore run including level of past use of ecstasy, amphetamines and LSD as additional regression blocks. This was felt to be important in view of the possibility, (data investigating this possibility for ecstasy and amphetamines were reviewed in Section 4.1.3.4), that these drugs could themselves impact on brain structure.

8.1.3 Results

8.1.3.1 Dose response relationships

Structures with significant or near significant relationships between level of exposure to a substance and raw volume in high risk subjects are shown in Table 8.2. Level of cannabis use correlated significantly and positively with volume of the left and right lateral ventricles ($P = .013$ and .007 respectively) and third ventricle ($P = .001$; association shown graphically in Figure 8.2). Level of alcohol consumption also correlated significantly and positively with volume of the left and right lateral ventricles ($P = .023$ and .005 respectively; right lateral ventricle association shown graphically in Figure 7.2) and third ventricle ($P = .031$). Conversely, the level of alcohol consumption showed a negative correlation with volume of the left and right prefrontal lobes ($P = .022$ and .049 respectively). No significant associations were found between tobacco use and volumes of any of the structures of interest. The associations between substance use and structure volumes outlined above were not apparent in the control group (see Table 8.3); though power to detect such an association was admittedly lower, it was absent even at trend level. Additionally, on application of Fisher's Z test, there were significant interactions between group membership and the structural consequences of both cannabis and alcohol use. For cannabis this was significant in the third ventricle ($Z = 3.05$, $P = .002$), while for alcohol this was significant in the right prefrontal lobe ($Z = -2.5$, $P = .02$) and third ventricle ($Z = 3.45$, $P = <.001$).

A striking feature of alcohol structure volume/exposure relationships in the high risk subjects is the existence of a 'J-shaped curve'; it seems that despite a clear tendency for a history of greater alcohol consumption to be associated with larger ventricular volume, third, fourth and lateral ventricular volumes in lifetime abstainers are greater than in those with a history of occasional use (e.g. Figure 8.2). A complementary relationship is seen in the frontal lobes. This pattern is not seen with tobacco and cannabis (e.g. Figure 8.3).

Brain region	**Level of exposure to cannabis**					
	Nil	Isolated	Regular	Frequent	Most days	Correlation analysis
Whole brain volume	1327.04 (135.51)	1341.76 (132.33)	1349.0 (139.26)	1317.06 (81.63)	1387.49 (109.09)	r = .129 P= .126
Left lateral ventricle	3.45 (2.21)	4.78 (3.11)	4.52 (3.20)	4.51 (4.00)	5.83 (5.70)	r = .208 P= **.013**
Right lateral ventricle	3.08 (1.81)	4.44 (4.09)	4.19 (2.53)	4.30 (2.63)	5.23 (5.70)	r = .226 P= **.007**
Third ventricle	0.31 (0.19)	0.34 (0.18)	0.45 (0.32)	0.53 (0.25)	0.47 (0.31)	r = .271 P= **.001**
Left frontal lobe	76.94 (12.37)	72.31 (13.46)	75.67 (11.59)	76.58 (10.40)	78.70 (10.77)	r = .044 P = .603
Right frontal lobe	79.44 (12.52)	77.60 (14.68)	79.52 (12.92)	79.97 (10.29)	81.38 (12.57)	r = .058 P = .494
	Level of exposure to alcohol					
	Teetotal	Occasional	Regular	Exceed safe limits	Dependent	Correlation analysis
Whole brain volume	1334.48 (90.99)	1384.96 (118.75)	1326.41 (136.61)	1324.84 (125.59)	1391.75 (66.98)	r = -.062 P= .465
Left lateral ventricle	4.97 (2.77)	2.89 (2.00)	4.07 (2.20)	5.37 (4.12)	7.06 (8.78)	r = .192 P= **.023**
Right lateral ventricle	3.92 (1.70)	2.75 (0.77)	3.78 (2.74)	4.79 (3.44)	7.31 (8.89)	r = .174 P= **.005**
Third ventricle	0.38 (0.21)	0.29 (0.14)	0.39 (0.24)	0.47 (0.29)	0.50 (0.42)	r = .183 P= **.031**
Left frontal lobe	77.6 (7.26)	81.51 (14.18)	75.00 (1.12)	72.15 (10.47)	78.38 (10.32)	r = -.194 P= **.022**
Right frontal lobe	78.65 (7.79)	84.65 (1.25)	79.31 (13.40)	75.76 (12.13)	79.84 (11.19)	r = -.166 P= **.049**
	Level of exposure to tobacco					
Smoking	Nil	0-10	10-20	20+		Correlation analysis
Whole brain volume	1357.37 (135.56)	1332.05 (121.08)	1319.07 (124.60)	1352.07 (109.29)		r = -.062 P= .173
Left lateral ventricle	4.36 (2.83)	4.19 (3.11)	4.32 (2.47)	6.94 (7.83)		r = .040 P = .639
Right lateral ventricle	3.81 (2.68)	4.07 (2.87)	4.01 (3.00)	6.04 (7.80)		r = .079 P = .361
Third ventricle	0.37 (0.27)	0.42 (0.26)	0.40 (0.15)	0.49 (0.35)		r = .142 P= .099
Left frontal lobe	77.33 (11.58)	75.81 (12.55)	73.34 (11.96)	75.80 (12.47)		r = -.146 P= .089
Right frontal lobe	80.80 (12.37)	80.63 (11.84)	76.04 (15.77)	75.34 (11.29)		r = -.151 P= .079

Table 8.2

Mean (SD) volumes (in cm^3) of regions of interest in high risk subjects with histories of increasing exposure to alcohol, cannabis and tobacco.

Data are displayed only for brain regions in which a significant correlation was observed in high risk subjects between volume and level of exposure to at least one substance.

For information on intake level which costitues each the use historic use groups see Section 5.2.1

Brain region	Level of exposure to alcohol					
	Teetotal	Occasional	Regular	Excessive use	Dependence[a]	Correlation analysis
Whole brain volume	1387.13 (320.96)	1379.91 (166.62)	1335.84 (153.90)	1299.45 (141.80)	1299.51	r = -.141 P= .290
Left lateral ventricle	2.17 (0.34)	6.33 (6.32)	4.09 (2.18)	3.94 (2.72)	3.52	r = .017 P= .924
Right lateral ventricle	3.09 (1.47)	3.64 (1.92)	3.51 (2.08)	3.61 (2.31)	7.41	r = .083 P= .637
Third ventricle	0.59 (0.07)	0.57 (0.14)	0.38 (0.19)	0.30 (0.16)	0.30	r = -.456 P= .006
Left frontal lobe	87.00 (28.39)	79.22 (12.15)	79.08 (13.94)	74.13 (9.20)	83.93	r = -.071 P= .551
Right frontal lobe	90.71 (32.47)	79.22 (12.15)	79.08 (13.94)	74.13 (9.20)	83.93	r = -.276 P= .109
	Level of exposure to cannabis					
	Nil	Isolated	Regular	Frequent	Most days[a]	Correlation analysis
Whole brain volume	1363.36 (207.57)	1300.06 (130.89)	1323.56 (85.23)	1349.95 (133.23)	1157.63	r = -.051 P= .772
Left lateral ventricle	4.10 (2.42)	4.10 (2.42)	3.12 (1.71)	5.58 (2.91)	3.23	r = .225 P= .193
Right lateral ventricle	3.55 (1.62)	3.15 (2.36)	2.91 (1.74)	5.29 (2.24)	2.24	r = .113 P= .519
Third ventricle	0.49 (0.13)	0.34 (0.18)	0.33 (0.28)	0.37 (0.16)	0.26	r = -.309 P= .071
Left frontal lobe	79.20 (17.75)	75.74 (10.93)	81.81 (12.06)	76.76 (73.05)	73.29	r = .104 P = .551
Right frontal lobe	84.15 (17.92)	75.74 (10.93)	81.81 (12.06)	80.33 (12.11)	64.70	r = -.059 P = .647
	Level of exposure to tobacco					
Smoking	Nil	0-10	11-20	21+		Correlation analysis
Whole brain volume	1386.82 (165.31)	1291.47 (144.10)	1256.34 (79.30)	1302.12 (90.41)		r = -.365 P = .028
Left lateral ventricle	4.61 (3.34)	3.30 (2.81)	4.41 (2.07)	2.74 (1.60)		r = -.100 P = .562
Right lateral ventricle	4.06 (1.89)	2.57 (2.20)	4.31 (2.34)	2.34 (0.90)		r = -.185 P= .279
Third ventricle	0.47 (0.16)	0.28 (0.16)	0.38 (0.21)	0.29 (0.25)		r = -.227 P= .182
Left frontal lobe	81.13 (13.58)	74.37 (10.69)	73.86 (5.20)	84.98 (29.66)		r = -.227 P= .182
Right frontal lobe	86.08 (13.58)	78.22 (15.69)	75.34 (9.59)	85.40 (17.15)		r = -.231 P= .175

Table 8.3

Mean (SD) volumes (in cm^3) of regions of interest in control subjects with histories of increasing exposure to alcohol, cannabis and tobacco.

Data are displayed only for brain regions in which a significant correlation was observed in high risk subjects between volume and level of exposure to at least one substance.

[a] No standard deviation as only a single subject.

For information on intake level which costitues each the use historic use groups see Section 5.2.1

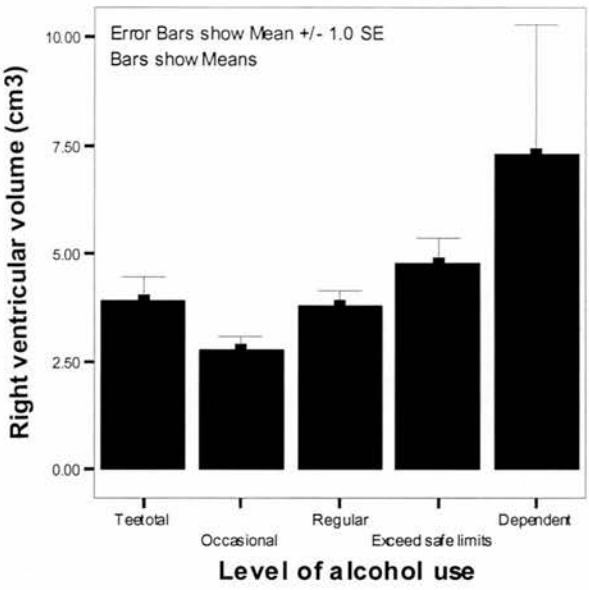

Figure 8.2
Bar graph illustrating increased volume (SE) of the right lateral ventricle (in cm^3) in association with increasing levels of alcohol exposure.

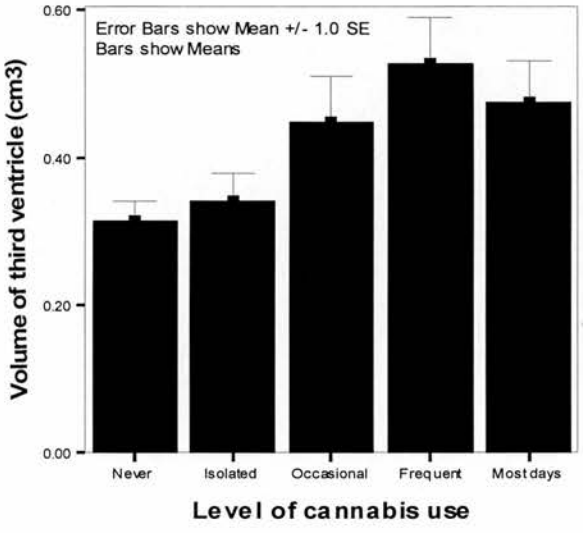

Figure 8.3
Bar graph illustrating increased volume (SE) of the third ventricle (in cm^3) in association with increasing levels of cannabis exposure.

8.1.3.2 Regression analyses

The correlation analysis outlined above did not control for potentially confounding factors or cross-correlation between the substances of interest. Blockwise multiple regression analysis was therefore undertaken, focusing on structures already demonstrating a significant relationship between volume and level of substance use. Only the 135 subjects for whom data were available on use of all substances under investigation were included in the regression analysis. Given low number of control subjects, (together with the absence of dose response relationship between historic substance use and structure volumes in this group), the regression analysis was not undertaken for them.

Output from the regression analysis is shown in Table 8.4. With a single exception (discussed below), the inclusion of IQ as an additional factor in the model did not significantly alter the results. Several findings are particularly noteworthy. Firstly, as expected from the dose response analysis above, level of alcohol consumption was a significant predictor of both right and left ventricular volumes. As well as higher levels of alcohol consumption being positively correlated with ventricular volume however, even relatively modest consumption of alcohol (at levels within government recommendations), was correlated with ventricular enlargement. Interestingly, lifetime abstinence also positively correlated with lateral ventricle volume.

Alcohol use exceeding UK government recommendations (more than 14 units/week for women or 21 units/week for men) correlated with reduced left frontal lobe volume. This correlation was significant on the right side only with inclusion of

IQ in the model ($R^2 = 0.54$, $P = .049$). No level of alcohol consumption was correlated with third ventricle volume (despite the strong dose response relationship). Third ventricular volume did however correlate positively with frequent use of cannabis. Left ventricular volume also correlated positively with use of cannabis most days. Though isolated use of cannabis correlated negatively with volume of the left frontal lobe, this was not within the context of a dose response relationship. Tobacco consumption of more than twenty cigarettes a day correlated negatively with volume of the right frontal lobe; again the dose response relationship between tobacco use and volume of the right frontal lobe had not reached significance.

Re-running the regression analysis excluding the three subjects with a history of opiate dependence did not alter the above findings. However, re-running the regression analysis with inclusion of highest use of amphetamines, ecstasy and LSD as additional regression blocks did. Although alcohol use exceeding safe recommendations remained a significant predictor of left lateral ventricle and left frontal lobe volumes ($R^2 = 0.226$, $P = .002$ and $R^2 = 0.590$, $P = .031$ respectively), it was no longer a significant predictor of right lateral ventricle volume. Frequent cannabis use remained a significant predictor of third ventricular volume ($R^2 = 0.148$, $P = .015$), but not left lateral ventricle volume. Most notably however in this expanded regression model a history of frequent (highest level of use greater than or equal to monthly) and repeated (highest level of use several times a year) amphetamine use were predictors of increased right and left lateral ventricular volume ($R^2 = 0.125$, $P = 0.005$ and $R^2 = 0.226$, $P = 0.011$ respectively), and repeated use of ecstasy was a predictor of increased left lateral ventricle volume ($R^2 = 0.226$, $P = 0.011$).

Despite the dose response relationships between alcohol and cannabis and structure volumes, it was not always the highest drug exposure level which came out of the regression model as being significant predictors. For this reason the regression analysis was repeated, combining the two highest exposure groups to each drug to create a single dummy variable representing all subjects with a history of substantial exposure to either substance. The combined cannabis exposure category correlated significantly with third ventricular volume, this being the case both with and without the inclusion of other illicit drugs in the regression model ($R^2 = 0.149$, $P = .014$ in both cases). Similarly on combining the two highest alcohol exposure groups, the resulting variable correlated significantly with left and right lateral ventricular and left frontal lobe volume, both with and without inclusion of other illicit drugs in the regression model. Output for each of the structures listed was $R^2 = 0.229$, $P = .008$; $R^2 = 0.161$, $P = .003$; and $R^2 = 0.614$, $P = .043$ respectively for each of the structures mentioned with other illicit drugs included, and $R^2 = 0.131$, $P = .001$; $R^2 = 0.167$, $P = <.001$; and $R^2 = 0.591$, $P = .025$ respectively for each of the structures without other illicit drugs being included in the regression model.

