# Acute and non-acute effects of cannabis in adolescents and adults

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*I, Claire Mokrysz, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis*

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**Abstract**

Preclinical research demonstrates that cannabinoids have differing effects in adolescent and adult animals, and human epidemiological research suggests that adolescent cannabis use has greater potential for harm than adult use. In **chapters 1 and 2**, I review this literature, describing the acute and non-acute effects of cannabis on memory, response inhibition and psychotic-like symptoms, with a focus on findings relating to adolescent populations and age of cannabis use onset. In **chapter 3**, I describe associations between adolescent cannabis use, IQ and educational performance, demonstrating that adjustment for potential confounders – most notably cigarette use – leaves cannabis use *not* associated with lower performance. **In chapter 4**, I describe the first study to compare the acute effects of cannabis in human adolescent (n=20; 16-17 years old) and adult (n=20; 24-28 years old) male cannabis users, in a placebo-controlled, double-blind cross-over design. After inhaling vaporised active or placebo cannabis, participants completed tasks assessing memory, inhibition, alongside physiological measures and subjective drug effects (e.g. "stoned"). Results showed contrasting profiles of adolescent resilience (blunted subjective, physiological and memory effects) and vulnerability (lack of satiety, impaired inhibitory processes). In **chapter 5**, in the same sample, I describe the acute psychotic-like effects of cannabis. Cannabis increased psychotic-like symptoms and the incidence of speech illusions in both adolescents and adults, though some self-rated effects were heightened in adults. In **chapter 6**, in a reduced sample, I describe the acute effects of cannabis on anhedonia (as indexed by reward responsivity, hedonic capacity and self-rated anhedonia) in adolescents (n=13) and adults (n=13). Cannabis did not affect reward responsivity or hedonic capacity in either group, though adults but not adolescents reported self-rated increases in anhedonia. In **chapter 7**, I integrate my findings, discuss their implications, consider limitations and suggest directions for future research into the effects of cannabis use in adolescence.

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**Mokrysz, C**., Freeman, T. P., Korkki, S., Griffiths, K., Curran, H. V. (2016). Are adolescents more vulnerable to the harmful effects of cannabis than adults? A placebo-controlled study in human males. *Translational Psychiatry*, in press.

Curran, H. V., Freeman, T. P., **Mokrysz, C**., Lewis, D. A., Morgan, C. J., & Parsons, L. H. (2016). Keep off the grass? Cannabis, cognition and addiction. *Nature Reviews Neuroscience*, *17*(5), 293-306.

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| | |
|---|---|
| 2-AG | 2-arachidonoylglycerol |
| AEA | N-arachidonylethanolamide (anandamide) |
| AES | Apathy Evaluation Scale |
| AKT1 | RAC-alpha serine/threonine-protein kinase |
| ALSPAC | Avon Longitudinal Study of Parents and Children |
| AUDIT | Alcohol Use Disorders Identification Test |
| AVH | Auditory-verbal hallucinations |
| BAI | Beck Anxiety Inventory |
| BDI-II | Beck Depression Inventory-II |
| BMI | Body-mass index |
| BOLD | Blood-oxygen-level dependent |
| BP | Blood pressure |
| Cann+CBD | Cannabis containing high-levels of THC and high-levels of CBD |
| Cann-CBD | Cannabis containing high-levels of THC and negligible levels of CBD |
| CAPE | Community Assessment of Psychic Experiences |
| CAST | Cannabis Abuse Symptomology Test |
| $CB_1R$ | Cannabinoid Receptor Type 1 |
| $CB_2R$ | Cannabinoid Receptor Type 2 |
| CBD | Cannabidiol |

| | |
|---|---|
| CIs | Confidence intervals |
| COMT | Catechol-O-methyl transferase |
| CPA | Conditioned place aversion |
| CPP | Conditioned place preference |
| DMTS | Delayed Matching to Samples |
| DSB | Digit Span Backwards |
| DSF | Digit Span Forwards |
| DTI | Diffusion Tensor Imaging |
| DZ | Dizygotic |
| eCB | Endocannabinoid |
| FTND | Fagerstrom Test for Nicotine Dependence |
| GABA | Gamma-aminobutyric acid |
| GEE | Generalized estimating equations |
| HR | Hazard Ratio |
| IV | Intravenous |
| LNS | WAIS-III letter-number sequencing |
| LTD | Long-term depression |
| LTP | Long-term potentiation |
| MZ | Monozygotic |
| OFC | Orbitofrontal cortex |

| | |
|---|---|
| OR | Odds ratio |
| PANSS | Positive and Negative Syndrome Scale |
| PFC | Prefrontal cortex |
| PND | Post-natal day |
| PRT | Probabilistic Reward Task |
| PSI | Psychotomimetic States Inventory |
| PSI_A | Psychotomimetic States Inventory – Anhedonia subscale |
| RDoC | Research Domain Criteria |
| S7 | Serial 7's subtraction |
| SD | Standard deviation |
| SDS | Severity of Dependence Scale |
| SE | Standard error |
| SHAPS | Snaith-Hamilton Pleasure Scale |
| SMT | Sternberg memory task |
| SPQ | Schizotypal Personality Questionnaire |
| SSRT | Stop-signal reaction time |
| SUPPS-P | Shortened version of UPPS-P Impulsive Behaviour Scale |
| SWM | CANTAB spatial working memory |
| TEPS | Temporal Experience of Pleasure Scale |
| THC | Delta-9-tetrahydrocannabinol |

| | |
|---|---|
| THC-COOH | 11-nor-9-Carboxy-THC |
| UNICEF | United Nations Children's Emergency Fund |
| WHO | World Health Organisation |
| WIN | WIN55, 212-2 |
| WN | White Noise |
| WTAR | Wechsler Test of Adult Reading |

# 1 Chapter 1. General Introduction

In recent decades it has become clear that the brain continues to develop throughout adolescence. Alongside clear behavioural and cognitive changes, dramatic functional and structural changes occur in the teenage brain, with development extending into early adulthood (Casey, Jones, & Hare, 2008). Longitudinal MRI studies with large samples have now documented structural changes throughout childhood and adolescence. Grey matter volume and thickness increases throughout childhood, peaking in the late pre-teen years before decreasing over the teenage years into early adulthood (Giedd et al., 1999; Raznahan et al., 2011). Meanwhile white matter volume and integrity follows a more linear increase with age from childhood into adulthood (Giedd et al., 1999; Peters et al., 2012). While evidence for the precise mechanisms of these changes remains limited, grey matter reductions in adolescence are thought to reflect synaptic reorganisation and pruning, while white matter increases are thought to reflect increased myelination (Paus, Keshavan, & Giedd, 2008).

With these revelations has come increased concern about the potentially harmful effects of adolescent substance use, and in particular cannabis use, on typical developmental trajectories (Lisdahl, Gilbart, Wright, & Shollenbarger, 2013; Lubman, Cheetham, & Yücel, 2015). Acutely, cannabis leads to pleasurable subjective feelings ("being stoned"), but also to transient cognitive impairment and psychotic-like experiences (Curran et al., 2016). Furthermore, cannabis users are often found to have impaired cognitive abilities relative to non-using controls when non-intoxicated, alongside an increased risk of psychotic disorders, and it has been suggested that earlier age of cannabis use may result in a greater risk of these putative harms (Crane, Schuster, Fusar-Poli, & Gonzalez, 2013).

But, what is the empirical evidence to suggest that adolescent cannabis use has greater potential for harm than adult use? This question is the focus of my thesis.

## 1.1 Cannabis and adolescence

An estimated 13% of 15-16 year olds in Europe and 23% of 15-17 year olds in the USA have taken cannabis in the previous year (Grucza et al., 2016; Hibell et al., 2012). Globally the median age of first cannabis use falls between 18-19 years old (Degenhardt et al., 2008; Degenhardt, Stockings, Patton, Hall, & Lynskey, 2016), indicating that approximately half of all cannabis users start before reaching adulthood. Worldwide, across all age groups cannabis is the most commonly used illicit drug (Degenhardt & Hall, 2012). Cannabis is disproportionately more prevalent in adolescence relative to other illicit drugs, as other drug use typically begins at an older age (for instance, globally the median age of first cocaine use falls between 21 and 24 years Degenhardt et al. (2016)).