Dependent variable	Independent variables	Beta	t (df)	*P* value	Adjusted R^2 (complete model)	Impact of including IQ in model
Left lateral ventricle	Teetotal	0.23	2.59 (134)	.011	.11	Regular use of alcohol becomes non-significant ($p = .066$)
	Regular use of alcohol	0.21	2.06 (134)	.042		
	Exceed safe limits of alcohol	0.33	3.32 (134)	.001		
	Use of cannabis most days	0.20	2.31 (134)	.022		
Right lateral ventricle	Whole brain volume	0.26	3.19 (134)	.002	.14	Nil
	Teetotal	0.19	2.13 (134)	.035		
	Regular use of alcohol	0.26	2.34 (134)	.021		
	Exceed safe limits of alcohol	0.40	3.74 (134)	<.001		
	Alcohol dependence	0.29	3.20 (134)	.002		
Left frontal lobe	Whole brain volume	0.69	12.00 (134)	<.001	.58	Nil
	Exceed safe limits of alcohol	-0.12	-2.18 (134)	.031		
	Isolated use of cannabis	-0.13	-2.31 (134)	.023		
	Age	-0.15	-2.64 (134)	.009		
Right frontal lobe	Whole brain volume	0.67	11.1 (134)	<.001	.54	Exceed safe limits of alcohol becomes significant ($P = .049$)
	Exceed safe limits of alcohol	-0.10	-1.6 (134)	.106		
	>20 cigarettes a day	-0.13	-2.2 (134)	.032		
	Age	-0.14	-2.3 (134)	.020		
Third ventricle	Frequent use of cannabis	0.20	2.6 (134)	.012	.15	Nil
	Gender	-0.33	-4.1 (134)	<.001		
	Age	0.15	1.9 (134)	.065		

Table 8.4

Correlation of variables of interest with regional brain volumes as determined by the primary regression analysis.

Logistic regression employed a backward elimination model, with the independent variables of interest allotted to 6 blocks. These blocks were gender, age, whole-brain volume, alcohol use, cannabis use, and tobacco use. This methodology is explained further in Section 8.1.2.

8.1.4 Discussion

Though the findings detailed above do seem robust, a number of points require further clarification. Firstly, as reported above, it is notable that for some substances the level of use that significantly correlated with structure volume in the regression model was not the highest level of exposure. For example, ''excessive use'' of alcohol rather than ''dependence'' positively correlated with right ventricular volume, despite mean ventricular volume in the latter group being greater than the former (4.79cm3 and 7.31cm3, respectively). In the case of alcohol exposure, this is explained by the number of subjects in the highest exposure group being relatively few compared with the excessive use category. This is not so for cannabis exposure

however, there being more subjects in the ''most days'' than ''frequent'' category, yet the latter emerging as having a significant correlation with third ventricular volume. It is for this reason that the analyses were repeated combining the two highest exposure groups. That these combined exposure category correlated significantly with ventricular volumes in each case reinforces the veracity of the association between cannabis exposure and third ventricular volume and alcohol use and lateral ventricular volume.

It is also the case that though the majority of findings from the regression analysis were robust to inclusion of past use of amphetamines, ecstasy, and LSD as additional regression blocks, this was not universally the true. Specifically, on inclusion of these additional variables heavy use of alcohol was no longer a significant predictor of right lateral ventricular volume and frequent use of cannabis was no longer a significant predictor of left ventricular volume. It seems most likely that this loss of significance is explained by the loss of power resulting from the inclusion of additional factors; clearly however these results do need to be viewed with a little more caution than associations which were robust to the inclusion of these additional covariates.

Data on control subjects have been included for completeness. Given their small numbers we have not focused on them in detail, but it is the case that the absence of structural associations comparable to those observed in high-risk subjects is in keeping with findings from other studies (see Sections 4.1.3.1 and 4.1.3.2). The finding of decreased third ventricular volume with increasing history of alcohol exposure is notable however and requires further comment. This was unexpected and may not be reproducible in a larger sample of control subjects. It is notable however that nonconsumption of alcohol is very unusual in Scotland,[5] and as such teetotal

individuals may possess characteristics atypical of the general population. As such, it is conceivable that some unidentified confounding factor and/or factors are driving this unexpected relationship. Such factors may also be of relevance in understanding the comparable trend toward third ventricular volume reduction observed in this group in association with cannabis consumption.

8.2 Voxel-based morphometric analysis

As discussed in Section 7.2.1, I believed the optimal approach for analyses of both baseline and follow up scans was to complement the hand-traced volumetric data with automated analyses. The baseline data were thus also subject to analysis using voxel-based morphometry. This required some modification of the contrasts made. This, together with other considerations important in planning the VBM analyses will be discussed below.

8.2.1 MRI acquisition

The MR images used in VBM analysis were the same dataset used for region-of interest measurements and full MR image acquisition details have been described previously (see Section 7.1).

8.2.2 Choice of subject groupings for exposure comparisons

Ideally the VBM analysis would replicate the volumetric analyses, enabling direct comparison of findings between the two methodologies. However, the scales at which the two methodologies operate at are very different, meaning that exactly equivalent comparisons are innately impossible. It is also the case that the logistic regression employed in the ROI analysis cannot be implemented in VBM, due to the constraints required to approximate the massively parallel general linear model

required for voxel wise analysis. It is thus the case that a different statistical model must be utilized for the VBM comparisons.

Ideally any contrast chosen should maximise the inclusion of subjects. This is important both in ensuring that any findings are scientifically robust and in maximising the power of any analyses undertaken. The latter point is particularly relevant given that VBM is generally recognised as a conservative technique.[613] The most parsimonious contrast model, which would clearly include all eligible subjects, is to compare those individuals with levels of exposure to a drug which could be postulated to be harmful against those who have never experienced this level of exposure. The point of cut off in level of substance use which determines inclusion in either of these two groups will need to be considered for each drug individually, taking into account any relevant pre-existing evidence of what may constitute potentially harmful use in this population. As research in this area is so sparse however, it is expected that in most instances this decision will be largely intuitive. Consideration of cut off points is discussed for each substance of interest to the current study below.

In the volumetric regression analyses variables that could reasonably be expected to influence structure volumes were included as covariates. It is equally important that this is also done when constructing the VBM analyses. It is the case however that inclusion of multiple covariates results in a substantial loss of degrees of freedom; this reduces the chance of finding differences between the two exposure groups through increasing chances of a Type II error. For this reason, though analyses will be undertaken with the inclusion of all relevant covariates, they will also be run without the inclusion of these covariates. The latter analyses are regarded as exploratory and the findings are viewed with caution.

8.2.3 Control group

Only 8 of the control subjects had a history of consumption of cannabis frequently or most days. Similarly 11 had histories of alcohol use exceeding government recommendations. We believed these numbers too small for contrasts to be sufficiently powered for meaningful analysis. This is particularly so given the conservative nature of VBM. Given this, VBM analysis was not undertaken in the control subjects. Any structural imaging abnormalities observed in association with substance misuse in the high risk group will thus be considered primarily with reference to findings in normal subjects reported in previously published work. In this way it can be ascertained if they are particular to this genetically at risk group.

8.2.4 Use of small volume corrections (SVCs)

The use of SVCs in VBM analyses was previously discussed in Section 7.2.2. The choice of which would be appropriate SVCs to use in the following VBM analyses needs now to be considered further. As discussed in Section 7.2.2, this choice will be informed by the findings from both the volumetric analysis and the wider neuroimaging literature. It is important that these comparisons are chosen selectively, to avoid multiple comparisons.

An AHC SVC will be employed in analyses investigating the structural imaging associations of use of all substances. I believe this is important, as though the baseline volumetric analyses did not find evidence of volume reduction in the AHC in association with use of any of the substances under investigation, the weight of

evidence demonstrating abnormalities of this region in schizophrenia is particularly robust. Other regions with particularly strong evidence for abnormalities in schizophrenia are the frontal lobes and thalami (see Sections 7.2.2 and 4.2.1). Exposure related reductions in pre-frontal lobe volume were observed in association with both alcohol and cannabis in the volumetric analyses, with high levels of alcohol consumption emerging from the regression analyses as predicting both right and left pre-frontal lobe volumes. Despite the absence of a dose response relationship, heavy tobacco consumption predicted left frontal lobe volume. Given these findings, application of a prefrontal lobe SVC also seemed justified in the exploration of the structural imaging consequences of all three substances.

The most robust structural abnormality observed in association with cannabis consumption in the volumetric analyses of baseline data was third ventricular enlargement. There was a strong dose response relationship, and cannabis consumption was the only substance to predict third ventricular volume in the regression analysis. These findings suggest that cannabis consumption may be associated with relatively localised deficits, particularly affecting areas surrounding the third ventricle. As the third ventricle is bounded by the thalamus and hypothalamus, on both the right and left sides, it is these structures which are most likely to have exhibited the tissue loss resulting in enlargement of the structure. Of the two structures it is the thalamus which constitutes the majority of this boundary, the walls of the third ventricle being formed predominantly by the anterior and dorsomedial thalamic nuclei.[607] It would thus be expected that any volume loss occurring in association with cannabis use would be most likely to have occurred in this region.

Given the above discussion, I believed specific exploration of cannabis associated volume loss in the regions surrounding the third ventricle to be justified. As the ROI analysis could not pinpoint where volume loss had occurred to give rise to third ventricular enlargement, I employed a 12mm spherical SVC centred at the centre point of the third ventricle. This enclosed all of the third ventricle and the immediately surrounding tissues; given the anatomy of this brain region the immediately surrounding structure was predominantly the dorsomedial thalamic nucleus. Given the absence of positive findings from ROI analyses investigating the impact of use of other substances on third ventricular volume, this SVC was not employed in investigating structural abnormalities associated with alcohol and tobacco.

The other two SVCs (for the AHC and frontal lobes) were constructed from anatomical designations taken from Duvernoy[614] and supplemented by readings from Talairach and Tournoux,[607, 614] these were previously employed by Moorehead *et al.*[615] The amygdala-hippocampal complex SVC comprised the right and left amygdala, hippocampus, entorhinal area and parahippocampal gyrus. These SVCs have been used in a number of previous publications deriving from this cohort.[493, 602, 616]

8.2.5 Scan preprocessing

This was undertaken using SPM (http://www.fil.ion.ucl.ac.uk/spm/spm99.html) running in Matlab 6.1 (The MathWorks, Natick, MA, USA). A study-specific template was first constructed from all high risk scans. Since this group

contained scans from subjects with all levels of substance exposure, it was believed to represent the entire study population and therefore minimised bias for spatial normalisation. The scans were normalised to the generic SPM T1 template using 12-point linear affine transformation. A study-specific T1 template was created from the mean image calculated from all the normalised T1 images and smoothed at 8-mm full-width at half maximum (FWHM).

To generate study-specific brain tissue a priori maps, the normalised images were segmented into grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) using SPM cluster analysis with a modified mixture model and the SPM GM, WM and CSF *a priori* probability maps. A brain tissue mask was produced using the "Xtract brain" function. This removes the extracerebral voxels from both the GM and WM segmented images using a series of dilation functions and adds together the segments forming a binary image of extracted brain tissue. Multiplication of this image with the original segmented images removes the extracerebral voxels. Mean images were calculated from the CSF and extracted GM and WM segments and smoothed at 8-mm FWHM to produce study-specific a priori maps.

To process the images using the study-specific template, extracted brains in native space were registered with the extracted brain template in MNI space. This registration used a combination of 12-linear affine transformations and a linear combination of smooth spatial basis functions to account for the non-linear global shape differences. The original T1 images were then normalised into stereotactic space by application of the same combination of linear and non-linear transformation parameters obtained for the extracted brains.

Successful spatial normalisation was determined by visual comparison of homologous regions of the template and normalised images using the 'check

registration' option in SPM; this function allows simultaneous visual inspection of anatomical regions on 2 or more images. Two images did not normalise correctly and so were not included in the analysis. This meant that, in the case of cannabis for example, the contrast was 68 people with a substantial cannabis use history against 72 who had had no/minimal exposure to the drug.

The GM optimally normalised images were then segmented into GM, WM and CSF using the study-specific templates and the same modified cluster analysis function, and the extracerebral voxels removed as previously described. Each of the GM and WM optimally normalised and segmented images were smoothed with a 12-mm FWHM kernel.

8.2.6 Statistical analysis

8.2.6.1 General

SPM5 was used for the statistical analysis as it was the latest version available. In all VBM analyses the statistical analysis was performed on a voxel by voxel basis, based on the general linear model. Analysis of grey and white matter was conducted separately. In each case a tissue appropriate mask was applied.

Voxel-wise statistical analysis performed on spatially normalised segmented images results in statistical parametric maps showing areas where tissue concentration differs significantly between groups. The hypothesis being investigated was that use

of each of these substances was associated with tissue loss. The reverse investigation (that use was associated with tissue gain) was not investigated. This was important in limiting the number of analyses. Additionally, as can be seen from Section 4.1.3, there is no significant evidence to suggest that use of cannabis, alcohol or tobacco is associated with tissue gain.

For the reasons discussed in Section 8.2.2 the contrasts of interest were between the high and low exposure groups to each substance. These groups were compared using a t-test comparison, both with and without inclusion of relevant covariates. All of the covariates which emerged from the volumetric regression analyses as being predictive of any structure volumes were included as covariates in these analyses. Covariates included were thus: (1) use of substances other than the specific substance under investigation (i.e four of past use of cannabis, alcohol, tobacco, amphetamines and ecstasy, each expressed as a binary measure); (2) age; (3) gender. IQ was not included as a covariate as it did not emerge from the volumetric regression analyses as a significant predictor of any structure volumes. Similarly, past use of LSD was not included as it did not predict any brain regional volumes in the volumetric regression analysis. Whole brain volume was not included as a covariate due to the inherent correction for brain volume provided by spatial normalisation.[617]

The statistical parametric maps are displayed on a 'glass brain' allowing visualisation of clusters in 3 orthogonal planes – axial, coronal and sagittal. For all analyses, significance was assessed using uncorrected height thresholds of $P<0.001$, and reported at $P<0.05$ corrected for multiple comparisons. This threshold has previously been used in VBM analysis.[618-620]

8.2.6.2 Statistical models for investigating imaging associations of specific drugs

Cannabis

For the cannabis analysis, the three highest cannabis exposure groups of high risk subjects, (those subjects who had used cannabis 'occasionally', 'frequently' or 'most days'), were compared to those who had either never used the drug, or used it only on isolated occasions (≤3 times in their life). The two latter groups were combined as it seemed highly unlikely that such minimal historic use of the drug would be associated with any identifiable sequelae; including these subjects in the 'substantially exposed' group would thus dilute any effects that were present. This choice of cut off could be regarded as somewhat at odds with the inclusion of people with any level of use of cannabis use in the inter-scan period in the 'exposed' group in the longitudinal analyses (see Section 6.2.1). I believe however that this slight difference in categorisation is justified for a number of reasons: (1) longitudinal analyses may be expected to be more sensitive to subtle interactions; (2) case-control comparisons undertaken by other groups have excluded subjects with a history of minimal use of cannabis from the exposure group;[537] and (3) the model chosen for the longitudinal analysis also has precedent.[539] In reality, (given that a dose response relationship between cannabis use and brain structural abnormalities is suggested by the volumetric analyses above), the consumption cut-off point chosen in determining exposed and unexposed groups should not make any fundamental difference to the results obtained.

By employing the approach outlined above the inclusion of all subjects was achieved and thus power maximised. Given the two images that did not normalise correctly, 140 scans were available; of these 72 subjects were in the non/minimally

exposed group, and 68 in the group with considerable cannabis exposure. It was thus the case that the two groups were numerically well balanced. Demographic and other relevant characteristics of these two groups are compared in Table 8.5.

	Never used cannabis/used on ≤3 occasions N = 72	**Consumed cannabis on >3 occasions** n = 68	***P***
Gender M:F	30:42	43:25	.011*
Age at t1 (SD)	21.2 (3.1)	21.2 (2.7)	.993
RISC at t1 (SD)	28.53	29.75	.511
Cigarette smoker Y:N	21:49	53:12	<.001*
History excessive alcohol use[a] Y:N	14:58	30:36	.001*
Past use ecstasy[a] Y:N	4:68	27:41	<.001*
Past use amphetamines Y:N	6:66	39:29	<.001*

Table 8.5
Demographic and drug use characteristics of the two groups with and without a significant history of cannabis exposure at point of entry in to the study.
P = independent t test, except * = chi squared

As discussed above, initial comparison was made without the addition of any covariates and at whole brain level. This was justified as a primary aim of this initial analysis was exploratory, to identify any brain regions not targeted with the ROI analysis but demonstrating structural abnormalities in association with cannabis use. As mentioned above, VBM is known to be a conservative method of analysis, meaning that addition of covariates would result in substantial loss of degrees of freedom. In this context this could result in the non-detection of important areas of tissue loss.

Supplementary analyses were also run with the inclusion of relevant SVCs; in the case of the cannabis analyses these were SVCs for the AHC, frontal lobes and a 12mm diameter sphere centred on the central point of the third ventricle. The volume of the sphere employed is comparable to those used in previous analyses of these data.[490] These analyses were run without the inclusion of any covariates in the initial

analysis. Given that these analyses had pre-decided areas of interest, exclusion of these covariates is more difficult to justify on the basis of it being an exploratory analysis and these findings must be interpreted with caution. It is the case however that the initial ROI regression analysis found that only cannabis consumption, gender and age were significant predictors of third ventricular volume; other variables were not predictive of volume of this structure. As when applying the third ventricular centred SVC we were particularly interested in changes in this region, it seemed reasonable to re-run the analysis covarying only for these relevant factors. This was particularly important in the case of gender, as this variable was unbalanced between the two groups.

Additionally, all the above analyses were re-run including all the covariates incorporated in to the logistic regression model (with the exception of whole brain volume). Given the substantial loss of degrees of freedom associated with this, it was viewed as unlikely to yield significant findings. It did however seem important to attempt as close a reproduction as possible of the ROI analysis.

Alcohol

The alcohol analyses were run in a manner comparable to those for cannabis, though with some important differences. In this analysis only the two highest alcohol exposure groups, (those subjects with a history of consuming greater than government recommendations or frank dependence) were combined to form the potentially risk associated group. They were contrasted against the 'occasional use' group, rather than 'teetotal' group. As discussed in Section 6.2.1, this was because of concerns that the 'teetotal' group may be atypical. The group consuming alcohol regularly but not reporting that they exceeded government recommendations were not included in these

analyses. This was because I did not believe it appropriate to include them in either the putative risk associated or control groups. Inclusion in the latter group was inappropriate as they had not consumed alcohol at a level generally believed to be associated with risk. Conversely, the fact that I suspected the genetically high risk group may have a particular susceptibility to the structural consequences of substance use, combined with concern that some within this group may actually have exceeded government recommendations (discussed in Section 6.2.1), meant it was not appropriate to include them in the comparator group. I believed the benefits of excluding these subjects outweighed the consequent loss of power. This meant that 28 individuals with a history of occasional use of alcohol were compared to 44 individuals with a history of alcohol use exceeding government recommendations. Demographic and other relevant characteristics of these two groups are compared in Table 8.5.

	History of occasional use of alcohol N = 28	**History of use exceeding government recommendations** n = 44	***P***
Gender M:F	14:14	24:20	.71*
Age at t1 (SD)	19.80 (3.19)	21.87 (2.69)	.004
RISC at t1 (SD)	26.57 (11.27)	29.73 (11.72)	.262
Cigarette smoker Y:N	8:19	31:12	<.001*
History excessive alcohol use[a] Y:N	5:23	30:14	<.001*
Past use ecstasy[a] Y:N	2:26	15:29	.009*
Past use amphetamines Y:N	3:25	21:23	.001*

Table 8.6
Comparison of demographic and relevant clinical details of high risk subjects with and without a history of hazardous or harmful alcohol consumption.
Past smoking history was not available for one subject from each category.
P = independent t test, except * = chi squared

Similarly to the cannabis VBM analysis, the initial analysis was run without any covariates. The analysis was also run including all potential covariates of interest,

again to try and replicate as much as possible the region of interest analysis. As before, the loss of degrees of freedom meant I believed it unlikely that this would yield significant findings.

Given that alcohol consumption emerged from the regression analysis as a significant predictor of frontal lobe volume, contrasts were also repeated including a frontal lobe SVC. Alcohol did not emerge from the regression analysis as a significant predictor of third ventricular volume; thus a thalamic/third-ventricular based SVC was not employed in the alcohol analyses. Again, given overwhelming evidence for amygdalohippocampal abnormalities as being important in the development of schizophrenia, an AHC SVC was included.

Other substances

There was no evidence of dose response relationships between tobacco consumption and volumes of any of the regions investigated in the ROI analyses. Consumption of greater than 20 cigarettes a day did however emerge from the regression analysis as predictive of right frontal lobe volume. This positive finding from the regression analysis justified using the frontal lobe SVC, and as in all drug contrasts the AHC SVC was also included. As there was no clear rationale for it, analyses were not repeated utilising the thalamic SVC.

In the VBM analyses smokers/former smokers were contrasted against those who had never smoked. In deciding on this cut off point I was aware that including those who had only ever been light smokers/were now non-smokers in the smoking group did risk diluting abnormalities only associated with heavier use. I did however believe this to be justified for two reasons: (1) given the young ages of the subjects, even those who were currently none smokers would have been unlikely to be

abstinent for long; (2) there is no obvious level of tobacco consumption which can be regarded as a cut off between harmful and non-harmful use. Demographic and other relevant characteristics of these two groups are compared in Table 8.6.

	Never smoked N = 61	**Smoker** n = 74	***P***
Gender M:F	31:30	41:33	.595*
Age at t1 (SD)	21.34 (3.11)	21.02 (2.75)	.525
RISC at t1 (SD)	27.77 (11.13)	30.31 (10.79)	.182
History of greater than isolated cannabis use Y:N	12:49	53:21	<.001*
History excessive alcohol use[a] Y:N	12:49	31:41	.004*
Past use ecstasy[a] Y:N	7:54	22:52	<.001*
Past use amphetamines Y:N	9:52	34:40	<.001*

Table 8.7
Comparison of demographic and relevant clinical details of high risk subjects with and without a history of smoking.
P = independent t test, except * = chi squared

As in previous analyses, the initial analysis was undertaken without inclusion of any covariates. The contrast was repeated including all covariates of interest. These analyses were then repeated utilising the frontal lobe and AHC SVCs.

Relatively small numbers of subjects had used ecstasy and amphetamines at a level exceeding isolated use; only 21 and 25 subjects respectively. Considering the relatively small numbers together with the relatively low power of VBM to detect differences, it did not seem feasible to undertake VBM contrasts for these variables. These analyses were therefore not undertaken.

8.2.7 Identifying Talairach Regions

The VBM whole brain and SVC analyses were implemented in MNI space. The voxel co-ordinates of the results are extracted from MNI standard space and I

also report the Talairach co-ordinates using the Matlab function mni2tal.[621] The anatomic location of significant results was manually checked using the Talairach atlas,[614] supplemented by use of Tailarach Daemon (http://ric.uthscsa.edu/projects/talairachdaemon.html). Talairach Daemon is a programme which provides anatomical labels for inputted Talairach coordinates.[622, 623]

8.2.8 Results

8.2.8.1 Cannabis

Demographics

Demographic details for the 140 subjects included in the initial analysis are detailed in Table 7.4. Sixty eight subjects who had used cannabis on more than three occasions were compared to 72 who had either never used the drug or used it on a maximum of three occasions. As information on past use of all drugs of interest was not available for a small number of subjects (see Section 5.2.3; complete data were available for 135), these subjects had to be excluded from analyses including these covariates.

VBM analysis

On the initial grey matter contrast, without application of a SVC, no significant regions of grey matter loss were observed. This was also the case on application of the AHC and frontal lobe SVCs. On applying the SVC encompassing

regions surrounding the third ventricle however, a region of density loss was identified in the medial thalamus and hypothalamus, with three single significant maximal voxel locations. One, at *P*_corrected = .005 (*t* contrast value = 4.12) was found at MNI coordinate [-6, -8, -2]. The second, at *P*_corrected = .008 (*t* contrast value = 3.85) was found at MNI coordinate [-6, -11, -5]. Finally, the third, at *P*_corrected = .017 (*t* contrast value = 3.72) was found at MNI coordinate [-2, 12, 5]. These were converted into Tailarach coordinates (detailed in Table 8.7) and are shown both on a 'glass brain' and overlayed on a structural image in Figure 8.4. When gender and age were included as covariates a significant result remained in this brain region, encompassing an area including the hypothalamus and inferior dorsomedial thalamic nucleus. There were two maximal voxel locations; one at *P*_corrected = .035 (*t* contrast value = 3.41) was found at MNI coordinate [-6, -8, -2], while the second, at *P*_corrected = .049 (*t* contrast value = 3.85) was found at MNI coordinate [-8, -12, -4].

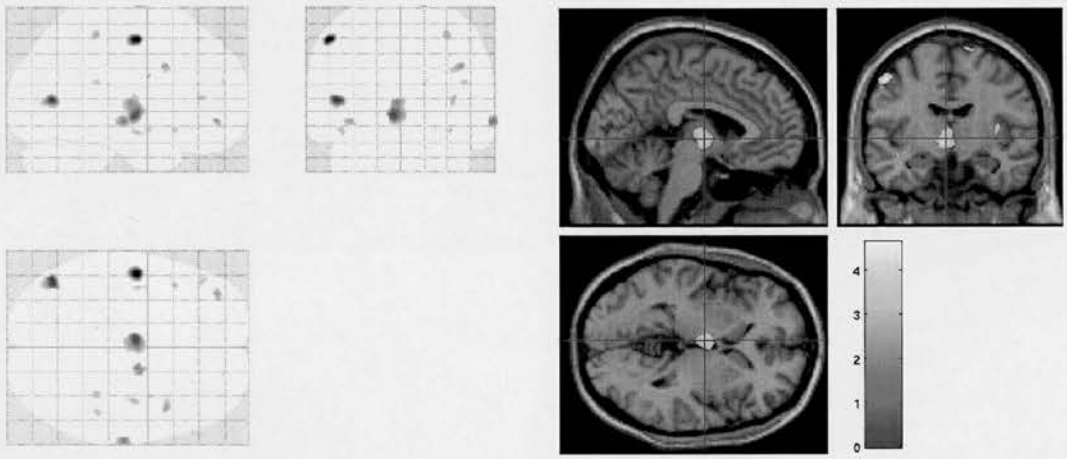

Figure 8.4.
SPM superimposed on a 'glass brain' showing voxels reduced density in cannabis exposed compared to non or minimally exposed subjects (left).
SPM overlay on a structural image demonstrating the region of density loss in the left thalamus (right).

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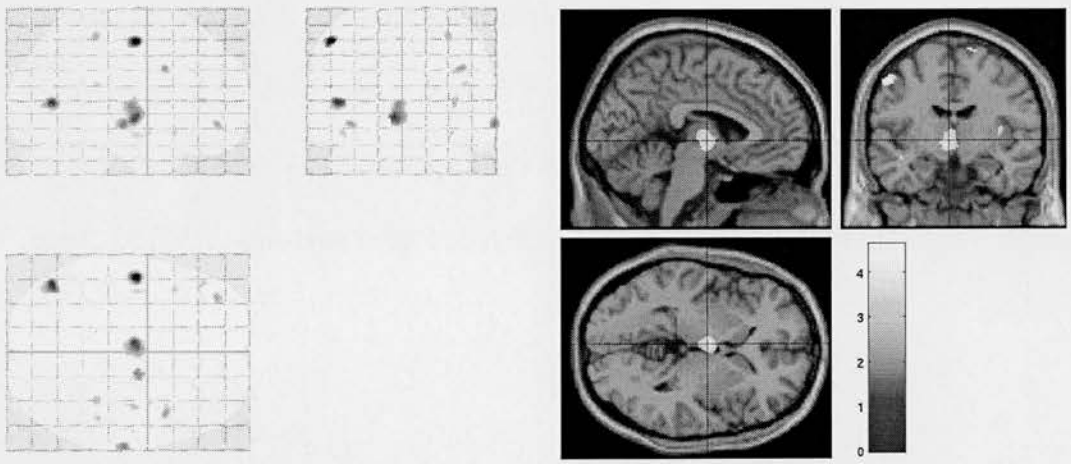

Figure 8.4.
SPM superimposed on a 'glass brain' showing voxels reduced density in cannabis exposed compared to non or minimally exposed subjects (left).
SPM overlay on a structural image demonstrating the region of density loss in the left thalamus (right).

When all the covariates incorporated into the ROI multiple regression analysis were included in the analysis both the above results became non-significant. In this analysis however a region of grey matter loss emerged in the right prefrontal lobe, in the region of the medial prefrontal gyrus (MNI coordinate 22, 33, -12). On application of the frontal lobe small volume correction this fell just short of significance, *P*_corrected = .060 (*t* contrast value = 4.32).

Voxel *P*(corr)	*X y z* (mm) Talairach coordinates	Point of maximal change
.005	-5.9 -8.7 -1.3	Left hypothalamus
.008	-5.9 -10.9 -3.7	Below left anterior thalamic nucleus
.017	-2.0 -11.4 5.2	Left dorsomedial thalamic nucleus

Table 8.8
Contrast of high risk subjects with >3 exposures to cannabis against those with minimal history of use of the drug. SVC for region around third ventricle has been applied.

The observed thalamic/hypothalamic result was robust to inclusion of all covariates found in the ROI regression analysis to predict third ventricular volume. It is the case however that on inclusion of additional covariates the finding became non-significant. Given this, I wished to try and confirm that this did represent a genuine effect. It is generally accepted that an observed effect such as this is regarded as more robust if the areas of maximal reduction are anatomically plausible when overlayed on the original scans included in the analysis.[624] With this aim, a random selection of scans were observed, with the location of maximum difference on the statistical maps (after application of the spherical SVC surrounding the third ventricle) being identified. A clear pattern emerged. In exposed subjects this point is on the thalamic/third ventricle boundary, whereas in controls it is in body of thalamus. This does seem to suggest that volume is being lost by the thalamus (in a fairly specific region that encompasses

the anterior and dorsomedial thalamic nuclei), and that this is resulting in the third ventricle result.

The white matter contrast revealed no significant areas of tissue density loss. This was also the case on inclusion of the relevant covariates.

Discussion

The findings outlined above suggest that the third ventricular enlargement observed on the ROI analysis in association with cannabis is due to loss of thalamic (and possibly hypothalamic) volume. Though not robust to inclusion of all covariates included in the ROI regression analysis (this likely being attributable to the consequent loss of degrees of freedom), it is robust to the inclusion of all covariates found to be predictive of third ventricular volume in the baseline regression analysis. These findings suggest that in this high risk population cannabis consumption may have a particularly thalamotoxic effect. Indeed, it even suggests that this effect may be specific to particular, paraventricular thalamic nuclei (namely the anterior and mediodorsal thalamic nuclei)

If thalamic volume loss has genuinely occurred in association with cannabis use, then why is this not seen on volumetric analysis? Though on first consideration this lack of consistency between the two methods seems contradictory, on reflection however it may not be entirely unexpected, a potential explanation arising from the intrinsic differences between the two techniques. Importantly, as discussed in Section 7.2.1, it is well recognised that volumetric and VBM techniques have different sensitivities; while VBM is less sensitive than volumetric techniques in some regions, it can be more sensitive to localised tissue loss.[604, 606, 613] The thalamic tissue loss observed by VBM has occurred in a small, relatively localised region within this

structure. Given that the thalamus is a relatively large structure, this will constitute only a small percentage of total volume, and thus the volume loss would be expected to be within the bounds of measurement error inherent in semi-automated hand-tracing techniques. It would thus be missed by the volumetric approach, even if detected by VBM. The resulting increase of volume of the third ventricle however, given that this is a smaller structure, will constitute a greater proportion of the total volume of this structure potentially lying out-with the boundaries of measurement error. It will thus stand a greater chance of being detected by hand-tracing.

These speculations are attractive, and do confer some coherence to the ROI and VBM results. Given the borderline significance of the result however, this explanation is certainly for from conclusive. What it does provide however is a hypothesis to guide analysis of the longitudinal data, suggesting that thalamic volume loss over time may be observed in association with cannabis exposure.

8.2.8.2 Alcohol

Demographics

Demographic details for the 72 individuals included in the analysis are detailed in Table 8.5. Twenty eight individuals with a history of occasional use of alcohol were compared to 44 individuals with a history of alcohol use exceeding government recommendations.