To put adolescent cannabis use in context, in the previous month, cannabis was used by 7% (Europe) and 17% (US) of 15-16 year olds. By comparison in the previous month, 57% (Europe) and 24% (US) of this same age group used alcohol and 28% (Europe) and 7% (US) used cigarettes (Hibell et al., 2012; Miech et al., 2015). The rates of alcohol and cigarette use by European adolescents is therefore much higher than for cannabis use. Nevertheless, in the past decade there has been a substantial rise in the numbers of under-18's receiving specialist treatment for cannabis use in the England, and many more adolescents receive treatment for cannabis than for alcohol problems (Figure 1.1) (NDTMS, 2014). Across Europe, an estimated 1.8% of 14-17 year olds meet clinical criteria for addiction to cannabis, six times the rate (0.3%) of adults (18-64 years) (Wittchen et al., 2011).

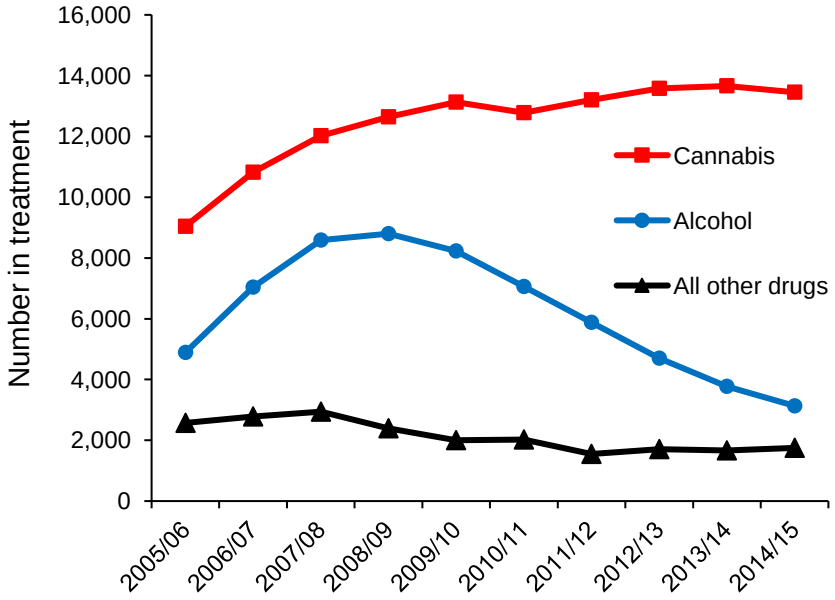


***Figure 1.1.*** *The numbers of under-18's in specialist drug treatment in England, according to primary drug - cannabis, alcohol and other illicit drugs - for which they are receiving treatment for. Data from Public Health England (NDTMS, 2014); figure provided by Tom Freeman (personal communication).*

## 1.2 Pharmacological effects of cannabis

The main psychoactive ingredient of cannabis, delta-9-tetrahydrocannabinol (THC) produces the "stoned" effects that users seek. THC acts on the endocannabinoid (eCB) system, primarily as a partial agonist at cannabinoid $CB_1$ receptors ($CB_1R$), but also at $CB_2R$, both of which are G protein-coupled receptors. $CB_1R$ are found mainly pre-synaptically at the terminals of central and peripheral neurons where they typically have inhibitory action on the release of excitatory and inhibitory neurotransmitters (Pertwee, 2005). $CB_1R$ are found throughout much of the brain, though particularly dense $CB_1R$ expression is seen in the hippocampus, prefrontal cortex (PFC), and amygdala, likely underlying the effects of cannabis on cognitive and memory function (Curran et al., 2016). $CB_2R$ meanwhile are found predominately in immune cells, where they

modulate immune cell migration and cytokine release. $CB_2R$ have more recently been found in neurons in the brain, though at much lower density than $CB_1R$, and the function of these receptors is not yet known (Pertwee, 2014).

In rodents, THC and other synthetic $CB_1R$ agonists alter levels of eCBs throughout many brain regions (Di Marzo et al., 2000; González et al., 2004), and studies with adult cannabis users have found altered eCB levels in cerebrospinal fluid (Morgan et al., 2013) and downregulated cortical $CB_1Rs$ (D'Souza et al., 2016; Hirvonen et al., 2012), relative to non-using controls. eCBs are neuroactive lipids that participate in a range of physiological processes including reward, motivation, emotional homeostasis, pain processing, and synaptic plasticity contributing to learning and memory. At present, the best-characterized eCBs are N-arachidonylethanolamide (anandamide; AEA) and 2-arachidonoylglycerol (2-AG) (Hillard, 2015; Mechoulam, Hanuš, Pertwee, & Howlett, 2014) and both of these lipids exert agonist activity at $CB_1Rs$ and $CB_2Rs$. eCBs are crucial in certain forms of neuronal plasticity, and THC has been shown to disrupt long-term potentiation (a model for learning and memory) and long-term depression in preclinical studies (Zhu, 2006).

While cannabis contains more than 100 cannabinoids alongside THC, in recent years particular focus has fallen on cannabidiol (CBD). CBD has a complex range of pharmacological actions. For example, although CBD has low affinity for $CB_1R$ it can attenuate $CB_1$ agonist effects in brain even at low concentrations (e.g. providing functional antagonism of $CB_1R$ signalling) (Pertwee, 2008). Conversely, CBD reduces the cellular reuptake and hydrolysis of the eCB AEA in the brain (Muniyappa et al., 2013; Pertwee, 2008). These findings, alongside human pharmacological work demonstrating that CBD can block some of the negative acute effects of THC (Englund et al., 2013; Morgan, Schafer, Freeman, & Curran, 2010), has led to increasing interest in CBD and its potential therapeutic value.

### 1.3 Cannabis and adolescent development

While research into adolescent development of the eCB system remains in its infancy, it appears to undergo dynamic changes throughout adolescence (Ellgren et al., 2008), with evidence of increasing $CB_1R$ density of grey and white matter continuing into late adolescence (Romero et al., 1997; Rubino & Parolaro, 2015; Verdurand et al., 2011) (although also see (Ellgren et al., 2008; N. L. Moore et al., 2010)), and changing levels of eCBs in the prefrontal cortex (PFC) and nucleus accumbens (NAc) throughout adolescence (Ellgren et al., 2008; Rubino & Parolaro, 2015). If there is indeed greater $CB_1R$ expression in adolescence than adulthood, this may represent a developmental period during which the brain is particularly sensitive to exogenous cannabinoids (Lubman et al., 2015). The eCB system is also thought to play an important role in neural reorganisation and maturational processes occurring during adolescence (Bossong & Niesink, 2010; Lubman et al., 2015), including synaptic pruning (Bossong & Niesink, 2010) and white-matter development (Solowij, Yücel, et al., 2011). Indeed, the eCB system has recently been implicated in the maturational pruning of glutamatergic synapses (Rubino et al., 2015) and development of GABA-ergic systems (Cass et al., 2014) in the PFC. Since exogenous cannabinoids affect the functioning of the eCB system, it is hypothesised that adolescent cannabis use may disrupt neurodevelopmental maturational processes during this period, such that adolescents are particularly susceptible to cannabis-related harms (Curran et al., 2016).

There is accumulating evidence in humans that aspects of brain architecture are more disrupted by cannabis when individuals start using it during adolescence, although there is a scarcity of direct comparisons with adult users. Some structural imaging studies in adolescent and young adult cannabis users have reported decreased volume in several cortical and sub-cortical regions (Batalla et al., 2013) but findings across different studies vary considerably (Jacobus & Tapert, 2014). For example, although structural differences between adolescent cannabis users and controls in orbitofrontal cortex (OFC) volume have been found, smaller volumes at 12 years of age were shown to *predict* cannabis use at 16, suggesting that differences in the OFC may be a vulnerability factor for use rather than a consequence (Cheetham et al., 2012). And although smaller hippocampal volumes in cannabis users have been associated with age of onset of use,

this association appears less consistent than does the association between reductions in the size of the hippocampus and the amount of use, suggesting that the structure of the hippocampus may be more affected by duration and intensity of exposure rather than by early use specifically (Lorenzetti, Solowij, Fornito, Ian Lubman, & Yucel, 2014).