Results

On the initial alcohol analysis (without any covariates), the strongest grey matter finding was loss of a region of grey matter in the left superior parietal lobe. This did not however reach significance; *P*_corrected = .580 (*t* contrast value = 4.22) was found at MNI coordinate [-32, -50, 63]. A frontal lobe SVC was applied, but even with this no significant regions of grey matter density loss were observed. This was also the case after application of an AHC SVC. No significant findings were observed after inclusion of all relevant covariates.

White matter findings were more striking. On the initial analysis (with no covariates), density loss was observed in a diffuse region extending in to much of the corpus callosum. Density loss was particularly pronounced in the splenium, the voxel location of maximum change being found at MNI coordinate [18, -25, 18], though this still fell short of significance; *P*_corrected = .291 (*t* contrast value = 4.36). This probability map is shown both on a 'glass brain' and overlayed on a structural image in Figure 8.5. This finding remained non-significant after inclusion of all relevant covariates. Even with application of a frontal lobe SVC no significant areas of white matter density reduction were seen in this region either with or without inclusion of covariates.

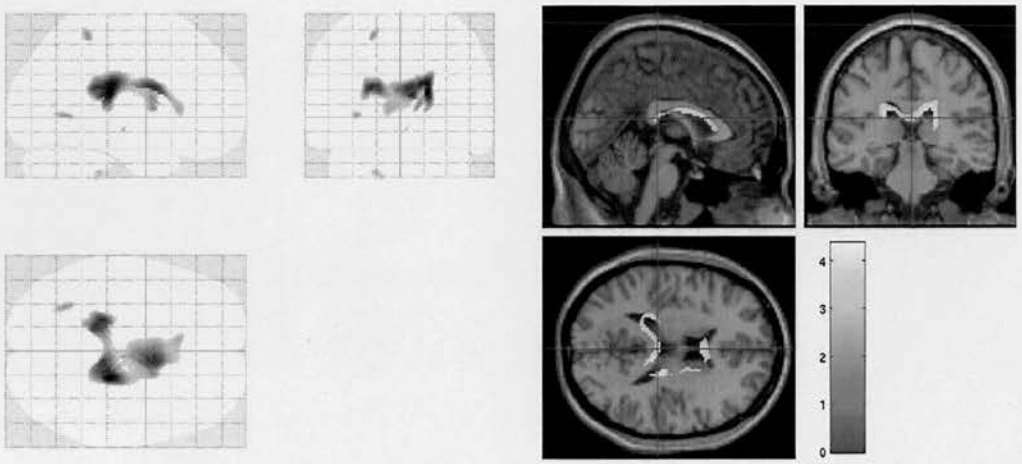

Figure 8.5
SPM superimposed on a 'glass brain' showing voxels demonstrating reduced white matter density in subjects with a history of excessive alcohol consumption compared to occasional drinkers (left).
SPM overlay on a structural image demonstrating the region of density loss in the corpus collosum (right).

Discussion

Abnormalities observed in association with excessive use of alcohol were seen predominantly in white matter. This is as we would have expected; the weight of evidence is that this tissue type is particularly vulnerable to the effects of alcohol.[377] Though it fell short of significance, the strongest grey matter result was in the left parietal lobe. There is in fact a body of evidence suggesting that the parietal lobe is particularly vulnerable to the effects of this drug.[397, 625] Indeed, this was a region demonstrated to be particularly vulnerable to volume loss in Mathalon *et al.*'s study of people with schizophrenia with alcohol use disorder comorbidity.[541]

8.2.8.3 Tobacco

Demographics

For the 135 people for whom smoking history was known, those with any history of smoking were compared to those who had never smoked. Demographic and relevant clinical details of these subjects are outlined in Table 8.6.

Results

On the initial tobacco analysis (without any covariates), the strongest grey matter finding was loss of a region of grey matter in the superior temporal gyrus, at MNI coordinate [41, -37, 15]. This did not however approach significance; *P*_corrected = .412 (*t* contrast value = 4.22). No significant regions of grey matter density loss were observed on applying either a frontal lobe or AHC SVC. Additionally, no significant findings were observed after inclusion of all of the covariates included in the ROI regression analysis.

White matter findings were more striking. On the initial analysis (with no covariates), a significant area of density loss was observed in the right cerebellum, maximal at MNI coordinate [24, -60, -43], *P*_corrected = .036 (*t* contrast value = 4.83). This probability map is shown both on a 'glass brain' and overlayed on a structural image in Figure 7.6. This finding remained significant after inclusion of all relevant covariates, *P*_corrected = .049 (*t* contrast value = 4.77). Even with application of a frontal lobe SVC no significant areas of white matter density reduction were seen in this region.

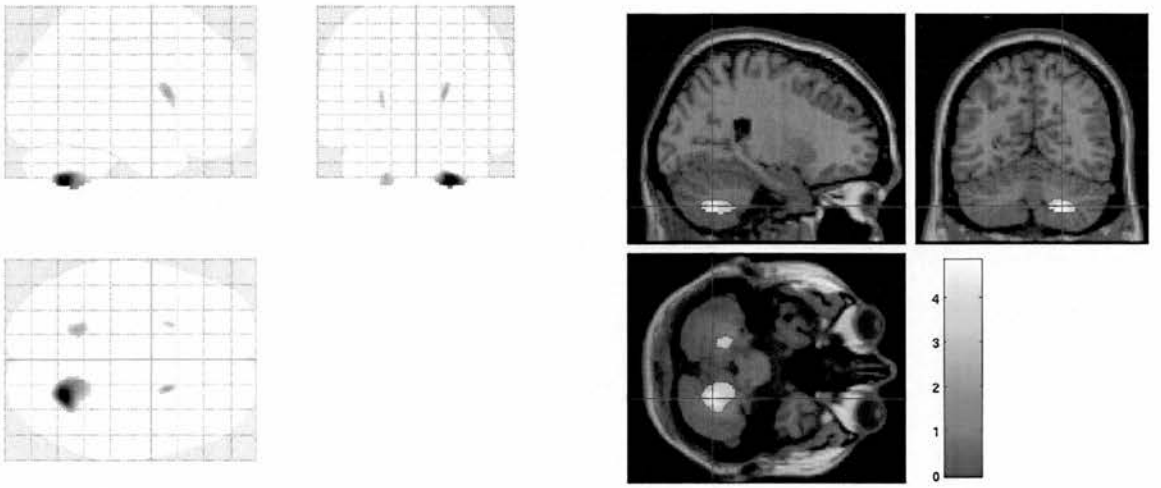

Figure 8.6
SPM superimposed on a 'glass brain' showing voxels demonstrating reduced grey matter density in subjects with a history of cigarette smoking compared to those without (left).
SPM overlay on a structural image demonstrating the region of density loss in the right cerebellum (right).

Discussion

Tobacco use was associated with reduced white matter density in the right cerebellum. This has not been reported in previous studies investigating brain structural abnormalities associated with tobacco use 4.1.3.3. The cerebellum was not an area which had been identified *a priori* as a brain region to be investigated in this study. There has been considerable interest in recent years however in the possibility that cerebellar abnormalities may be important in the pathophysiology of schizophrenia.[626] Clearly this finding does raise intriguing possibilities that an interaction between tobacco use and cerebellar structure does exists in those at high risk for schizophrenia. As this was a brain region which was not a primary focus of this study however (and for which volumetric analysis was not undertaken), it will not be specifically investigated further; it is of course feasible however that longitudinal changes may be apparent in subsequent automated analyses (see next Chapter).

8.3 Summary of findings from baseline analyses

As had been expected, there were some differences between findings from the semi-automated volumetric analyses and from VBM. These seem to be explicable by the differing sensitivities of the two methodologies however, and it is the case that results of the two methodologies are overall generally reasonably compatible. Findings from these two methodologies will now be synthesised, with the aim of providing the most accurate picture possible of the structural abnormalities associated with substance by this at-risk group. The structural abnormalities associated with alcohol, cannabis, tobacco and the other drugs of interest will be discussed in turn.

8.3.1 Cannabis

Among the strongest findings from the volumetric analyses of the high risk group was that of third ventricular enlargement in association with cannabis consumption. A dose response relationship was observed between level of cannabis consumption and volume of this structure, and frequent use of cannabis emerged from the regression analysis as a predictor of third ventricular volume. In contrast, there was no suggestion of third ventricular enlargement in association with cannabis consumption in the controls. The thalamus surrounds the third ventricle, and would be the most obvious structure from which tissue was lost to give rise to this third ventricular enlargement. In keeping with this, VBM analysis identified a region of tissue density loss localised to the anterior and mediodorsal nuclei of the left thalamus. Though this result was not robust to the inclusion of all the covariates

included in the ROI analysis, it does indeed suggest that the third ventricular enlargement observed in association with cannabis consumption is attributable, (at least in part), to thalamic volume loss. Such an effect seems to be specific to individuals at genetically high risk of schizophrenia.

8.3.2 Alcohol

Alcohol use history exhibited a dose response relationship both with ventricular enlargement and frontal lobe volume loss in those individuals at high genetic risk of schizophrenia. Once again, a comparable relationship was not apparent in controls. Alcohol use exceeding government recommendations also emerged from the regression analysis as predictive of lateral ventricular volume and left frontal lobe volume reduction in high risk subjects. As discussed in Section 4.1.3.2, brain structural abnormalities associated with alcohol use would be expected to be diffuse; as outlined in Section 6.2.1, this would make them difficult to detect at a significant level using VBM. This was indeed the case, VBM indicating loss of grey matter in the prefrontal lobes and loss of white matter in the corpus callosum, but neither of these findings approaching significance.

8.3.3 Tobacco

Though tobacco use did not demonstrate a dose response relationship with volume of any of the brain regions investigated, consumption of greater than 20

cigarettes a day did emerge from the regression analysis as predictive of right frontal lobe volume. There was no suggestion of tissue loss in this region in association with tobacco use on VBM analysis. VBM comparisons did however reveal an area of reduced white mater density in association with a history of tobacco use in the right cerebellum.

8.3.4 Other drugs

It is of substantial interest that when entered in to the regression model as additional factors, amphetamine and ecstasy use were predictors of either left or right lateral ventricular volume. Unfortunately, users with substantial histories of use of these drugs were too few to justify running VBM analyses to further elucidate any structural imaging abnormalities associated with their use in schizophrenia. In the case of amphetamines at least, there is considerable evidence that substantial use of these substances is associated with structural brain abnormalities (see Section 4.1.3.4). The possibility that people at risk of, (or even with), schizophrenia are particularly vulnerable to brain structural sequelae on using these drugs, (in addition to alcohol and cannabis), is an important consideration for future research.

Chapter 9

Analysis of longitudinal data

9.1 Planning of longitudinal analyses

9.1.1 Integration of baseline findings in to planning of longitudinal analyses

At the outset of this study it was hoped that the longitudinal analysis could be guided by baseline findings. Given this, consideration of the data presented in Chapter 7 will be important in planning which brain regions on which to focus investigation in the longitudinal analyses.

Findings from the baseline analyses were summarised in Section 8.3. As discussed in this section, synthesis of the volumetric and VBM analyses suggested that thalamic volume loss was associated with cannabis consumption. Other structural abnormalities observed in association with cannabis use, particularly lateral ventricular volume loss, are suggestive of diffuse change rather than implicating a particular structure. It is the case however that although no dose response relationship was observed, VBM analysis (on inclusion of all relevant covariates employed in the volumetric regression analysis) did reveal a near significant region of grey matter density loss in the right medial frontal gyrus.

In the case of alcohol, a dose response relationship was seen between frontal lobe volume loss and use of this drug. The association also emerged as significant in the regression analysis. No significant regions of density loss were seen in association with alcohol use on VBM analysis, a fact likely attributable to the nature of this change being diffuse. Synthesis of the baseline analyses did not suggest that thalamic volume loss had occurred in association with this drug.

Associations between tobacco use and brain structural abnormalities were generally less obvious than was the case with cannabis and alcohol. An exception was the identification of an area of white matter density loss in the right cerebellum with VBM.

Though it is important to incorporate baseline findings in to the planning of longitudinal analyses, this should not be relied on exclusively. It is possible that genuine associations between use of a particular substance and specific brain regional volume loss could be undetectable by a case-control study design, but detectable within a longitudinal study design. Thus, if there is strong reason to suspect that a particular brain region may be vulnerable to the effects of a particular substance, it may be reasonable to investigate this putative association even in the absence of data from the baseline analyses suggesting such a relationship.

Given the above considerations, a number of putative effects will be of particular interest in the longitudinal analysis. Firstly, given the data outlined above, it will be important to investigate if thalamic volume loss occurs in association with cannabis use. Additionally, investigation of frontal lobe volume loss in association with either cannabis or alcohol will also be a priority. No associations were observed between use of any of the substances investigated and hippocampal volume loss in the baseline analysis. It is the case however that hippocampal volume loss is one of the most robust findings in schizophrenia (see Section 4.2.1); as a consequence, and despite the paucity of findings involving this structure on baseline analysis, I believe it is appropriate that this structure is also singled out *a priori* for investigation of any longitudinal changes occurring in it in association with use of either cannabis or alcohol.

As discussed above, the only significant association observed between tobacco use and structural imaging abnormalities in the baseline analysis was the finding of an area of reduced white matter density in the right cerebellum in association with smoking seen on VBM. The cerebellum was not a region subject to hand tracing analysis; given this, longitudinal changes in this structure can not be investigated by ROI methodology. Given the absence of any findings from baseline analyses strongly suggestive of structural abnormalities in association with tobacco use in any of the brain regions actually subject to volumetric analysis, volumetric techniques were not used to investigate if longitudinal structure volume loss occurred in association with tobacco use. Any grey or white matter loss over time in association with smoking will however be investigated with TBM.

Relatively few subjects used ecstasy or amphetamines between scanning points. Given the small number of subjects with this exposure, and to limit the total number of comparisons, abnormalities associated with these drugs will also not be directly investigated. They will however be included as covariates in other analyses.

9.1.2 The biological rationale for focusing on these structures in the longitudinal analysis

Utilising findings from the baseline analyses to guide the longitudinal analyses has numerous advantages. It provides coherence to the study design, reduces the total number of comparisons undertaken and can hopefully facilitate the detection of abnormalities where they exist. As alluded to above however, it is also important that the choice of regions being investigated is underpinned by a clear biological rationale.

This should reflect the existing evidence base, with there being good reason to suspect (in this population at least), that any abnormalities observed could reasonably be attributable to substance use. With this in mind, I will now examine how each of the potential interactions between substance misuse and brain structure being singled out for investigation in the longitudinal analyses could be conceptualised as biologically feasible.

9.1.2.1 Regions in which the effects of cannabis will be specifically investigated

Thalamus

As reviewed in Section 4.2.1, thalamic volume reduction is in fact one of the most consistently demonstrated structural abnormalities in schizophrenia.[627, 628] The point in illness development at which this abnormality arises has however been more difficult to ascertain. Previous reports from the EHRS have indicated that people who are clinically well but at genetically high risk of schizophrenia have reduced thalamic volume compared to controls, (see Section 4.2.2.1 and Lawrie *et al.*[629]) and replicated studies have established this reduction as a measure of genetic liability to psychosis.[630, 631] In contrast however, a VBM-based meta-analysis of high risk studies failed to detect thalamic deficits on combining eight (admittedly heterogeneous) reports; conversely, the same meta-analysis detected left thalamic deficits in first episode compared to high risk subjects, and bilateral deficits in those with chronic schizophrenia compared to controls.[632] As discussed in Section 4.2, synthesis of the available data suggests that, though some thalamic abnormalities may indeed exist

long before the development of schizophrenia, it is likely that further structural change does occur during transition to illness.

Central to the stress-diathesis model of schizophrenia is the belief that the condition arises when a vulnerable individual is exposed to environmental stressors that increase the risk of transition from vulnerability phenotype to frank psychosis.[633] As discussed in Section 3.2.2.1.1, cannabis is an environmental factor with considerable evidence of risk modifying effect. The findings from our baseline analysis may thus suggest that cannabis exposure could provide one potential mechanism by which thalamic structural changes arise during the process of development of schizophrenia.

Independent of our earlier findings, there are data suggesting the thalamus is directly influenced by exposure to cannabis. Though CB1 receptors are not as densely expressed in this brain region as some others, they are present. Indeed, highest levels of binding are found in the mediodorsal and anterior complex nuclei,[634] regions that connect to cortical association areas consistently implicated in schizophrenia.[635] Changes in thalamic regional cerebral blood flow secondary to cannabis consumption have also been reported, though these findings have been inconsistent and not all studies have reported effects.[636, 637] Interestingly, this is despite animal studies finding robust and reproducible effects of cannabis consumption on the function of several thalamic subregions.[638, 639] Structural imaging studies have not reported thalamic volume loss secondary to cannabis consumption in cannabis users. Indeed, as reviewed in Section 4.1.3.1, in the non-schizophrenic population there have been no consistent reports of *any* structural abnormalities in association with use of this drug. In contrast, a number of studies have reported grey matter loss in association with cannabis use in people with established schizophrenia (see Section 4.3.1.1). In the

longitudinal study of Rais *et al.* this occurred in the context of third ventricular enlargement.[539]

Considering the above it does therefore seem biologically plausible that cannabis could be associated with thalamic abnormalities. If such abnormalities did occur, we would expect them most likely to be detectable in a longitudinal study of vulnerable individuals, such as those in the EHRS. It does thus seem that investigation of longitudinal thalamic volume change in association with cannabis consumption is clearly justifiable.

Frontal lobes

As reviewed in Section 4.2.1, frontal lobe volume loss is again one of the most robust findings in schizophrenia. Very subtle deficits in the region may be present even prior to the onset of illness, but it seems likely that the transition from at risk state to frank psychosis is associated with significant prefrontal grey matter loss. Identification of environmental exposures associated with prefrontal loss in at-risk individuals is thus of paramount importance.

As discussed above and in Section 4.1.3.1, in the 'normal' population cannabis consumption is not associated with any robust structural imaging abnormalities. It is the case however that expression of the CB1 receptor, the principle cannabinoid receptor in the brain, is particularly pronounced in the prefrontal cortex.[640] Thus, if cannabis consumption was to have brain structural consequences, this would be one of the prime candidate regions. The finding of a near significant region of tissue density reduction in the right prefrontal lobe in association with cannabis on baseline VBM analysis must also be considered in this context. This, together with the accumulated data outlined above, does suggest that further exploration of the

possibility that cannabis use is associated with longitudinal loss of frontal lobe grey matter in individuals at genetically high risk of schizophrenia is warranted. Thus, this possibility will also be specifically investigated in the longitudinal analyses.

Amygdalo-hippocampal complex

The AHC is also a region consistently identified as being reduced in volume in people with chronic schizophrenia (see Section 4.2.1.1). It is also the structure most consistently reported as reduced in first episode subjects. Evidence that more subtle hippocampal abnormalities are present in people at high risk of schizophrenia are particularly robust, and it does seem that further reductions of hippocampal volume may occur in individuals who are at particularly elevated risk of making the transition to psychosis (see Section 4.2.3).

As discussed above, no structural brain abnormalities are consistently identified in 'normal' cannabis using subjects. It is the case however that the limited data available do suggest that people with schizophrenia who have used cannabis exhibit additional grey matter reductions in left hippocampus (see Section 4.3.1.1). It is also the case that, together with the frontal lobes, CB1 receptor expression is particularly dense in the hippocampus.[640] On considering these data it does thus seem that this is a further structure in which the possibility of longitudinal structural changes in association with cannabis should be specifically explored.

9.1.2.2 Regions in which the effects of alcohol will be specifically investigated

Frontal lobes and AHC

The structural abnormalities seen at baseline in association with cannabis consumption appear to be predominantly localised around the third ventricle. In contrast, the abnormalities associated with alcohol seem to be more diffuse, manifesting as lateral ventricular enlargement and frontal lobe volume loss. As discussed in sections 4.1.3.2.1 and 4.2.1, frontal lobe volume loss is strongly associated with both alcohol use and schizophrenia. As discussed in Section 4.1.3.2.3, if alcohol-associated abnormalities are detectably anywhere in young alcohol abusers it is likely either here or in the hippocampi. There is thus a strong rationale for investigating if further frontal lobe volume loss is observed in association with excessive alcohol use between scans.

Aside from the frontal lobe effects, there was little suggestion from baseline analyses that specific brain regions were predominantly affected by alcohol consumption. As however hippocampal volume loss is one of the most robust findings in schizophrenia (see Section 4.2.1), as well as possibly being associated with alcohol use in young people, (see above), it is important to investigate if heavy alcohol consumption by this group is associated with progressive structural abnormalities of this structure. Longitudinal analyses investigating changes overt time in association with use of alcohol in the high risk subjects will thus be limited to these two regions.

9.1.2.3 Regions in which the effects of tobacco will be specifically investigated

Aside from cerebellar white matter loss there was little evidence from baseline analyses that tobacco use was associated with brain structural abnormalities in schizophrenia. Similarly (in the absence of either concurrent alcohol absue or cerebrovascular disease at least), there was little evidence of smoking being associated with substantial brain structural abnormalities in the general popualties (see Section 4.1.3.3). In the single longitudinal study investigating the brain structural consequences of smoking in schizophrenia, it was reported that smoking could not explain the brain structural changes observed (see Section 4.3.1.3).

Given the above data the association of smoking with brain structure volume loss in those at high risk of schizophrenia will not be investigated for any specific brain regions. It will however be investigated with automated whole brain methodology, in an exploratory manner.

9.2 Volumetric analysis

9.2.1 Semi-automated volumetric methodology

This was as described for the baseline analyses (see Section 8.1.2). The same tracing protocols were employed, and tracing was undertaken by the same operators. Follow up scans were obtained using the same scanner, scanning protocol and tracing protocol as the baseline images.

All thalamic nuclei were grouped together, as MRI resolution makes distinction between the individual thalamic nuclei difficult.[641] The intraclass correlation coefficient between raters was 0.84 for the thalami, 0.82 for the amygdalo-hippocampal complex, and 0.98 for the prefrontal lobes.

9.2.2 Statistical analysis of volumetric data

Statistical testing was conducted with the SPSS 14. Demographic and clinical variables were compared between high risk subgroups in the two exposure groups for both cannabis and alcohol, as previously outlined in Section 5.2.2.4 (Tables 5.10, 5.11 and 5.12). These data are reproduced in the tables below together with baseline volumes both of the whole brain and specific brain regions being compared in the analyses undertaken for each substance. Volumes were compared between exposure groups using the independent t-test.

Mean absolute change in regional volumes was calculated as the mean difference between the two scans (scan 2 – scan 1), such that a negative value indicates volume reduction. Rate of change in volume was determined by dividing this value by the time (in days) between the two scans.

	No cannabis use N = 32	**Cannabis use** N = 25	***P***
Mean age at first assessment (SD)	21.11 (2.87)	21.76 (2.52)	.38
Gender (male/female)	15:17	15:10	.33 (a)
Handedness (R:L:both)	28:4:0	21:2:2	.24 (a)
IQ (SD)	100.59	100.12	.90
Exceed recommended max alcohol consumption	4	7	.18 (b)
Smoke tobacco	8	18	<.001 (a)
Use ecstasy	0	9	<.001 (b)
Use amphetamines	0	10	<.001 (b)
Days between assessments (SD)	648.38 (128.06)	679.12 (206.85)	.49
Rate of change in whole brain volume	-13.49 (43.74)	-0.27 (68.20)	.40
Baseline whole brain volume (cm^3)	1349.13 (127.07)	1362.85 (138.86)	.70
Baseline right prefrontal lobe volume (cm^3)	79.11 (13.88)	77.13 (11.95)	.58
Baseline left prefrontal lobe volume (cm^3)	75.36 (13.41)	74.93 (10.22)	.90
Baseline right AHC volume (cm^3)	4.74 (0.68)	48.54 (0.97)	.52
Baseline left AHC volume (cm^3)	4.60 (0.52)	4.60 (0.63)	.98
Baseline right thalamic volume (cm^3)	6.01 (0.77)	6.23 (0.87)	.29
Baseline left thalamic volume (cm^3)	6.15 (0.79)	6.29 (0.91)	.55
Rust Inventory of Schizotypal Cognitions baseline score	25.44 (9.94)	27.92 (11.70)	.39

Table 9.1
Demographic and clinical characteristics of high risk subjects who did and did not consume cannabis between timepoints 1 and 2.
P = independent t test, except (a) = chi squared and (b) = Fisher's exact test

	Within recommended limits N = 46	**Exceed recommended limits** N = 11	***P***
Mean age at first assessment (SD)	21.31	21.73	.650
Gender (male/female)	5:6	25:21	.596 (a)
Handedness (R:L:both)	39:5:2	10:1:0	.762 (a)
IQ (SD)	101.93 (13.52)	93.91 (11.04)	.008
Use cannabis	18:28	7:4	.184 (a)
Smoke tobacco Y:N	17:29	9:2	.007 (a)
Use ecstasy Y:N	6:40	3:8	.354 (b)
Use amphetamines Y:N	7:39	3:8	.387 (b)
Days between assessments (SD)	656.09 (144.05)	686.00 (246.35)	.597
Rate of change in whole brain volume	-10.11 (58.48)	2.42 (42.51)	.507
Baseline whole brain volume (cm^3)	1359.62 (125.61)	1336.42 (158.48)	.603
Baseline right frontal lobe (cm^3)	78.49 (12.32)	72.07 (10.63)	.773
Baseline left frontal lobe volume (cm^3)	75.91 (12.32)	72.07 (10.63)	.346
Baseline right AHC volume (cm^3)	4.74 (0.68)	4.99 (0.63)	.264
Baseline left AHC volume (cm^3)	4.59 (0.75)	4.66 (0.55)	.712
Rust Inventory of Schizotypal Cognitions baseline score	24.80 (10.12)	33.73 (10.60)	.012

Table 9.2
Demographic and clinical characteristics of high risk subjects who do and do not report hazardous or harmful levels alcohol consumption between timepoints 1 and 2.
P = independent t test, except (a) = chi squared and (b) = Fisher's exact test

9.2.2.1 Longitudinal changes associated with cannabis consumption

Any differential volume change in high risk subjects exposed and not exposed to cannabis in the interim period was examined for right and left thalami, amygdalo-hippocampal complexes and frontal lobes separately, using repeated measures analysis of variance and looking for cannabis exposure × time interactions. Structure volume at t1 and t2 were entered as the dependent variable, gender and exposure status to cannabis, alcohol, tobacco, ecstasy and amphetamines were included as fixed factors, and age at first assessment and days between assessments 1 and 2 were entered as covariates. A small number of subjects had used illicit drugs other than those listed above. Repeated measures analysis was therefore rerun excluding any

individuals with a history of use of any substance other than alcohol, cannabis, tobacco, ecstasy and amphetamines. None of the subjects who did not consume cannabis between the two timepoints had used either ecstasy or amphetamines, whereas substantial numbers of cannabis using subjects had used one of these drugs. Given this imbalance of variables, and the evidence that ecstasy in particular may be specifically thalamotoxic,[642] repeated measures analyses was also run excluding any subjects who had used either of these two drugs in the inter-scan period.

It was conceivable that any volume change observed to occur in the structures investigated in association with cannabis consumption was simply part of a more generalised effect. To ascertain if any volume changes were specific to these structures, repeated measures analyses was re-run with addition of rate of change of whole brain volume ([WBV2-WBV1]/time between assessments) included as an additional covariate.

9.2.2.2 Longitudinal changes associated with alcohol consumption

This employed the same methodology as above, with the exception that only associations with the prefrontal lobes and AHC were investigated. Thalamic volume changes were not investigated as it was important to limit the total number of analyses, and there was no reason to suspect from baseline analyses that alcohol consumption was associated with thalamic volume abnormalities in this population. As alcohol consumption was the variable of interest, it was obviously not included as a covariate; cannabis consumption in the intervening period was included instead.

9.2.3 Results

9.2.3.1 Cannabis

As can be seen in Table 9.1, at baseline there was no significant difference in whole brain, thalamic, AHC complex or frontal lobe volumes between people who did and did not subsequently use cannabis. Additionally, the two groups were reasonably balanced at baseline in terms of age, gender and scores on the Rust Inventory of Schizotypal Cognitions.[643]

The mean raw absolute volume change in the thalami, AHCs and frontal lobes together with mean rate of volume change ([volume at t2 – volume at t1]/time between scans) in both the cannabis exposed and non-exposed groups is shown in Table 8.3. Cannabis exposure × time interactions are also shown, after inclusion of the covariates detailed in the accompanying text. As can be seen, cannabis exposure is associated with bilateral thalamic volume loss, this effect being significant on the left ($F = 4.47, p = .04$) and highly significant on the right ($F = 7.66, p = .008$). This effect was not observed in the frontal lobes or AHC.

Analysis was repeated with the 7 subjects who consumed illicit drugs other than cannabis, ecstasy or amphetamines in the period of interest excluded; the cannabis exposure × time interaction remained significant on both the right and left side. It was also re-run excluding the 14 people who had used either ecstasy or amphetamine in the period of interest; again the cannabis exposure × time interaction remained significant bilaterally. Additionally, the primary analysis was also re-run with the inclusion of rate of change of whole brain volume as an additional covariate.

Once again, the cannabis exposure × time interaction remained significant on both sides, the p value increasing marginally ($p = .01$ on the right side and $p = .046$ on the left).

	Absolute change (scan 2 – scan 1) Mean (SD)		**Rate of change ([scan 2 – scan 1]/time) Mean (SD)**		**Exposure × time***
	No cannabis exposure (mm^3)	**Cannabis exposure (mm^3)**	**No cannabis exposure (mm^3/day)**	**Cannabis exposure (mm^3/day)**	
Right frontal lobe	136.36 (5717.37)	1947.12 (6978.63)	0.13 (9.63)	2.70 (10.97)	$F = 0.07, p = .79$
Left frontal lobe	-1284.44 (4633.68)	564.15 (6237.21)	-1.74 (7.42)	-0.05 (11.37)	$F = 0.08, p = .77$
Right AHC	147.77 (563.94)	175.31 (432.19)	0.18 (0.95)	0.19 (0.66)	$F = 0.71, p = .40$
Left AHC	-121.37 (488.70)	-4.43 (384.77)	-0.23 (0.81)	-0.03 (0.59)	$F = 0.01, p = .92$
Right thalamus	32.84 (509.35)	-264.48 (621.70)	0.06 (0.80)	-0.36 (0.92)	$F = 7.66, p = <.01$
Left thalamus	26.66 (667.58)	-181.39 (621.70)	0.07 (1.00)	-0.22 (1.01)	$F = 4.47, p = .04$

Table 9.3
Comparison of specific structure volume changes over time in cannabis exposed and non-exposed high risk individuals.
*investigation of exposure × time interaction has been undertaken with inclusion of the following covariates: gender, age at first assessment, time between scans and use of other substances in inter-scan period (alcohol exceeding government recommendations, tobacco use, use of ecstasy and use of amphetamines each being included as separate factors).

9.2.3.2 Alcohol

As can be seen in Table 9.2, at baseline there was no significant difference in baseline whole brain, thalamic, AHC complex or frontal lobe volumes on comparing people who did and did not subsequently use cannabis. Additionally, the two groups were reasonably balanced at baseline in terms of age and gender. The two groups did differ substantially in scores on the Rust Inventory of Schizotypal Cognitions [643] however, an observation which will be further investigated in Section 9.4.

Those who consumed alcohol in excess of governemt recommended limits were more likely than those who did not to smoke in the inter-scan period. They were

however no more likely to use cannabis, amphetamines or cannabis. In addition to the substances detailed in Table 9.2, two subjects used opiates, two cocaine and six LSD in the period between scans. The LSD and cocaine using subjects were in the 'within recommended limits' group, while those who used opiates were in the hazardous/harmful drinking group.