Diffusion tensor imaging (DTI) studies have found poorer white-matter integrity (indexed by both lower fractional anisotropy and higher mean diffusivity) in adolescents who use cannabis frequently compared with non-users (Clark, Chung, Thatcher, Pajtek, & Long, 2012; Jacobus et al., 2009; Jacobus, Squeglia, Infante, Bava, & Tapert, 2013; Jacobus & Tapert, 2014; Lubman et al., 2015), and reductions in those indices of white-matter integrity correlate with deficits in measures of cognitive performance. However, cannabis users were often also heavy users of alcohol or other drugs so whether these effects related to cannabis use specifically is unclear. Indeed, in a longitudinal study, Bava et al found that alcohol use but not cannabis use between scans predicted reduced white matter integrity at follow-up (Bava, Jacobus, Thayer, & Tapert, 2013). Whether such findings represent causal relationships between adolescent cannabis use and brain structure is therefore difficult to determine.

Perhaps the most compelling evidence for increased adolescent risk from cannabis use comes from associations between younger age of cannabis use onset and negative outcomes. However, while some have found such associations, as I will describe in this chapter (for cognition) and chapter 2 (for mental health), findings are limited and such studies are rarely able to rule out alternative explanations for their findings. Indeed, younger age of onset will typically result in a longer duration of cannabis use prior to study participation, and younger onset of cannabis use is often associated with heavier and more frequent use (Gruber, Sagar, Dahlgren, Racine, & Lukas, 2012) and cannabis dependence (Chen & Anthony, 2003; Chen, O'Brien, & Anthony, 2005; von Sydow, Lieb, Pfister, Höfler, & Wittchen, 2002), all of which have also been demonstrated to predict poorer outcomes (Di Forti et al., 2014; Gruber et al., 2012; McGrath et al., 2010; Schoeler, Kambeitz, Behlke, Murray, & Bhattacharyya, 2016). Moreover, there are many vulnerability factors (for instance, social and economic disadvantage) that may contribute

to younger age of cannabis use onset (Degenhardt et al., 2016), such that it is inherently difficult to separate out the individual contribution of early cannabis use from other overlapping risk factors. Thus, while theoretically it can be argued that adolescent cannabis users may be particularly at risk of cannabis-related harms, the evidence to date is far from clear.

### 1.4 Cannabis and cognition

Acutely, cannabis consumption leads to a variety of intoxication effects (i.e. "feeling stoned"), including the positive effects that users seek (for instance, euphoria, laughter, and enhanced sensory perception) alongside more negative experiences that may not be desired (for instance, paranoia and anxiety). The extent of these different effects are thought to vary widely between users (Atakan et al., 2013; Green, Kavanagh, & Young, 2003), and while evidence is currently limited they are likely to be influenced not only by dose (Curran, Brignell, Fletcher, Middleton, & Henry, 2002) and route of administration (Ohlsson et al., 1981), but also by genetics (Morgan, Freeman, Powell, & Curran, 2016), cannabis use history (D'Souza, Ranganathan, et al., 2008), but also see (Ramaekers et al., 2016)), and the context in which cannabis is taken (Carlin, Bakker, Halpern, & Post, 1972). Alongside these subjective effects, cannabis also impacts on cognitive functioning. Acute effects on cognition are transient and likely contribute to the overall experience of feeling stoned. There is good experimental evidence that acutely cannabis leads to transient deficits of verbal and working memory, attention, inhibition and psychomotor function. However, whether there are more long-lasting cognitive effects of cannabis, outlasting the intoxication period, remains hotly debated.

Throughout this thesis I will use the term "acute" to refer to transient effects resulting from cannabis or THC administration that are apparent during the period of subjective intoxication, and will use the term "non-acute" to refer to effects that outlast the intoxication period. While the terms chronic or persistent imply that these effects are long-lasting, my definition of non-acute makes fewer assumptions about the underlying cause of such effects. Non-acute may refer to temporary effects from recent cannabis use (which nevertheless outlast subjective

intoxication), or to longer-lasting effects that persist after all cannabinoids are cleared from the body but are reversible, or to long-lasting effects that do not recover. The terms residual and sub-acute are often used to describe temporary effects from recent cannabis use, that are thought to recover once all cannabinoids are cleared from the system. However, it is often impossible to separate the different possible types of effect throughout much of the non-acute cannabis literature, and to my mind such terms make assumptions about the longevity of the effects.

### 1.4.1 Associations between cannabis and cognitive function

Cannabis use, and particularly frequent use, has been associated with longer lasting impairment of similar cognitive domains to those affected by acute doses. Furthermore, prospective cohort data suggests cannabis use is associated with lower IQ and poorer educational attainment (as will be covered in more detail in chapter 3). While the acute findings have been well-replicated, particularly for verbal memory effects, the evidence regarding non-acute effects is mixed and often insufficient to allow us to draw robust conclusions. While in the future, data from randomised controlled trials of medicinal cannabis may be able to provide strong causal evidence for or against non-acute cognitive effects of cannabis, to date our knowledge is dependent upon epidemiological observational evidence and preclinical work with non-human primates and rodents.

A recent systematic review of the cognitive effects of cannabis in humans came to the following conclusions regarding non-acute use: 1) there is good evidence of associations between cannabis use and impairments of verbal learning and memory, and attention and attentional bias; 2) there is limited evidence of associations between cannabis use and impairments of psychomotor function; and 3) there is mixed evidence of associations between cannabis use and impairments of executive functions (including inhibitory processes) and decision-making (Broyd, van Hell, Beale, Yücel, & Solowij, 2015).

In the following section I will focus my coverage more specifically on the evidence relating to the effects of cannabis on verbal learning and memory, working memory and response

inhibition. These domains have arguably received the most attention in terms of acute effects, and as such were chosen for my acute study described in chapter 4, though there is also limited evidence that cannabis may impact upon other cognitive functions, including psychomotor control, attention & executive function (Broyd et al., 2015). For each domain in turn (verbal learning and memory, then working memory, then response inhibition), I will introduce the tasks typically used to index it and then review the literature, in the following format. I will first review the evidence for the acute effects of cannabis on the domain in human adults. Given that, to date, no studies have assessed the acute effects of THC or cannabis in adolescents, I will then focus on preclinical studies in which the acute effects have been assessed in adolescent animals. Next, I will review the human epidemiological evidence linking cannabis use to non-intoxicated impairments in the domain, before reviewing the evidence to suggest that adolescent onset of use is more likely to lead to these impairments.

### 1.4.2 Memory

#### *1.4.2.1 Verbal learning and memory*

Verbal learning and memory impairments are the most consistently reported effects of cannabis, both acutely and non-acutely. Such effects on memory are consistent with the extensive preclinical evidence of: the amnestic effects of cannabis in animal models; the high density of cannabinoid receptors in memory-associated brain regions such as the hippocampus, amygdala and PFC; and observations that THC induces disruption of plasticity (including long-term potentiation (LTP) and long-term depression (LTD)) in the hippocampus and decreases acetylcholine release in both the hippocampus and the PFC (Curran et al., 2016).

Typically tasks indexing verbal learning and memory require participants to learn word lists or prose, followed by immediate and delayed recall and/or recognition tests. The pattern of performance across tests can tentatively indicate the specific memory processes that have been impaired. Impaired immediate recall (when free recall of the words or prose is tested immediately following stimuli presentation) suggests encoding deficits, while intact immediate

recall but impaired delayed recall (when free recall of the words or prose is tested after a delay since stimuli presentation) suggests consolidation (storage) deficits. Further, impaired recall of stimuli but intact recognition suggests retrieval deficits, but may also represent lower levels of encoding. For instance, recognition of previously presented stimuli likely requires a lower level of processing than free recall- that is, impaired recall of stimuli but intact recognition may instead suggest lower level encoding rather than retrieval deficits. Such interpretations should therefore be made with caution.

#### 1.4.2.1.1 Acute effects of cannabis on verbal learning and memory

Acutely THC (Curran et al., 2002; D'Souza, Braley, et al., 2008; D'Souza et al., 2004; D'Souza, Ranganathan, et al., 2008; Englund et al., 2013; Liem-Moolenaar et al., 2010; Morrison et al., 2009; Ranganathan et al., 2012; Theunissen et al., 2015) and cannabis (Morgan et al., 2010) reliably (though see (McDonald, Schleifer, Richards, & de Wit, 2003; Vandrey et al., 2013)) produce deficits of verbal learning and memory in minimal and frequent users, typically impacting upon both immediate and delayed free recall but not recognition memory. A number of early studies also demonstrated that delayed recall of stimuli presented prior to cannabis exposure was not affected (Abel, 1971; Darley, Tinklenberg, Roth, Hollister, & Atkinson, 1973; Dornbush, 1974). Together such findings suggest that THC impacts upon encoding of verbal information but not retrieval.