Rate of change of frontal lobe and AHC volumes between t1 and t2 scans were compared in those whose alcohol consumption exceeded government recommendations to those whose did not. The covarites included in this anlaysis were gender, age at first assessment, time between scans and use of other substances in inter-scan period (cannabis use, tobacco use, use of ecstasy and use of amphetamines each being included as separate factors) In neither structure and on neither side was the alcohol exposure × time interaction significant.

9.3.4 Discussion

It is thus the case that the only significant finding to arise from the volumetric analyses investigating longitudinal changes in association with substance misuse in the high risk individuals is thalamic volume loss in association with cannabis use. Such a finding is clearly compatible with the baseline analyses which were discussed in Section 9.1.2. Notably, baseline volumetric analysis identified third ventricular enlargement in association with cannabis use, and VBM analysis localised tissue density reductions associated with use of the drug to the thalamic dorsomedial and anterior nuclei. The longitudinal findings add substantially to this, confirming that

thalamic volume loss is a dynamic process, occurring in association with exposure to cannabis in this vulnerable group.

These longitudinal findings do seem robust, but a number of potential limitations must be considered. Firstly, given the diffuse boundaries of the thalamus, it is a structure which is generally accepted as challenging to trace. Despite this however, inter-rater reliabilities for thalamic volume were acceptable (0.84). The possibility of 'rater drift' could be proposed to explain the apparent volume loss; it is the case however that rate of change was compared *in two subgroups* (i.e. thalami in the two groups contrasted were both measured at the two timepoints), thus negating this as a possible explanation for the findings. Equally, the possibility of the cannabis effect arising due to confounding by use of other substances was addressed by inclusion of use of other substances as covariates. Indeed, this latter possibility is further undermined by the fact that the effect was actually robust to the exclusion of all subjects who had used illicit drugs other than cannabis in the between-scan period. It is thus the case that there is no obvious explanation for the thalamic volume loss obsereved other than it being attributable to the effects of cannabis.

9.3 Tensor-based morphometric analysis

As discussed earlier, in addition to semi-automated ROI techniques, fully automated VBM was applied to baseline scans of subjects recruited to the EHRS. Subjects with the highest degree of exposure to alcohol and cannabis were compared to those with the lowest, and those with a history of tobacco smoking were compared to non-smokers.

Given the potential advantages of automated techniques (Section 7.2.1), it would seem optimal to also apply an automated technique to the analysis of the longitudinal data. Conventional VBM studies have been used in schizophrenia to study changes over time.[644] Concerns have been raised about this approach however, it being unable to distinguish the effects that are due to imperfect image realignment from changes in tissue density.[615] A refinement to VBM is Tensor-Based Morphometry (TBM), which uses the deformation field created when warping a subjects' follow-up brain scan to their baseline scan. Unlike VBM, this technique is able to distinguish intrinsic changes in brain anatomy from translational shifts caused by imperfect image registration.[615] This is essentially the basis of TBM; by application of a high dimensional warp it corrects for slice misalignment between timepoints, and thus ensures that the same voxels are being compared in successive scans from the same subject. This opens up the possibility of applying an automated image analysis technique more suited to the analysis of longitudinal data than VBM. As it can be applied to standard, T1 weighted images, it is feasible to employ this methodology in the analysis of longitudinal data from the EHRS.

In a manner comparable to VBM, TBM is more sensitive to localised tissue loss than region of interest techniques. Thus, while diffuse changes may not be detected by it (but may be by ROI), for localised tissue loss the converse is true.[604, 606]

As discussed in relation to the ROI analysis, a subset of subjects were rescanned at time 2. Further scanning points were undertaken, but as the first two scans used the same scanner, these two scan points are of particular interest. The difficulties posed by a change of scanner would make the application of TBM to elucidation of longitudinal changes substantially more complicated, if not completely unfeasible; it would thus be outwith the scope of this study.

When running TBM analyses, similarly to VBM, corrections are made for multiple comparisons at whole brain level. In the context of TBM it is common practice to restrict the analysis to regions which have been determined *a priori* to be of particular interest. As discussed previously, in relation to VBM analyses, this is achieved by using the small volume correction function in SPM, significance being correcting for the voxels included in this restricted analysis rather than the whole brain. As with the VBM baseline analyses, the regions in which use of a SVC will be justified in the TBM contrasts will be determined by combination of literature review and findings from previous analyses in this study. These considerations were discussed in Section 9.1.2, and the regions for which an SVC will be applied in the TBM analysis will be the same as those regions investigated with hand-traced ROI techniques. Thus, thalamic, AHC and frontal lobe SVCs will be applied in the cannabis analysis, and AHC and frontal lobe SVCs in the alcohol analysis. The case for employing a thalamic SVC is clearly strengthened by the finding of thalamic volume reductions over time in association with cannabis consumption in the ROI analysis (Section 9.2.3.1).

9.3.1 Background information

MR image acquisition was as discussed previously. Contrasts made were as undertaken for the analyses of volumetric data, described above (Section 9.2). As will be discussed below however, two fewer scans were available for the automated longitudinal analyses.

9.3.2 Scan preprocessing

We implemented the TBM protocol released for the Statistical Parametric Mapping (SPM2) application by John Ashburner (http://www.fil.ion.ucl.ac.uk/spm/). This protocol was implemented by and discussed in Kipps *et al.*[645] and implemented by Whitford *et al.*[646] We implemented this staged protocol as described below and in Moorhead *et al.*'s 2007 publication.[615]

1. The SPM brain extract function was used to recover the first- and second-round native space brains from the participants' T1 scans and the SPM2 default T1-weighted single subject using raw space segmentations. These extractions were used to exclude non-brain tissue from the analysis.

2. The SPM coregister function was used to register the first- and second-round extracted brains with the extracted brain from the SPM single subject, without rescaling. This provided a coregistration mapping of the brain tissue for each subject and with alignment along the MNI template anterior–posterior commissure axis. The mappings to obtain these anterior–posterior commissure axis registrations were then

applied to the T1 raw images (non-brain-extracted) to obtain coregistered native space T1 images. The SPM segment function was then used to extract gray matter (GM) segments for the native space coregistered first- and second-round scans.

3. The SPM deformations toolbox was used to implement a high-dimensional (HD) warp between the second- and first-round coregistered brains given by step 2. The resultant warp was then used to implement a HD registration of the second-round GM segment with the first-round GM segment. This HD warp is used to minimize local registration differences between the first- and second-round tissue segments. We subtracted the first-round GM segment from the second-round HD warped GM segment to give a native space GM difference image. The Jacobian determinants for the HD warp were then evaluated.

4. In a procedure analogous to modulated-VBM, localized tissue change is recovered in the form of a GM and WM modulated difference images. In this the Jacobian determinants from HD deformation between the 1st and 2nd rounds are used to ensure that the assessments of tissue changes over time are corrected for MRI sampling noise.

5. To obtain subject-to-subject coregistration, we applied nonlinear warping to normalize the first-round extracted brain from step 2 with the SPM2 single-subject T1-extracted brain acquired from step 1. The normalization warp was then applied to the modulated difference image from step 4. Steps 1–5 were also repeated for white matter.

Unfortunatley scans from two subjects could not be successfully preprocessed for the TBM analysis. This was the reason why the total number of subjects available for the automated longitudinal analyses was 55 rather than 57. Both of these individuals were in the cannabis consuming group.

9.3.3 Statistical analysis

9.3.3.1 Statistical design for cannabis contrast

As the two subjects whose scans could not be successfully preprocessed for the TBM analysis were both in the cannabis using group, in the automated analyses 23 subjects who consumed cannabis between scan points were contrasted against 32 who did not. Demographic and relevant clinical details of these two groups are compared in Table 9.4.

Differences in the modulated difference images (from step 5) between high risk subjects who did and did not consume cannabis between timepoints 1 and 2 were compared in SPM5 using the general linear model. A grey matter mask was included. Age, interscan interval, sex, and use of alcohol, cigarettes, ecstasy and amphetamines were included in the model as covariates. As these were not exploratory analyses, but guided by findings from earlier analyses, I did not feel that running the analysis without the inclusion of appropriate covariates was justified. T-contrasts were thresholded at T = 0.0001 (uncorrected). Whole brain analysis was supplemented with

a small volume correction for the thalamus, frotal lobes and amygdala and hippocampus combined (the AHC). These SVCs were the same as those used in the baseline VBM analysis (see Section 8.2.4). As was the case with the VBM analyses, the TBM whole brain and SVC analyses were implemented in MNI space. Again as before, the voxel co-ordinates of the results are extracted from MNI standard space and I also report the Talairach co-ordinates using the Matlab function mni2tal.[621] The anatomic location of significant results was manually checked using the Talairach atlas,[614] supplemented by use of Tailarach Daemon (http://ric.uthscsa.edu/projects/talairachdaemon.html).

Two additional steps were taken to confirm that results were genuine and exclude possibilities such as that, for example, they were due to the presence of an artefact. Firstly, statistical parametric maps were superimposed on scans from the study to ensure that regions of maximal difference were indeed arising in anatomically feasible locations. Additionally, the identical analysis was run on expansion images to investigate volume gain in association with cannabis consumption. This would only be expected to be seen in the presence of an artefact.

9.3.3.2 Statistical design for alcohol contrast

This was undertaken as for the cannabis analysis, but with the 11 hazardous/dependent drinkers contrasted against the 44 who did not exceed government recommendations. Demographic and relevant clinical details of these two groups are compared in Table 9.5. Obviously alcohol consumption was not included

as a covariate, with use of cannabis in the inter-scan period being substituted instead. The alcohol analyses did not include a thalamic SVC, but did include frontal lobe and AHC SVCs. As in the cannabis analysis, (as a safeguard against any possibility that results arose from artefacts), in the event of any positive findings the same analysis was run on the expanded images.

9.3.4 Results

9.3.4.1 Cannabis

Demographic and relevant clinical details for the 55 subjects included in the TBM analysis are detailed in Table 9.4. Participants who used and did not use cannabis between the scan points did not differ significantly in terms of gender, age at time of initial scan, rating on the RISC at scan timepoint 1, or the proportion who exceeding government recommended maximum weekly alcohol consumption between scanning points. Significantly more of the cannabis using group did however smoke cigarettes and consume ecstasy and amphetamines in the inter-scan period. All the aforementioned variables, including those not significantly different between the groups, were included as covariates in the TBM analysis.

	No cannabis between scans N = 32	**Consumed cannabis between scans** N = 23	***P***
Gender M:F	15:17	15:8	.18
Age at t1 (SD)	21.1 (2.9)	21.8 (2.6)	.34
IQ (SD)	100.59 (14.73)	100.09 (12.10)	.89
Days between scans (SD)	648.4 (128.1)	679.6 (214.9)	.50
RISC at t1 (SD)	25.4 (9.9)	27.9 (11.4)	.40
Cigarette smoker Y:N	6	17	<.01
Excessive alcohol Y:N	4	7	.10 (a)
Ecstasy	0	9	<.01 (a)
Amphetamines	0	9	<.01

Table 9.4
Comparison of demographic details and other substance use in inter-scan period in those who did and did not consume cannabis between timepoints 1 and 2.
The t test was used for comparison of age, RISC and time lapse between scans. For all other comparisons chi squared was used, aside from (a) which used Fischer's exact test.

The initial analysis, conducted at whole brain level, compared grey matter loss in cannabis consumers versus non-consumers between timepoints 1 and 2. Though they did not reach statistical significance at the level of whole brain analysis, three regions of prominent grey matter loss were observed in the former compared to latter group. These were located left prefrontal lobe, left caudate and right anterior hippocampus. The caudate was not part of our initial hypothesis, and thus this area of grey matter loss was not investigated any further. The analysis was however rerun with the inclusion of both amygdala-hippocampal and frontal lobe SVCs.

Using the bilateral amygdala-hippocampal complex SVC, greater GM tissue loss was found in the cannabis using group compared to the cannabis non-using group. The group differences in tissue change over time were estimated using t-contrasts; the df in these contrasts was 46. A single significant maximal voxel location at P_corrected = .029 (t contrast value = 3.88) was found at MNI coordinate [-35, -8.0, -24]. This was converted into Tailarach coordinates, detailed in Table 8.5, and is seen on saggittal, coronal and transverse views on Figure 9.1.

Voxel *P*(corr)	*X y z* (mm) Talairach coordinates	Point of maximal change
.029	-34.7 -8.8 -20.6	Right anterior hippocampus

Table 9.5
Maximum voxel results for contrast of cannabis consumers versus cannabis non-consumers between time1 and time2. The amygdala-hippocampal complex SVC has been applied.

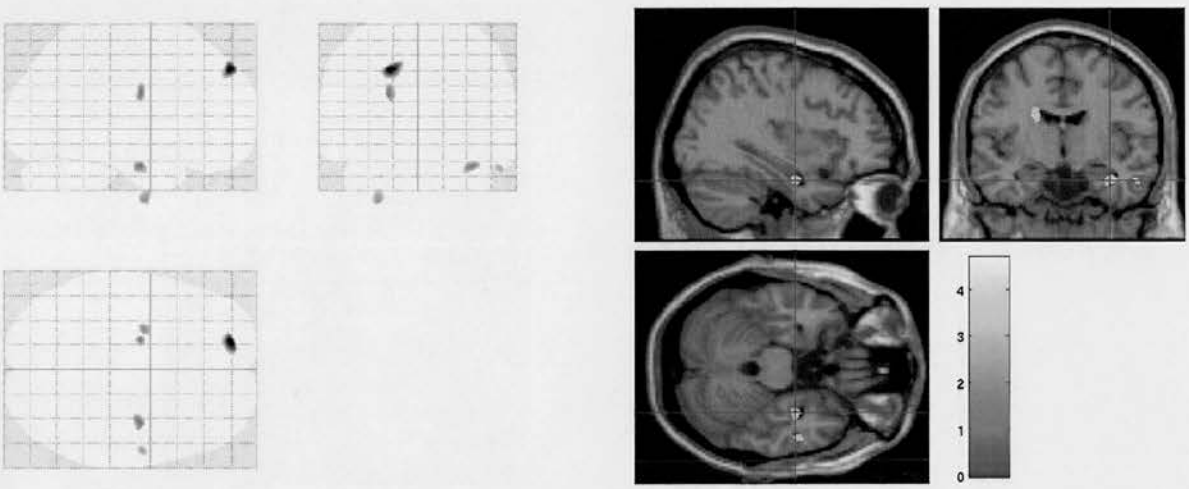

Figure 9.1
SPM superimposed on a 'glass brain' showing voxels of reduced density in those exposed to cannabis in the interim period when compared to those not exposed (left). SPM overlay on a structural image demonstrating the region of density loss in the right anterior hippocampus (right).

When a bilateral frontal lobe SVC was applied, greater GM tissue loss was again found in the cannabis using group compared to the cannabis non-using group. As before, the group differences in tissue change over time were estimated using t-contrasts, the df in these contrasts being 46. A single significant maximal voxel location at *P*_corrected = .026 (*t* contrast value = 4.68) was found at MNI coordinate [-18.0, 55.0, 42]. This was again converted into Talairach coordinates (detailed in Table 9.6) and is marked by cross-hairs on saggittal, coronal and transverse views on Figure 9.2.

Voxel *P*(corr)	*X y z* (mm) Talairach coordinates	Point of maximal change
.026	-17.8 55.2 35.9	Left superior frontal gyrus

Table 9.6
Maximum voxel results for contrast of cannabis consumers versus cannabis non-consumers between time1 and time2. The frontal lobe SVC has been applied.

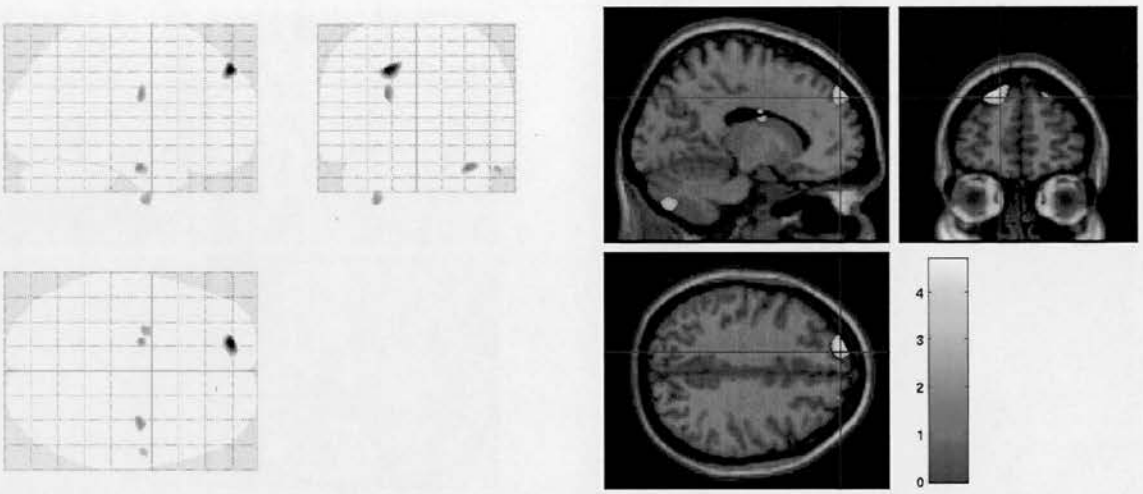

Figure 9.2
SPM superimposed on a 'glass brain' showing voxels of reduced density in those exposed to cannabis in the interim period when compared to those not exposed (left). SPM overlay on a structural image demonstrating the region of density loss in the left superior frontal gyrus (right).

It is notable that none of the subjects who did not consume cannabis in the inter-scan period consumed ecstasy or amphetamine, while substantial numbers of the cannabis consuming subjects did. This raises concerns that structural abnormalities associated with use of these substances could potentially confound the results outlined above. This emphasises the importance of having included these variables as covariates. Additionally however, given that this imbalance of variables was so marked across the comparator groups, I felt it important to take further steps to confirm that this imbalance was not resulting in a spurious finding. I thus repeated the above analyses excluding the 11 subjects who consumed either amphetamine, ecstasy or both drugs in

the inter-scan period. The analysis included all the other covariates in the previous analyses.

On repeating this analysis an area of significantly greater grey matter loss was again seen in the region of the anterior hippocampus. This was again statistically significant on applying an amygdala-hippocampal SVC (*P*_corrected = .039, *t* contrast value = 3.90, Talairach coordinates 31.7, -10.7, -19.7). The region of grey matter loss observed in the left prefrontal lobe did not remain significant after removal of these subjects. Given the persistence of the hippocampal result however, this seems most likely to be due to loss of power rather than the initial finding being spurious.

9.3.4.2 Alcohol

Demographic and relevant clinical details for the 55 subjects included in the TBM analysis are detailed in Table 9.7. Participants who did and did not exceed government alcohol recommendations did not differ significantly in terms of gender, age at time of initial scan, or level of use of the illicit drugs of interest. They did however differ on levels of tobacco use and, as was previously noted in the volumetric analyses, rating on the Rust Inventory of Schizotypal Cognitions (RISC). As before, all the aforementioned variables, including those not significantly different between the groups, were included as covariates in the TBM analysis.

The initial analysis, conducted at whole brain level, compared grey matter loss in the 11 subjects whose alcohol consumption exceeded government recommendations in the inter-scan period again those whose consumption did not. A

region of increased grey matter loss was identified at MNI coordinate [1, -52, -4], but this fell far short of significance [*P*_corrected = .812 (*t* contrast value = 3.82)]. This was located in the anterior lobe of the right cerebellum. On rerunning the analysis with the frontal lobe and AHC SVCs no significant regions of density loss were identified.

	Did not exceed recommendations between scans N = 44	**Did exceed recommendations between scans** n = 11	***P***
Gender M:F	25:19	5:6	.498
Age at t1 (SD)	21.33 (2.79)	21.72 (2.73)	.676
IQ (SD)	102.00 (13.77)	93.91 (11.04)	.077
Days between scans (SD)	655.27 (146.41)	686.00 (246.35)	.594
RISC at t1 (SD)	24.64 (9.83)	33.73 (10.60)	.009
Cigarette smoker Y:N	16:28	9:2	.007
Cannabis Y:N	16:28	7:4	.170 (a)
Ecstasy	6:38	3:8	.362 (a)
Amphetamines	6:38	3:8	.362 (a)

Table 9.7
Comparison of demographic details and other substance use in inter-scan period in those who did and did not consume alcohol at levels exceeding government reccomendations between timepoints 1 and 2.
The t test was used for comparison of age, RISC and time lapse between scans. For all other comparisons chi squared was used, aside from (a) which used Fischer's exact test.

9.3.4.3 Tobacco

This was undertaken as for the above analyses, but with the 25 subjects who smoked between scans contrasted against the 30 who did not. Levels of use of other substances were included as covariates. Given the lack of evidence for effects in any specific brain regions, only an AHC SVC was included. As in the other analyses, (with the aim of guarding against any possibility that results arose from artefacts), in the event of any positive findings the same analysis was run on the expanded images.

	Non-smoker between scans N = 30	**Smoker between scans** n = 25	***P***
Gender M:F	16:14	14:11	.843
Age at t1 (SD)	21.53	21.27	.735
IQ (SD)	101.23 (15.34)	99.36 (11.32)	.61
Days between scans (SD)	659.97	663.16	.945
RISC at t1 (SD)	23.27	30.28	.013
Excess alcohol Y:N	2:28	9:16	.015
Cannabis Y:N	6:24	17:8	<.001
Ecstasy Y:N	1:29	8:17	.008 (a)
Amphetamines Y:N	2:28	7:18	.064 (a)

Table 9.8
Comparison of demographic details and other substance use in inter-scan period in those who did and did not smoke between timepoints 1 and 2.
The t test was used for comparison of age, RISC and time lapse between scans. For all other comparisons chi squared was used, aside from [a] which used Fischer's exact test.

The initial analysis, conducted at whole brain level, compared grey matter loss in the 30 subjects who did not smoke in the inter-scan period against the 25 who did. A region of increased grey matter loss was identified at MNI coordinate [-41, 23, 49], but this fell far short of significance [*P*_corrected = .868 (*t* contrast value = 3.74)]. This was located in the left middle frontal gyrus. On rerunning the comparison with the AHC SVC no significant regions of density loss were identified. Though not a planned contrast, given the location of the identified region of grey matter density loss, the comparison was also repeated with the frontal lobe SVC; it remained non-significant even after application of this SVC.

9.3.5 Discussion

As was the case with the volumetric analysis, the automated approach did not identify any areas of significant structural change associated with alcohol use by the high risk subjects. Similarly, there was no evidence of significant structural change in association with smoking. By contrast however, significant areas of tissue loss were

identified in the right anterior hippocampus and left superior frontal gyrus in association with cannabis use. As discussed in Section 9.1.2.1, both structures exhibit a high density of CB1 receptors; they are thus structures which, if a cannabis/risk interaction was to occur anywhere in the brain of this group of high risk individuals, would be regarded as a definite candidate regions in which to observe such an effect. The case for the hippocampus is particularly strong in this regard, there being previous evidence of this structure being vulnerable to the effects of cannabis in people with schizophrenia.

9.4 RISC rating as a predictor of substance use

As discussed in Section 6.2.3 and above, a higher RISC rating at point of entry in to the study was associated with greater use of both tobacco and alcohol between scan points 1 and 2. This was despite RISC rating not being significantly different on comparing groups with different historic levels of exposure to alcohol and tobacco at entry in to the study (see Table 6.6). As discussed in Section 6.2.3, to ascertain if RISC rating independently predicted use of either or indeed both drugs, a logistic regression analysis was planned.

Logistic regression was first undertaken in separate analyses to establish if RISC rating predicted use of alcohol or tobacco. In the alcohol analysis level of use of alcohol between scanning points (either exceeded or did not exceed government recommendations) was entered as the categorical outcome variable. Age and gender were included as covariates. This was repeated for the tobacco analysis, with whether or not the person was a smoker at the second assessment replacing alcohol use as the categorical outcome variable. Results are shown in Table 9.8.

Dependent variable	**Significance of RISC as a predictor of substance use**			
	B (SE)	Wald	*P*	Exp(B) (CI)
Alcohol	0.094 (0.041)	5.256	.022	1.10 (1.01-1.19)
Tobacco	0.077 (0.030)	6.839	.009	1.08 (1.02-1.15)

Table 9.9
RISC rating as a predictor of alcohol and tobacco use between scan points

When both alcohol and tobacco use were simultaneously entered in to the analysis, only tobacco use emerged as significantly predicted by RISC rating (Wald statistic 3.49, $P = .040$). These results were unaltered by the inclusion of IQ as an additional covariate.

On the basis of the above it does thus seem that, in a population at genetically increased risk of developing schizophrenia at least, a greater weight of schizotypal symptoms is associated with an increased tendency to either continue or begin using tobacco. It may also be associated with an increased tendency to use alcohol, though some of this association may be explained by the overlap between tobacco and alcohol use.

These findings are particularly interesting when considered with the baseline data investigating the association between historic levels of substance use and rating on the RISC. Though RISC ratings were higher in cannabis consumers in this baseline data, excessive alcohol consumption and smoking were *not* associated with higher RISC ratings. This dichotomy may have implications for understanding the association between schizophrenia and smoking, but first I must explore the actual changes in substance use which brought it about.

The fact that higher RISC ratings are associated with subsequent but not current excessive alcohol use and tobacco smoking clearly implies that the prevalence of these behaviours changes over time in the subset of high risk subjects for whom longitudinal data are available. Specifically, those high risk subjects with higher RISC ratings are either less likely to cease the activities of excessive drinking and smoking tobacco and/or are more likely to take up these behaviours. Conversely, people with higher RISC ratings at baseline do not seem more likely to consume cannabis in the follow-up period. The extent of these behavioural changes, and how they relate to RISC ratings are detailed in Table 9.9.

Substance	Subjects commencing behaviour between scan points		Subjects ceasing behaviour between scan points		Significance of difference in RISC ratings between groups
	No.	RISC rating (SD)	No.	RISC rating (SD)	
Alcohol	4	34.3 (3.2)	10	26.1 (10.3)	.154
Tobacco	4	23.8 (7.1)	4	18.5 (10.0)	.425

Table 9.10
Comparison of baseline RISC ratings in those who do and do not commence substance use between scans.
Comparisons are made using the independent t-test.

As can be seen from the table, it is only in relatively few cases that a subject's substance using behaviour between the scan points differs from their pattern of use up to the point of inclusion in to the study. This makes the comparisons of RISC ratings in the starting/stopping use groups very low powered, a fact that likely explains the fact that the RISC rating comparisons detailed above are not significant. These data do however suggest that, in the case of both smoking and excessive alcohol consumption, higher RISC ratings are indeed associated with subsequent use of these substances, and lower ratings with cessation of use. It does thus seem to be the case that a change in substance use patterns between scan points does indeed contribute to higher RISC ratings at baseline being associated with subsequent use of tobacco and, (though the relationship seems weaker), with excessive use of alcohol. This would seem to provide evidence that the experience of psychotic-type symptoms may predispose to use of these substances, this association being particularly strong for tobacco. This suggests that self-medication is indeed a relevant consideration in explaining the association between tobacco smoking and schizophrenia; it can also not be discounted as contributing to the association between alcohol use and schizophrneia.

9.5 Substance misuse at point of entry in to the study and the subsequent development of schizophrenia

The analyses outlined above investigate the association between the experience of psychotic-type symptoms and subsequent substance use. Given the nature of the current study however, it is of course also important to explore if a history of drug use is associated with the subsequent development of schizophrenia.

Outcome (i.e. whether or not an individual developed schizophrenia during the course of the study), is known for all high risk subjects recruited. Additionally, level of use of various substances up to the point of recruitment is known for the vast majority of partipants; specifically, cannabis history is known for 145 individuals, alcohol history for 143, and tobacco use for 137. A total of 19 of the subjects for whom these data are available developed schizophrenia during the duration of the study. By contrast, none of the control subjects developed schizophrenia. As described in Section 6.3, availability of these data means that the association between a history of use of each of these substances and the subsequent development of schizophrenia can be explored. The contrasts chosen were previously discussed in Section 6.3.

Results of these contrasts are detailed in Table 8.10. When the proportion of high risk subjects with a history of cannabis exposure greater than isolated use were compared to those whose use history was below this cut off, the former individuals had an elevated rate of developing schizophrenia (P = .029, OR 3.18; 95% CI 1.08-9.36). A similar result was obtained when subjects with a history of alcohol dependence were compared to all individuals with lesser histories of use. Again, those with a history of heavy use of the substance were at elevated risk of developing

schizophrenia (P = .017, OR = 6.35; 95% CI 1.53-26.26). In contrast, when smokers were compared to non-smokers, the rate of development of schizophrenia was not significantly elevated in the former group (P = .129).

Substance under contrast	**Levels of use being contrasted**	**Develop schizophrenia N (% of subjects with that level of use)**		**Chi squared test**
		Yes	No	
Cannabis	Nil/isolated	5 (6.9)	67 (93.1)	χ^2 (1, N = 115) = 4.76
	Occasional/frequently/most days	14 (19.2)	59 (80.8)	p = .029
Alcohol	Non-dependent use	15 (11.2)	119 (88.8)	χ^2 (1, N = 143) = 5.74[a]
	Dependent	4 (44.4)	5 (55.6)	p = .017

Table 9.11
Comparison of proportion of subjects developing schizophrenia at different levels of exposure to cannabis and alcohol.
[a] Likelihood ratio test

Chapter 10

Discussion

10.1 Main findings

The main findings from the image analysis sections are summarised in Tabe 10.1 below. On considering the totality of the imaging data in this study, there is support for the contention that certain brain structural abnormalities are associated with the use of specific substances by people at genetically high risk of schizophrenia. It must be acknowledged however that some findings do initially present as contradictory. Themes and contradictions that emerge from the synthesis of these findings will be discussed below. Where there do seem to be inconsistencies, potential explanations for these will be postulated. This will be followed by integration of these findings into the wider literature base, and consideration of what they may tell us about the role of substance misuse in the aetiology of schizophrenia.

Data	Image analysis methodology	Main findings in high risk individuals
Baseline	ROI	Dose response relationship between levels of alcohol use and (increased) volume of lateral ventricles and (decreased) volume of frontal lobes. Heavy alcohol use emerges from regression analysis as a significant predictor of (increased) lateral ventricular volumes and (decreased) left frontal lobe volume. Dose response relationship between level of cannabis use and (increased) third ventricular volume and frequent cannabis use emerges from regression analysis as a significant predictor of (increased) third ventricular volume.
	Voxel-based	Cannabis use associated with reduced grey matter density in the left anterior and dorsomedial thalamus and left hypothalamus
Longitudinal	ROI	Cannabis use in the inter-scan period associated with bilateral thalamic volume loss
	Voxel-based	Cannabis use in the inter-scan period associated with grey matter loss in the right anterior hippocampus and left superior frontal gyrus

Table 10.1.
Summary of findings from image-analysis section.

10.1.1 Cannabis use is associated with thalamic volume loss

This is strongly supported by baseline findings. Specifically, a dose response relationship exists between level of cannabis use and third ventricular volume, and regions of tissue loss are identified in association with a history of significant cannabis consumption in the anterior and mediodorsal thalamic nuclei by VBM (see Sections 8.1.3 and 8.2.8.1). Furthermore, longitudinal ROI methodology demonstrates progressive thalamic volume loss in association with ongoing cannabis consumption (see Section 9.3.4.1). It is the case however that volumetric analysis does *not* identify reduced thalamic volume on the baseline analysis, and TBM does not identify regions of thalamic grey matter loss over time in association with cannabis use (see Sections 8.1.3 and 9.3.4.1).

As discussed in Section 7.2.1 the sensitivities of the semi-automated volumetric and automated techniques do differ; the former methodology has greater sensitivity for the detection of diffuse change, while the latter is more likely to detect localised abnormalities. Given these considerations it is not entirely surprising that findings of the two approaches do not exactly tally, the differing sensitivities of the two techniques conceivably going some way to explaining the apparent inconsistencies seen. It may be, for example, that cannabis use by a high risk population at a younger age is associated with effects localised to the anterior and mediodorsal thalamic nuclei; such localised change will be apparent at the time of the baseline scans, and is most likely to be detected by VBM. Conversely, cannabis use in older high risk individuals may be associated with more diffuse effects; this effect would occur between scans 1 and 2 and would be more likely to be detected by a volumetric rather than voxel-based approach. Hence, a combination of cannabis

having subtly different effects on thalamic structure at different stages of development and the different sensitivities of volumetric and voxel-based approaches offers a viable explanation for the findings obtained.