Additionally, a number of studies report increased intrusions and false positives (that is, false recall of a word that was not presented) following THC (D'Souza, Braley, et al., 2008; D'Souza, Ranganathan, et al., 2008). Similar results appear for intravenous (IV), intrapulmonary (smoked or inhaled) and oral (ingested) administration of THC, though there have been no direct comparisons of task performance between administration routes. Deficits do not appear to be as a result of reduced information processing (Belmore & Miller, 1980) or deficient rehearsal during encoding (Darley, Tinklenberg, Roth, & Atkinson, 1974).

A small number of studies have assessed whether higher doses of THC lead to greater impairments, and typically support a dose-response relationship. Curran and colleagues (Curran et al., 2002) administered 0.0mg, 7.5mg or 15.0mg of THC orally to 15 cannabis users (using no more than once per week) in a within-subjects design, finding no impairment to word or prose recall following 7.5mg of THC, but considerable impairment of both immediate and delayed recall following 15.0mg of THC. Impairments peaked at 2 hours, coinciding with peak subjective effects and plasma THC levels. D'Souza and colleagues (D'Souza et al., 2004) administered 0.0mg, 2.5mg or 5.0mg IV THC to 22 cannabis users with no history of cannabis use disorder, in a within-subjects design, finding greater impairment to immediate and delayed recall following the larger dose. D'Souza et al subsequently replicated this dose-response finding for immediate recall in both frequent (n=30) and infrequent users (n=22), but for delayed recall only in non-users (D'Souza, Ranganathan, et al., 2008). Intriguingly the frequent users were minimally affected by both active doses on delayed recall, and even improved (non-significantly) following the 2.5mg dose.

A small number of studies have compared the effects of THC in frequent users to non-users (typically those with no recent use and a small number of lifetime exposures), with mixed findings. D'Souza, Braley, et al (2008) found no differences in the effect of IV THC on word recall between frequent (n=11) and non-users (n=17), while D'Souza, Ranganathan, et al (2008; described above) found that THC impaired frequent users on immediate and delayed recall to a lesser extent than non-users. While difficult to interpret with limited studies, this discrepancy may be related to the larger doses administered in the latter study (2.5mg and 5.0mg THC) relative to the former (0.03mg/kg, equivalent to 2.1mg at the mean body weight (72.3kg) of their participants).

While most studies have administered THC alone, Morgan et al (Morgan et al., 2010) investigated the impact of cannabis content (THC, CBD) in a naturalistic within-subjects study of 'at least monthly' cannabis users. Participants were assessed in their own homes on both a drug-free day and after smoking their own cannabis. Two groups were compared, according to

the CBD content of their smoked cannabis. Despite no group difference in cannabis THC content, those who smoked cannabis with low levels of CBD (samples with less than 0.14% CBD; n=22) performed worse on immediate and delayed prose recall relative to those who smoked CBD-rich cannabis (samples with more than 0.75% CBD; n=22). Further Englund et al (Englund et al., 2013) administered 1.5mg IV THC following an oral pre-dose of placebo (n=26) or CBD (n=22). While CBD did not attenuate the impairing effect of THC on immediate recall, exploration of a trend level interaction showed that delayed recall was only impaired following the placebo pre-dose, but not following CBD. Together these studies suggest a potentially protective effect of CBD on the verbal memory impairing effects of THC.

#### 1.4.2.1.1.1 Age-related findings: acute

To date, no studies have assessed the role of age of user, or age of cannabis use onset, on the acute effects of THC or cannabis on verbal memory or learning in humans. Verbal tasks are clearly not directly translatable to animal models, and as such much animal work on memory and learning has focused on spatial learning and novel object recognition paradigms.

Preclinical evidence for increased adolescent vulnerability to acute effects of cannabis is mixed, with some suggesting acute cannabinoid treatment has a greater impairing effect on spatial and non-spatial learning (THC) (Cha, Jones, Kuhn, Wilson, & Swartzwelder, 2007; Cha, White, Kuhn, Wilson, & Swartzwelder, 2006) and object recognition (WIN) (Schneider, Schömig, & Leweke, 2008) in adolescent compared to adult rats. Others however report the opposite, with evidence of greater acute impairments in adult rodents - including impaired novel object recognition (WIN) (Fox, Sterling, & Van Bockstaele, 2009), and spatial learning (WIN) (Acheson, Moore, Kuhn, Wilson, & Swartzwelder, 2011). While the direct translation of such findings is limited given the differing constructs indexed, such findings do suggest that there may be age-related differences in the acute effects of cannabis on hippocampal-dependent memory in humans. Though, the use of the full $CB_1R$ agonist WIN rather than THC (a partial agonist with relatively weak $CB_1R$ affinity) also restricts translation to human cannabis use.

#### 1.4.2.1.2 Non-acute associations between cannabis use and verbal learning and memory

In 2003 Grant and colleagues performed a meta-analysis across broad neurocognitive domains of 11 studies comparing non-intoxicated cognitive functioning in cannabis users and non-users (I. Grant, Gonzalez, Carey, Natarajan, & Wolfson, 2003). The authors combined data from 623 cannabis users, and 409 controls who had no or very limited cannabis exposure. Importantly while the analysis demonstrated no evidence of robust group differences on attention, simple reaction time, language, executive function, motor, and perceptual-motor performance, they did find small but robust impairments of learning (including immediate recall on word recall tasks) and retrieval (including delayed recall on word recall tasks) in cannabis users. The effect size (Cohen's standardised mean difference, *d*) for learning was -0.24 (99% CIs: -0.41, -0.06) and for retrieval was -0.27 (99% CIs: -0.49, -0.04). Interestingly, meta-regression revealed that duration of cannabis use prior to study participation did not moderate the effect sizes.

Since 2003 a large number of studies have further assessed associations between non-acute cannabis use and verbal learning and memory. In 2012 Schreiner and Dunn (Schreiner & Dunn, 2012) conducted an updated meta-analysis, with matching inclusion criteria to Grant et al (I. Grant et al., 2003), though only including studies published since 2000. The authors combined data from 33 independent samples including adolescent and adult samples (1,010 cannabis users, 839 controls) and calculated effect sizes for the same eight domains as Grant and colleagues (2003), and also calculated a global effect size which summarised data across all domains. The analysis revealed a small overall group difference on global cognitive performance, alongside domain specific group differences for learning (Hedge's *g*; -0.35 (95% confidence intervals (CIs): -0.55, -0.15)), retrieval (-0.25 (95% CIs: -0.47, -0.02)), attention, language, executive function, and motor performance. While Grant et al (2003) used Cohen's *d* as an estimate of effect size and Schreiner and Dunn (2012) used Hedge's *g* (*g* corrects for potential overestimation of effect that can occur in small samples by weighting effects by sample size), the magnitudes of *d* and *g* are comparable; similar estimates for learning and retrieval impairments were found in both the 2003 and 2012 meta-analyses. Schreiner and Dunn

(2012) further replicated the finding that duration of cannabis use prior to study participation did not moderate these relationships.

Given concern throughout the literature that cannabis may have residual or sub-acute effects on cognition, potentially resulting from lingering cannabinoids stored in lipid cells (cannabinoids can often be detected in blood and urine one month or more following abstinence), Schreiner and Dunn (2012) then conducted a second meta-analysis, now including only those studies with a minimum abstinence period of 25 days (leaving 13 of 33 samples in the analysis). This second analysis revealed attenuated effect sizes for all individual domains and global performance, with all confidence intervals now crossing the null. This finding suggests that impairments may be temporary or reversible with abstinence, potentially resulting from residual circulating cannabinoids or from cannabis withdrawal.

While there are limitations to the meta-analytic approach here, not least the combining of different tasks into one measure of a broad cognitive domain such as learning, this result highlights an important debate about the reversibility of putative impairments following abstinence from cannabis. Given that the non-acute cognitive deficits most often found in cannabis use groups mirror those typically found in acute studies, it is important to interpret the non-acute literature with the caveat that residual effects may be influencing findings. Though, to my knowledge the hypothesis that residual effects are due to cannabinoids still circulating in the body has yet to be directly tested.

#### 1.4.2.1.2.1 Age-related findings: non-acute

A number of studies have compared verbal learning and memory in adolescent cannabis users relative to non-users or lighter users. Broadly these can be separated into those requiring short-term abstinence (typically 12-24 hours) and those who monitored abstinence across a longer period of up to 1 month prior to assessment.

Short-term abstinence

Harvey et al compared current weekly (n=34) to less than weekly (n=36) cannabis users aged 13-18 years, following 12 hours of self-reported abstinence; weekly users had poorer immediate but not delayed word recall relative to placebo (Harvey et al., 2007). Dougherty et al compared current cannabis users (using at least 4 days per week for the past 6 months; n=45) to non-users (n=48) aged 14-17 years, following a minimum 18 hours monitored abstinence (confirmed by declining THC in urine between screening and testing sessions) (Dougherty et al., 2013). Cannabis users has poorer word recall (whether this reflected immediate/ delayed recall was not specified) relative to the non-users. Solowij et al (2011) assessed verbal memory in adolescent and adult (aged 16-20 years) regular cannabis users (n=52), regular alcohol users with no history of regular cannabis use (n=67), and controls with no regular substance use histories (n=62; (Solowij, Jones, et al., 2011)). Relative to both the alcohol users and controls, cannabis users (following at least 12 hours of abstinence, self-reported and corroborated via THC saliva levels) had poorer immediate and delayed recall, and recognition memory. These group differences persisted following covariate adjustment for premorbid functioning (as indexed by age 12 school verbal and numerical ability test results), and for group differences in mental health, tobacco and alcohol use and gender.

Studies assessing verbal memory in adolescent cannabis users following short-term abstinence therefore typically demonstrate impairments relative to healthy controls, alcohol users, and less frequent cannabis users. However, without longitudinal studies assessing verbal learning and memory before cannabis exposure, these cross-sectional studies cannot indicate whether impairments occurred following cannabis exposure.

Long-term abstinence

A number of adolescent studies have monitored abstinence over an extended period of time before assessment. Hanson et al conducted a key longitudinal study over 3 weeks of monitored abstinence (abstinence confirmed by decreasing THC metabolites over regular serial urine tests) with weekly or more adolescent cannabis users (n=19) relative to non-user controls (n=21; aged

15-19 years) (Hanson et al., 2010). Immediate word recall impairments (they did not report delayed recall assessment) apparent in cannabis users after 2 days of abstinence, normalised to non-user performance following 2 weeks of abstinence and remained normalised after 3 weeks. A key strength of this study was the longitudinal design, while other studies have rarely taken baseline assessments to assess change over the period of abstinence.

Mahmood et al similarly monitored abstinence for 22 days in cannabis users (n=65) and non-user controls (n=65) aged 15-19 years, finding no group differences on immediate and delayed recall performance when assessed following abstinence (Mahmood, Jacobus, Bava, Scarlett, & Tapert, 2010). Winward et al monitored abstinence over 4 weeks, finding no differences between cannabis users (n=20) and non-users (n=55; aged 16-18 years) on immediate or delayed free recall, though cannabis users did have impaired delayed cued recall performance (Winward, Hanson, Tapert, & Brown, 2014). Medina et al similarly monitored abstinence over at least 23 days in monthly cannabis users (n=31) and non-user controls (n=34; aged 16-18 years), finding no difference between groups on word recall (a composite measure including immediate and delayed recall and recognition memory), but poorer performance in the cannabis users on prose recall (composite measure as above) (Medina et al., 2007).

Of interest, Jacobsen et al monitored cannabis abstinence (requiring a negative urine screen after one month to participate) in 20 daily tobacco smokers who used cannabis, relative to 25 daily tobacco smokers who did not use cannabis (aged 13-18 years) (Jacobsen, Pugh, Constable, Westerveld, & Mencl, 2007). Participants attended two sessions following 1 month of cannabis abstinence; on one occasion they smoked cigarettes as usual and on the other they abstained from nicotine for 24 hours prior to assessment. When tobacco smoking as usual, no differences were found on immediate and delayed recall between the cannabis users and non-users. Interestingly however, following 24 hours of nicotine abstinence, the cannabis users but not the non-users showed impairment of delayed recall. The authors therefore suggest that nicotine, acutely a cognitive enhancer (Heishman, Kleykamp, & Singleton, 2010), may be masking verbal memory deficits in cannabis users who also consume tobacco on a daily basis (that is, if a

participant smokes a cigarette soon before memory assessment, the nicotine dose may subsequently mask an impairment).

Therefore, while assessing cannabis users after minimal periods of abstinence reliably detects verbal learning and memory impairments relative to non-users, assessments following two weeks or more of abstinence tend to report no or fewer performance impairments. While promising, a number of limitations of such studies must be considered. Sample sizes are typically small, particularly for monitored abstinence studies and the degree of cannabis use in both the cannabis use and control groups varies widely across studies. For instance, some included those who fulfil criteria for cannabis use dependence, while others excluded these users; others defined non-use as less than 5 lifetime uses, while others defined non-use as less than 40 lifetime uses. Another key issue for monitored abstinence studies is whether cannabis users who are motivated to quit for an extended period differ from typical cannabis users, potentially questioning the generalisability of such findings to other cannabis users.

Associations with age of cannabis use onset

It is therefore apparent that adolescent findings for verbal learning and memory (at least for immediate and delayed recall) are typically similar to those reported in adults (I. Grant et al., 2003; Schreiner & Dunn, 2012). However, I am aware of no studies to date directly comparing adolescent and adult cannabis user groups; though studies have assessed whether a younger age of cannabis use onset predicts greater verbal memory problems.

Solowij et al (as described above) found correlations between word recall (immediate and delayed) and both age of first cannabis use and age of first regular cannabis use (Solowij, Jones, et al., 2011) in 16-20 year old cannabis users (n=52). After adjustment for frequency and quantity of cannabis use, younger age of first regular cannabis use continued to predict poorer immediate and delayed recall; though they did not adjust for duration of cannabis use. Wagner et al assessed verbal word recall in 142 current and former cannabis users (self-reported abstinence of 12 hours minimum required; mean time since last use= 134 days) aged 18-35,

performing a median split by age of onset creating a group of early-onsetters (aged 11-14 years) and late-onsetters (aged 15-35 years) (Wagner, Becker, Gouzoulis-Mayfrank, & Daumann, 2010). Participants were also classified by duration of use (short (0-30 months), long (36-240 months)) and frequency of use (low (0-9 days per month), high (10+ days per month)). The authors report a three-way interaction of age of onset x frequency of use x duration of use for immediate recall. In the early on-setters, long relative to short duration of use predicted poorer immediate recall in both low and high frequency users; however, in the late-onsetters, long relative to short duration of use only predicted poorer immediate recall in high frequency users. However, alone, age of onset did not predict worse recall performance. These findings suggest there may be complex interactions between age of onset and cannabis use patterns, though the exploratory interaction finding requires replication before strong conclusions can be made. Recently Crane et al assessed verbal memory in 69 cannabis users aged 18-24 years (self-reported abstinence on day of testing; time since last use ranged from 1-45 days) (Crane, Schuster, Mermelstein, & Gonzalez, 2015). The authors report an interaction between age of first regular use and gender, with earlier age of regular use predicting immediate and delayed recall performance in women but not men.

Others however have found no relationship between age of cannabis use onset and verbal memory (Gruber et al., 2012; Tait, Mackinnon, & Christensen, 2011; Winward et al., 2014), and age of cannabis use onset did not moderate the relationship between cannabis use and impairment for any cognitive domain in the 2012 meta-analysis described above (Schreiner & Dunn, 2012). Though as the findings of Wagner et al (Wagner et al., 2010) and Crane et al (Crane et al., 2015) demonstrate, there may not be a simple relationship between age of onset and verbal memory, with gender differences and interactions with frequency and duration of use potentially influencing the relationship. Importantly associations between age of onset and verbal memory also cannot suggest causality, since there may be other common factors that influence both earlier age of substance use and poorer verbal memory. Moreover, as with all retrospective drug history measures, recall of age of cannabis use onset may be unreliable.