10.1.2 Cannabis use is associated with right anterior hippocampus and left superior frontal gyrus grey matter loss

Both of these effects were seen only on the longitudinal TBM analysis (see Secion 9.3.4.1). It is the case however that a region of grey matter loss which approached significance was also seen in association with a history of substantial cannabis use in the baseline VBM analysis (in the right medial prefrontal gyrus, see Section 8.2.8.1). As discussed above, the sensitivity of the automated image analysis methodologies is greater for localised abnormalities. The fact that frontal lobe deficits are observed only with these methodologies (and not volumetric approaches) could thus be explained by the frontal lobe abnormalities that occur in association with cannabis use being very localised. Given that the frontal lobes are relatively large structures, this will constitute only a small percentage of total volume, and thus the volume loss would be expected to be within the bounds of the measurement error inherent in semi-automated hand-tracing techniques. It would thus be missed by volumetric approaches, even if detected by voxel-based techniques.

A similar explanation may of course be relevant when considering that longitudinal anterior hippocampal grey matter loss was seen in association with cannabis use with TBM but not volumetry (see Sections 9.3.4.1 and 8.2.8.1). In explaining why such an effect was not seen in the baseline VBM analysis however,

the influence of age may once again be important. That hippocampal volume loss precedes transition to schizophrenia is one of the most robustly supported dynamic changes believed to occur in those destined to develop the condition (see Sections 4.2.3 and 9.1.2.1). It may be that the period between scans 1 and 2 captured the window in which individuals are vulnerable to cannabis exposure influencing these changes, whereas the majority of those assessed in the baseline analyses had not yet passed through this risk period; consequently the effect is only observed on the longitudinal analyses.

10.1.3 Substantial alcohol use is associated with lateral ventricle enlargement and frontal lobe volume loss

The effects of alcohol on brain structure in high risk individuals were only apparent in the baseline volumetric analyses. They were however very marked. A strong dose response relationship was apparent bilaterally relating historic alcohol consumption to lateral ventricular enlargement and frontal lobe volume loss; additionally, volumes of both the lateral ventricles and the left frontal lobe emerging from the regression analysis as predicted by a history of heavy alcohol use.

As discussed in Section 4.1.3.2 brain structural abnormalities associated with alcohol use would be expected to be diffuse. As discussed above and in Sections 7.2.1 and 8.3.2 this would make them difficult to detect as significant findings using voxel-based approaches; thus a tenable explanation as to why comparable effects were not detected with VBM and TBM is provided.

On considering why effects of alcohol were not observed on volumetric analysis of longitudinal data, age may again be important. It is conceivable that the effects of alcohol on brain structure in those at high risk of schizophrenia are most apparent at earlier ages; hence, they would be detected on the baseline but not longitudinal analysis. It is the case however, that in contrast to the findings with cannabis, there were *no* significant longitudinal brain structural changes observed in association with heavy alcohol use. Given that only 11 subjects on whom longitudinal data were available drank at levels exceeding government recommendation this may of course be explained by a lack of power; other potential implications for this finding will however be discussed further below.

10.2 Implications of these findings for our understanding of the relationship between drug use and schizophrenia

These are the first findings to demonstrate that within a population at high risk of schizophrenia for familial reasons, but clinically well, those who abuse alcohol and/or cannabis have structural imaging findings distinguishing them from those who do not. In contrast, use of these substances by people not at elevated risk of schizophrenia was not associated with comparable imaging findings. Furthermore, within the high risk group both alcohol dependence and regular cannabis use were associated with the subsequent development of schizophrenia (see Section 9.5). We believe that the most likely explanation for these findings is that people at high risk of schizophrenia are more vulnerable to the effects of these substances on the brain, and that these brain structural consequences influence subsequent risk of schizophrenia.

This, together with alternative explanations for the findings of this study will be considered below. In examining these possibilities different substances of abuse will be considered separately; this is essential as it is of course the case that the nature of the relationship between drug use and schizophrenia may differ for different substances.

10.2.1 The relationship between structural imaging abnormalities and substance use is driven by self medication

The self-medication hypothesis was discussed in Section 3.2.1. On reviewing these data it was clear that the hypothesis that people with schizophrenia use substances to relieve psychotic symptoms had negligible support. Nonetheless, it was conceivable that some features of the broader syndrome could be transiently relieved by specific substances; specifically stimulants and tobacco may alleviate cognitive deficits, and alcohol may elevate mood and relieve anxiety. For self-medication to be a feasible explanation for the current findings, it would require that those with greater structural abnormalities were more unwell and consumed more substances. This possibility will be explored for those two substances to which the majority of findings from this study relate; specifically cannabis and alcohol. In undertaking this discussion it is important to emphasise that the high risk individuals who took part in this study were *all well* at the time of assessment/s, none actually having schizophrenia. It is the case however that some individuals did experience subclinical psychotic-type symptoms, the tendency towards this being encapsulated by scores on

the RISC. As higher scores on the RISC would be expected to represent a greater need for self medication, consideration of the relationship between RISC scores and subsequent substance use will be useful in exploring the feasibility of the self-medication hypothesis in this population.

Cannabis

As discussed in Section 3.2.2.1.1, evidence for self medication driving the relationship between cannabis and either schizophrenia or the extended phenotype of the condition is particularly weak. This is further supported by the present study. Specifically, if self-medication was a viable explanation for the association, then those with a greater weight of subsyndromal psychopathology at the baseline assessment would be expected to be more likely to use cannabis in the subsequent follow up period. As can be seen from Section 9.4 however, such as association is not seen; those with higher RISC scores at baseline are in fact no more likely to subsequently use cannabis than those with lower scores on this measure. Also relevant to this discussion is a previous analysis of this sample, which has demonstrated that partial or transient psychotic symptoms were not related to the brain structural abnormalities.[491] Those with greater partial or transient psychotic symptoms at baseline do not have more pronounced brain structural abnormalities, and thus self medication is clearly not a tenable explanation for the associations seen in this study.

Alcohol

The finding that partial or transient psychotic symptoms were not related to brain structural abnormalities is equally relevant when considering the alcohol results.

Clearly this finding also undermines any suggestion that the structural abnormalities that were associated with alcohol use in the baseline analyses are explicable by self medication. It is the case however that the suggestion that some of the association between alcohol use and schizophrenia is mediated by self medication is more credible than is the case for cannabis. Firstly, as outlined in Section 3.2.1.2, though alcohol use may also worsen psychotic symptoms it may genuinely provide transient relief from anxiety and dysphoria. Furthermore, (though admittedly the association was rendered non-significant on controlling for tobacco use), high RISC scores at baseline were associated with subsequent heavy use of alcohol by the high risk subjects. This provides prospective support for the supposition that those with a greater weight of subsyndromal psycopathology are indeed more likely to use alcohol to excess. Thus, though it clearly has limited utility in explaining the structural associations observed in this study, that self medication mediates some of the association between schizophrenia and alcohol use cannot be completely dismissed.

10.2.2 Brain structural abnormalities predispose to substance misuse

A potential explanation for the observed association between brain structural abnormalities and substance misuse is that, independent of any associations with schizophrenia, these abnormalities predispose to alcohol and cannabis use. The compatibility of this explanation with Chambers' theory that cortical and hippocampal dysfunctions in schizophrenia are responsible for the greater reinforcing properties of drugs of misuse (and hence development of drug problems) in this population is evident (see Section 3.2.3.1).

Cannabis

As demonstrated in Chapter9, it is the case that cannabis use is associated with progressive grey matter loss in several brain regions in people at high genetic risk of schizophrenia. Given the progressive nature of these strutural abnormalities it is clearly the case that their association with cannabis use is not explicable by such abnormalities simply predisposing to use of the drug.

Alcohol

When data from this study are considered alone, the above possibility is however difficult to refute in relation to alcohol use; specifically, though baseline associations between alcohol use and structural abnormalities were robust, significant progressive changes attributable to the drug were not demonstrated in the longitudinal analyses (see Chapter 9). Potential explanations for the absence of such findings are discussed in Section 10.1.3. It is the case however that previous longitudinal studies have demonstrated the progressive nature of brain structural abnormalities with ongoing alcohol use (see Section 4.1.3.2.1). Additionally, data that pre-existing brain structural abnormalities predispose to alcohol use are not very convincing (see Section 4.1.1) Thus, though the possibility that structural abnormalities predispose to alcohol misuse cannot be discounted on the basis of the data presented in this study alone, this is clearly not the whole story; the evidence is strong that (in a high risk population such as this at least), both alcohol and cannabis use do impact on brain structure.

10.2.3 The observed findings are an expected consequence of substance misuse

This hinges on the structural abnormalities observed being a ''normal'' consequence of the reported levels of drug use. Two sources of information are relevant in ascertaining if this is the case. The first and most direct of these are the comparisons made between the brain structural associations of substance use observed in the high risk populations and those observed in the controls. As discussed in Section 8.1.3.2, there was no suggestion that associations comparable to those observed in the high risk group were present in controls even at trend level. It could be argued that is potentially attributable to the small size of the control group. It is the case however that Fischer Z test (which accounts for the relative size of each group) demonstrated a significant difference between high-risk and control subjects in the effect of alcohol on the right prefrontal lobe and cannabis on the third ventricle.

The second important source of information in assessing if the effects observed are a 'normal' consequence of drug use are studies investigating the brain structural consequences of such behaviours in individuals without (and not at particularly elevated risk of) mental illness. This will now be considered for both alcohol and cannabis.

Cannabis

The brain structural abnormalities associated with heavy cannabis use were reviewed in Section 4.1.3.1. As was apparent from this and previous reviews, in the normal population there is no consistent evidence of brain structural abnormalities being associated with cannabis use. It is thus clear that the structural abnormalities observed in association with cannabis sue in this study are not a 'normal'

consequence of this exposure.

Alcohol

The brain structural abnormalities identified in chronic alcoholics were reviewed in Section 4.1.3.2, the extent of these abnormalities in individuals under 40 being the subject of a systematic review. Though abnormalities where identified in some of these studies, these were subtle, inconsistent, and certainly not of the magnitude identified in the present study. If present they were most commonly localized to the frontal cortex and hippocampi. In all of these studies individuals included had diagnoses of alcohol abuse or dependence, and all of the non-adolescent subjects were recruited from treatment groups. Reflecting the more pronounced drinking histories of the individuals in these studies, when quantified (e.g. Fein *et al*, Pfefferbaum *et al*[378, 381]) lifetime alcohol intake was greatly in excess of even the heaviest drinking high risk subjects. It does thus seem to be the case that although subtle structural changes, possibly including a degree of frontal lobe volume loss, may be observable in ''uncomplicated'' alcoholism in this age range, the gross structural abnormalities we observed would not be expected in a healthy population.

10.2.4 People at elevated familial risk of schizophrenia are particularly vulnerable to the structural imaging consequences of substance use

As discussed above, the brain structural abnormalities observed in this population in association with use of alcohol and cannabis, (and indeed amphetamine and ecstasy), are all of greater magnitude than would be expected in healthy

individuals with a comparable level of exposure. This is in keeping with previous evidence that brain volume loss is more pronounced in individuals with established schizophrenia who use either cannabis or alcohol, (reviewed in Sections 4.3.1.1 and 4.3.1.2 respectively), findings which suggested that individuals with schizophrenia have a particular sensitivity to brain volume loss on exposure to these substances. The current study builds on this work, being the first to provide structural imaging evidence that such a process may occur in a population at high risk of schizophrenia but currently clinically well. This provides biologically plausible evidence of how use of these substances may be contributing to the aetiology of schizophrenia and clearly underlines the potential hazards of a high-risk population using them.

Cannabis

That people at high risk of schizophrenia for familial reasons are particularly vulnerable to the brain structural consequences of cannabis use is a particularly robust finding of this study. As well as a dose response relationship between cannabis use and structural brain abnormalities being demonstrated in baseline data, (which is not present in controls), longitudinal data provide evidence of progressive changes in association with use of the drug. The findings presented in this report thus demonstrate for the first time the structural brain changes that occur when people vulnerable to schizophrenia use this drug. This has important implicatios for the ongoing debate as to whether or not cannabis use can increase the risk of an individual subsequently developing schizophrenia. For the first time evidence is provided of cannabis having effects on brain structure which are specific to those at high risk of the condition. When this is combined with data from cohort studies implicating cannabis as a risk factor for schizophrenia (reviewed in Section 3.2.2.1.1),

the case for cannabis use playing a role in the aetiology of the psychosis seems remarkably robust. The current findings have implications which go beyond this however. By identifying the brain regions in which cannabis potentiates the development of brain structural abnormalities, the data presented in this report yield insights in to *how* cannabis mediates its risk modifying effects. Specifically, it seems that cannabis use by this population promotes grey matter loss in the thalami, anterior hippocampus and regions of the frontal lobes. As reviewed in Section 4.2, these are precisely those regions in which changes are believed to occur during the transition from at risk state to psychosis.

Alcohol

In contrast to the data relating to cannabis, only cross-sectional findings confirmed an association in those at high risk of schizophrenia between brain structural abnormalities and heavy use of alcohol. Consequently inferences about direction of causality do remain speculative. Nonetheless, and as discussed above, it is the case that the most likely explanation for these associations is that, in a manner comparable to the effects of cannabis, those at elevated risk of schizophrenia are particularly susceptible the brain structural consequences of alcohol use. When taken together with the markedly elevated rate of schizophrenia in subjects with a history of alcohol dependence, this does raise the rather controversial possibility that alcohol consumption could itself be interacting with vulnerability factors for schizophrenia to increase some individuals' risk of developing the condition. As discussed in Section 3.2.2.1.2, a role for alcohol in the development of schizophrenia can be conceptualized within the framework of the stress vulnerability theory of schizophrenia. The findings outlined in this report thus raise the intriguing possibility

that not only cannabis, but also alcohol may, in a vulnerable population at least, contribute to the aetiology of schizophrenia. Though the structural abnormalities associated with alcohol do seem rather more diffuse than is the case with cannabis, the available evidence does suggest that effects on the frontal lobes may be particularly important (see Section 8.1.3.1).

10.3 Limitations

Though the findings detailed above do seem robust, a number of limitations must nonetheless be acknowledged. Firstly, it is the case that ascertainment of drug use did rely entirely on self report. Though this may be regarded as some as a weakness, it is our opinion that the nature of the exposures that were ascertained means that drug testing would in fact have added little to the study. Additionally, and as discussed in Section 6.4.2, self report has been shown to be a reliable measure of drug use in the research context. A more important limitation is the small number of controls available for the baseline analyses, and absence of a normal control comparator group in the longitudinal analyses. It is notable however that despite the relatively small numbers of controls, a significant difference in the effect of both cannabis and alcohol on high risk and control subjects was nonetheless demonstrated in the baseline analyses (see Section 8.1.3.2). It is my belief that the weakness of not having a control group available for the longitudinal analyses is substantially obviated by the comprehensive review of brain structural imaging findings in 'normal' drug

and alcohol users which is reported in Section 4.1. Thus, though longitudinal comparator data in normal controls are not available from the study itself, data from studies undertaken by multiple other groups can be considered when interpreting the longitudinal findings.

A further limitation of the study is the fact that it was not feasible to explore the possibility of specific gene-drug interactions impacting on brain structure. As discussed in Section 4.2.4, it is unfortunately the case that the cohort was simply not large enough to enable this. Furthermore, the nature of the study also means that it can offer little insight into the processes occurring at a cellular or receptor level that result in the observed brain structural consequences. Nonetheless it is the case, (as was discussed above), that by localizing the structures on which the effects of these substances are mediated, the current findings can direct future research designed to elucidate this information. The particular impact of cannabis on thalamic structure, for example, may offer some clues as to the circuits involved in mediating the propsychotic effects of this drug.

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