Relatedly, in a recent meta-analysis Schoeler and colleagues compared cannabis users and non-users on various measures of verbal and working memory (Schoeler, Kambeitz, et al., 2016). They found no difference between the global memory effect size for early-onset (<17 years) relative to late-onset (≥17 years) cannabis users, suggesting that earlier onset of cannabis use was not associated with increased memory impairment.

In summary, whether adolescent cannabis users have poor verbal learning and memory outcomes remains for debate, though promisingly a number of studies have demonstrated no evidence of deficits following abstinence of as little as two weeks. However, few studies have followed adolescent cannabis users into adulthood to assess any long-term effects adolescent use may be having on verbal memory functioning.

#### *1.4.2.2 Working memory*

Working memory refers to a cognitive system for the temporary storage and manipulation of remembered information (Baddeley, 1992). Such a system is clearly essential for successful day-to-day functioning, supporting many other cognitive functions including decision making, problem solving and selective attention. Whether cannabis affects working memory function has been widely assessed, though with very mixed findings, particularly in regard to the acute effects.

Working memory has been assessed with a large number of different tasks, including the N-back (0- 1- 2- or 3-back), digit span forwards and backwards (DSF, DSB), serial 7's subtraction (S7), delayed matching to samples (DMTS), WAIS-III letter-number sequencing (LNS), CANTAB spatial working memory (SWM) and the Sternberg memory task (SMT). The 0-back, DSF and SMT, arguably require only maintenance and recognition of information. While specific task procedures vary, the other tasks have in common the requirement to maintain information and manipulate it 'online'. Tasks also vary in their memory load (for instance, the 1-back requires comparison of a stimulus to the stimulus immediately preceding, while the 2-back requires comparison to the stimulus presented two before) and complexity (for instance,

‘easy’ and ‘hard’ DMTS versions requires memory of simple and complex shapes). Tasks are typically either spatial (spatial N-back, DMTS, SWM), requiring memory of the location of stimuli, or verbal (verbal N-back, DSF, DSB, S7, LNS, SMT), requiring memory of words or numbers presented either visually or auditory. Task performance is typically measured by accuracy and/or reaction time.

As a quick aside, clearly working memory and verbal learning and memory are not fully dissociable domains. Indeed, simple maintenance of words or letters as demanded by a number of working memory tasks described above has a conceptually similar memory demand as immediate verbal recall tasks described previously, and working memory likely contributes to recall of recently processed stimuli on such tasks. However, working memory is distinct from other short term memory processes such as verbal learning and memory, since it enables the manipulation, rather than simple recall or recognition, of stored information.

#### 1.4.2.2.1 Acute effects of cannabis on working memory

##### Maintenance

Findings for the SMT consistently report an increased recognition reaction time following THC (Bossong et al., 2012; Hunault et al., 2009; Schoedel et al., 2011; Theunissen et al., 2015) or nabilone (Wesnes et al., 2009) relative to placebo, with some also reporting decreased accuracy (Bossong et al., 2012; Hunault et al., 2009; Wesnes et al., 2009). Meanwhile, Morrison et al (2009) reported decreased accuracy in 22 male cannabis users for the DSF following IV THC, while McDonald et al (2003) found no effect following oral THC in 37 cannabis users. Findings for the 0-back are more mixed, with some finding no impairment to accuracy or reaction time following THC administration on both verbal (Kollins et al., 2015) and spatial (Vandrey et al., 2013) 0-backs, though Ilan et al found that THC increased reaction times on the spatial 0-back in 10 cannabis users (Ilan, Smith, & Gevins, 2004). Together these findings suggest that under some circumstances THC impairs maintenance of information even without any manipulation task demands, though inconsistent findings preclude strong conclusions. Of note, the 0-back has

a particularly low memory demand, while the SMT typically consists of a number of trials with increasing memory load, potentially accounting for the discrepancies between results on these tasks.

Manipulation

Mixed findings have also been reported for SWM, with increased errors following THC in minimal users but not in frequent users (D'Souza, Braley, et al., 2008) or occasional users (Ranganathan et al., 2012); potentially suggesting tolerance to the effects following even irregular cannabis use. Surprisingly, Makela and colleagues reported a decrease in errors following THC in 19 cannabis users, though this may represent practice effects, since this improvement only occurred when participants were randomised to receive placebo on the first occasion (Makela et al., 2006).

THC is typically found to impair reaction times on the N-back task, though often not consistently across different memory loads (1- 2- and 3-back). Kollins et al (2015) found in 16 cannabis users that THC increased reaction time on the verbal 3-back but not the 1- or 2-back (or as described above, the 0-back), though accuracy was not affected at any load. Meanwhile Vandrey et al found in 13 daily cannabis users that THC increased reaction time on the spatial 1-back but not the 2- or 3-back (or as described above, the 0-back), and did not report accuracy (Vandrey et al., 2013). Ilan et al (2004) found that THC increased reaction time and decreased accuracy for the spatial 2-back (and as described above, increased reaction time on the 0-back), and Hart et al (n=24) found that THC increased reaction times but did not affect accuracy for the spatial 1- and 2-backs (Hart et al., 2010). Meanwhile, both Morrison et al (2009) and McDonald et al (2003) reported impaired DSB following IV THC.

In summary, the acute effects of cannabis on working memory abilities, both maintenance and manipulation, remain unclear as a result of inconsistent findings. Such inconsistencies may be due to methodological differences- including differing doses, routes of administration, sample characteristics and small sample sizes. Moreover, different tasks pertaining to index similar

constructs nevertheless have differing components, memory demands, and difficulty levels, thus confusing comparison of effects found on different tasks.

#### 1.4.2.2.1.1 Age-related findings: acute

As described above, to date no studies have administered cannabis to adolescents in a controlled study. Preclinical evidence for working memory effects of cannabis in younger animals is also limited, though in 14 adolescent rhesus monkeys Verrico and colleagues found that acute doses of THC but not placebo led to impaired spatial working memory, but not the earlier maturing object working memory (Verrico et al., 2012). Such findings suggest that the effects of cannabis may be dependent upon developmental stage of the cognitive function being measured.

### 1.4.2.2.2 Non-acute associations between cannabis use and working memory

In a recent meta-analysis Schoeler, Kambeitz et al (2016) compared working memory ability for cannabis users and non-users (including both adult and adolescent samples). They reported robust evidence of non-acute impairment in cannabis users on tasks of verbal working memory (Cohen's d= 0.11 (95% CIs: 0.04, 0.17)) but not for visual/spatial working memory (d= -0.02 (95% CIs: -0.26, 0.21)). Of note however, the global memory effect size (that is, including all memory measures for verbal memory, working memory and prospective memory) reduced by 50% once the authors exclude studies including participants who had been abstinent for fewer than 10 days.

#### 1.4.2.2.2.1 Age-related findings: non-acute

There are mixed findings for working memory impairments in adolescent cannabis users. Most studies have found no differences between users & non-users on various working memory tasks, even after short periods of abstinence (Jager, Block, Luijten, & Ramsey, 2010; Padula, Schweinsburg, & Tapert, 2007; Alecia Dager Schweinsburg et al., 2010; Whitlow et al., 2004). As described above, Hanson et al followed cannabis users aged 15-19 years over 3 weeks of monitored abstinence. Similar to their findings for verbal recall, at baseline the cannabis users

(n=19) performed lower on the LNS (a verbal working memory task) relative to non-using controls (n=21), but after three weeks of abstinence no groups differences were detected. However, some have found evidence of working memory impairments detectable following an extended period of abstinence. In a sample of 31 cannabis users aged 16-18 years, Medina et al found lower DSB performance after at least 23 days of monitored abstinence, relative to 34 non-using controls (Medina et al., 2007). Similarly, as described above, Jacobsen et al (2007) monitored abstinence in 20 daily tobacco smokers who used cannabis, relative to 25 daily tobacco-only smokers who did not use cannabis (aged 13-18 years). Following one month of cannabis abstinence, the cannabis users showed a greater decrease in N-back performance with increasing memory load (for the 2-back relative to 1-back) than the tobacco-only smokers.

Associations with age of cannabis use onset

To my knowledge, one study has assessed the links between age of cannabis use onset and non-acute working memory ability. Gruber et al (2012) found no difference in working memory performance (as assessed by both the DSF and DSB) between non-users (n=28), early-onset (<16 years; n=19) or late-onset (≥16 years; n=15) cannabis users, after 12 hours' abstinence. Relatedly, as described above, in a recent meta-analysis Schoeler, Kambeitz et al (2016) compared cannabis users and non-users on various measures of verbal and working memory. They found no difference between the global memory effect size for early-onset (<17 years) relative to late-onset (≥17 years) cannabis users, suggesting that earlier onset of cannabis use was not associated with increased memory impairment.

Repeated administration studies with adolescent animals

Repeated administration studies with animals further suggest greater vulnerability to cannabis-related harm in adolescents. Repeated adolescent THC exposure has been shown to lead to adulthood deficits in spatial working memory in rats (Rubino, Realini, Braida, Alberio, et al., 2009; Rubino, Realini, Braida, Guidi, et al., 2009), though intriguingly a recent study instead found improved working memory in adult rats who had repeatedly self-administered the

synthetic $CB_1R$ agonist WIN55,212-2 (WIN) in adolescence (Kirschmann, Pollock, Nagarajan, & Torregrossa, 2016). Furthermore, in adolescent rhesus monkeys Verrico and colleagues found that repeated doses of THC led to impaired spatial but not object working memory (mirroring their acute findings described above), and prevented the maturational improvement in spatial working memory typically seen at that age, but did not affect the earlier developing object working memory Verrico, Gu, Peterson, Sampson, and Lewis (2014). However, there have been no direct comparisons of the working memory effects of repeated cannabinoid doses in adolescent versus adult rats, precluding conclusions about whether repeated adolescent exposure is particularly damaging.

### 1.4.3 Response inhibition

Response inhibition, and recently its putative association with age of cannabis use onset, has been a key area of research in the cannabis literature. However, whether cannabis use leads to impaired response inhibition remains unclear, with mixed evidence both acutely and non-acutely. Response inhibition is an aspect of impulsivity, and can be broadly defined as "the process by which a prepotent, routine or dominant response is deliberately withheld" (Hampshire, Chamberlain, Monti, Duncan, & Owen, 2010).

Response inhibition is typically assessed by one of three tasks: Stroop, go/no-go, or stop-signal. The Stroop colour-interference task has been used on occasion throughout the cannabis literature, though specific interpretation of the key outcome of interest (time to complete reading of list of color-incongruent words) is problematic since increased latency may reflect impairment of attentional processes or processing speed, rather than specifically impaired inhibitory function. As a result, the stop-signal and go/no-go tasks are now much more widely used across the response inhibition literature. However, while these two tasks are often interpreted interchangeably, the tasks fundamentally measure different functions.

The stop-signal task measures "action cancellation", requiring participants to inhibit already initiated responses. The key variable of interest is the theoretical speed at which an already

initiated response can be stopped (SSRT), calculated according to the horse-race model which conceptualises action as a competition to finish first between stopping processes and reaction processes, such that whichever process finishes first determines outcome (Logan & Cowan, 1984). The SSRT represents the total time taken to attend to, process and execute an inhibitory response to the stop-signal. The stop-signal task can either utilise a tracking or non-tracking procedure. For the tracking procedure, the delay between stimulus and stop-signal is altered according to the participant's performance with the aim of finding the delay at which the participant is able to inhibit their responses on approximately 50% of trials. The SSRT can then be calculated from this delay. For the non-tracking procedure, a number of different delays are pre-defined and utilised throughout the task, and the delay at which the participant is able to inhibit their responses on approximately 50% of trials has to be estimated from task performance.

The go/no-go task meanwhile measures "action prevention", requiring participants to prevent the initiation of a planned response. The key outcome of interest is the rate of commission errors (that is, failures to prevent initiation of the response). Stop-signal and go/no-go tasks have been shown to have both common and distinct networks of neural activation, suggesting action cancellation and action prevention engage similar but not identical functions.

#### *1.4.3.1 Acute effects of cannabis on response inhibition*

Acutely, the stop-signal task (both the tracking and non-tracking versions) has been most widely used, with most (Metrik et al., 2012; Ramaekers, Kauert, Theunissen, Toennes, & Moeller, 2008; Ramaekers et al., 2006; Ramaekers et al., 2016; Van Wel et al., 2013) but not all (Ramaekers et al., 2011) studies reporting an impairing effect of THC or cannabis on task performance, though not always on the SSRT which is the task's key measure of response inhibition. The wide number of dependent variables produced by this task can be problematic; studies typically report some combination of SSRT, accuracy on go-trials (that is, trials without a stop-signal), reaction time on go-trials, and additionally for the non-tracking version,

commission errors and omission errors. This can prevent meaningful comparison between different studies, and the option of choosing from many outcomes increases the possibility Type 1 errors. The go/no-go task has also been used to a lesser extent, though contrary to the stop-signal findings, some (Atakan et al., 2013; McDonald et al., 2003) but not all (Bhattacharyya et al., 2015) studies reported no effect of THC or cannabis on task performance (as measured by commission errors, omission errors and reaction time on go-trials).

As with verbal memory, a limited number of studies have assessed whether there is a relationship between THC dose and inhibition task performance. Ramaekers et al administered the stop-signal (non-tracking) following two high doses of cannabis (equivalent THC: 250μg/kg, 500μg/kg) or placebo to 20 occasional cannabis users (mean frequency of cannabis use= 3 days per month; aged 19-29 years; within-subjects design) (Ramaekers et al., 2006). The drug was administered via cannabis cigarettes, which were smoked. Placebo cigarettes contained only tobacco, while cannabis cigarettes contained both cannabis and tobacco.. Both active doses increased omission errors, though to a greater extent in the higher dose, suggesting a general reduction in task engagement and ability that increased with dose. Neither dose affected go-trial reaction time. The higher dose but not lower dose increased SSRT relative to placebo, suggesting impairment of response inhibition at the higher dose. Surprisingly, the lower dose but not higher dose increased commission errors, apparently contradicting the SSRT results. Relatedly, McDonald et al administered the stop-signal (tracking) and go/no-go tasks following two active THC doses (7.5mg, 15.0mg) or placebo (oral capsules) in 37 current cannabis users (mean frequency of cannabis use= 1.6 days per week; aged 18-45 years; within-subjects design) (McDonald et al., 2003). THC increased SSRT on the higher but not lower dose, though mean values of SSRT at each dose suggest a linear dose-response relationship. Neither dose affected go-trial reaction times. THC did not affect performance on the go/no-go task (i.e. no increase in commission or omission errors and no increase in go-trial reaction time).

Of particular importance, for instance to better inform drug-driving legislation, is the question of whether tolerance develops to the putative impairing effects of cannabis on response

inhibition following prolonged or heavy use. Ramaekers et al (Ramaekers et al., 2008) administered cannabis or placebo cigarettes (as described above (Ramaekers et al., 2011); active dose equivalent THC: 500μg/kg) to 12 occasional and 12 heavy cannabis users. Mean number of cannabis use exposures per year was 55 by the occasional users, and 340 by the heavy users. Performance on the stop-signal task (non-tracking) was affected by cannabis in both groups; SSRT increased and overall accuracy decreased following THC, but there was effect on go-trial reaction time. No differences on task performance were found between heavy and occasional users, suggesting a lack of tolerance to inhibitory effects of cannabis. Additionally, in a large study of 122 cannabis and cocaine users, vaporised cannabis (THC equivalent: 300μg/kg), oral cocaine, or placebo was administered in a double-blind cross-over study (Ramaekers et al., 2016). Cannabis (and cocaine) increased commission errors, relative to placebo. (While not reported in this paper, previously published results from a subset of this larger sample (n=61) reported that cannabis did not affect SSRT or reaction time on go-trials (Van Wel et al., 2013)). Similar to findings above, cannabis use history (as defined by total number of cannabis use exposures in the past 3 months) did not correlate with commission error rate, again suggesting a lack of tolerance following heavier use. However, recent cannabis use did negatively correlate with subjective intoxication and psychomotor impairment, suggesting tolerance to these effects does occur with increased cannabis use. Meanwhile, an earlier study from the same group found no impairment of SSRT or commission errors on the stop-signal task following smoked cannabis in 21 heavy (mean number of cannabis use exposures= 374 per year) cannabis users (Ramaekers et al., 2011), though no relationships between cannabis use history and task performance were explored.

The go/no-go task has been administered to a lesser extent following acute THC or cannabis, with more mixed findings. As described above, McDonald et al (2003) found no effect of oral THC on go/no-go performance. Similarly Borgwardt et al found no behavioural effect of oral THC (10mg) or CBD (600mg) relative to placebo on go/no-go performance in 15 minimal cannabis users (≤15 lifetime exposures) (Borgwardt et al., 2008). Meanwhile, Bhattacharyya et

al compared oral THC (10mg) to placebo in 36 minimal cannabis users (≤25 lifetime exposures), reporting an increase in commission errors and a decrease in go-trial reaction time following THC (Bhattacharyya et al., 2015). Similarly, Atakan et al found a trend-level increase in commission errors alongside no increase in go-trial reaction time, following oral THC (10 mg THC) relative to placebo in 21 minimal cannabis users (≤25 lifetime exposures) (Atakan et al., 2013).

In summary, THC and cannabis appear to influence stop-signal task performance; though this is not consistently reflected in impairment of SSRT. Increased commission errors alongside unaffected SSRT are difficult to interpret, possibly suggesting lack of task engagement or motivation rather than impaired response inhibition. Go-trial reaction time is typically unaffected by cannabis, suggesting that findings do not merely reflect a general slowing of responses following cannabis. Meanwhile, findings for the go/no-go task are more mixed, though methodologically these studies are more similar to each other (in terms of dose, route of administration and cannabis use history of participants). While this difference between tasks may reflect a specific impairing effect of THC on action cancellation but not action prevention, there are key differences to note between studies using each task. All studies described above utilising the go/no-go task administered THC orally, while the majority of studies assessing stop-signal performance administered THC via inhalation (smoked or vaporised). Given that route of administration has been shown to influence the time-scale and magnitude of some effects of cannabis (Chait & Zacny, 1992; Hart et al., 2002), such differences complicate comparability between task findings. Moreover, while studies of stop-signal performance have tested recreational cannabis users ranging from occasional to heavy users, studies of go/no-go performance have typically used participants with minimal cannabis exposure. Nevertheless, in the only study to date (to my knowledge) to administer both the stop-signal and go/no-go tasks (oral THC; recreational cannabis users), the authors reported no effect of drug on go/no-go performance, but increased SSRT on the stop-signal task (McDonald et al., 2003).

#### 1.4.3.1.1 Age-related findings: acute

To date, no studies have assessed the influence of age on the acute effects of cannabis or THC on response inhibition, either in humans or animals. Translational tasks to measure similar constructs to the stop-signal and go/no-go tasks i.e. response inhibition (rather than, for instance, the 5-choice serial reaction time task, which measures the ability to wait before executing a planned response (Eagle & Baunez, 2010)) have been developed for rodents in the past decade (Eagle, Bari, & Robbins, 2008)). However, the large number of sessions required to train animals on the task (for instance, see (Pattij et al., 2007)) is likely to preclude testing of adolescent rodents, given the short duration of rodent adolescence (e.g. in rats, adolescence is considered to last 15 days, from postnatal day (PND) 28 to 42, inclusive (Spear, 2000)).

To my knowledge only one study to date has assessed the effect of cannabinoids on such tasks in adult rodents (Pattij et al., 2007). Adult Wistar rats were administered the synthetic $CB_1R$ agonist WIN at three doses (0.3mg/kg, 1.0mg/kg, or 3.0mg/kg) or vehicle, and performance on the stop-signal task was measured. WIN did not affect SSRT, but did decrease the rate of correct inhibitions (i.e. increased commission error rate) at the lowest dose. WIN did not affect the number of omission errors, but did increase reaction time on go-trials (though only in the two higher doses). While replications are required, preferably with THC rather than the more potent $CB_1R$ agonists often used in preclinical work (for instance, WIN is a full agonist), this study suggests that in a rodent model, cannabinoid agonists do not impair the ability to inhibit an already initiated response.

#### *1.4.3.2 Non-acute associations between cannabis use and response inhibition*

There is little evidence that adult cannabis users have non-acute impairment of response inhibition. While a few small studies have found impaired response inhibition in cannabis users relative to controls (Lisdahl & Price, 2012; Moreno et al., 2012), most have found no group differences across a range of response inhibition tasks (Gonzalez et al., 2012; J. D. Grant et al., 2012; Gruber & Yurgelun-Todd, 2005; Hester, Nestor, & Garavan, 2009; Jutras-Aswad et al., 2012; Price et al., 2015), even in a large sample of heavy cannabis users seeking treatment, after

just one day of abstinence (Solowij et al., 2002). Indeed, such studies have either not imposed any abstinence period (J. D. Grant et al., 2012; Gruber & Yurgelun-Todd, 2005; Hester et al., 2009; Jutras-Aswad et al., 2012), or imposed short self-reported periods of abstinence ranging from less than 24 h (Gonzalez et al., 2012; Solowij et al., 2002) to seven days (Price et al., 2015).

A recent meta-analysis of stop-signal and go/no-go performance in non-intoxicated heavy and/or dependent cannabis users, found no evidence of impaired SSRT and no-signal reaction times for the stop-signal task, or impaired commission errors, omission errors, and no-signal reaction times for the go/no-go task (Smith, Mattick, Jamadar, & Iredale, 2014). Smith et al combined data from six stop-signal studies, including 136 cannabis users and 326 non-users, resulting in an effect size, g = 0.112 (95% CIs: -0.120, 0.343). Combining data from five go/no-go studies, including 144 cannabis users and 147 non-users, resulted in g = 0.004 (95% CIs: -0.230, 0.239).

Given the consistency of tasks used across studies, and the small sample size of individual studies (only three of the studies included more than 20 cannabis users), the meta-analytic method is particularly useful in this instance. However, the majority of included studies assessed adult-only populations (with one stop-signal study including those aged 16 years or older (Huddy et al., 2013), and one go/no-go study (Tapert et al., 2007) including only 16-18 year olds), so whether these findings generalise to adolescent populations is not yet clear.

#### 1.4.3.2.1 Age-related findings: non-acute

A few studies to date have assessed response inhibition in an adolescent population, with mixed findings. Behan et al administered the go/no-go task to adolescent (aged 14-19 years) cannabis users (n=17) in treatment for cannabis dependence and non-using controls (n=18; (Behan et al., 2014)). Participants were asked to not use cannabis the night before testing. Cannabis users had lower no-go trial accuracy (which indicates a higher commission error rate), with no change to omission error rate or go-trial reaction time. Dougherty et al administered a non-tracking stop-

signal task to adolescent (aged 14-17 years) cannabis users (at least 4 days per week, for a minimum of 6 months; n=45) and non-users (n=48), finding a trend-level increase in commission errors in the cannabis users (Dougherty et al., 2013). Participants were required to be abstinent for at least 18 hours, which was confirmed by decreased urinary levels of THC metabolite 11-nor-9-Carboxy-THC (THC-COOH) from the previous day. Tapert et al recruited adolescent (aged 16-18 years) cannabis users (mean frequency= 14 days per month; n=16) and non-users (n=17; (Tapert et al., 2007)). Abstinence was monitored for a period of 28 days with bi- or tri-weekly urine tests; THC-COOH levels were required to have declined at each test. Participants completed the go/no-go task after successfully completing the abstinence period. No differences in task performance were reported for accuracy or no-signal reaction times.

While it is hard to make any strong conclusions based on these few studies, a similar pattern to verbal memory findings is apparent: the longer the abstinence period, the weaker the evidence for impaired response inhibition. Furthermore, the only study reporting strong evidence of impaired response inhibition was from a sample of dependent cannabis users receiving treatment (Behan et al., 2014); as such, impairment may be related to their substance use disorder rather than a specific effect of cannabis use, or indeed may reflect cannabis withdrawal symptoms following overnight abstinence.

#### 1.4.3.2.1.1 Associations with age of cannabis use onset

While in adult cannabis users there is little evidence of non-acute impaired response inhibition, given the small number of studies to date in adolescent samples it is difficult to say whether the same is true for adolescent cannabis users.

Whether impaired response inhibition is associated with an earlier age of cannabis use onset has been explored in a number of studies, with some demonstrating that early-onset cannabis users had lower response inhibition performance than late-onsetters (Gruber et al., 2012; Sagar et al., 2015; Tamm et al., 2013), but other finding no difference between early-onset users and both late-onset groups or non-using controls (Fontes et al., 2011; Hester et al., 2009; Pope et al.,

2003). For instance, Gruber et al compared early-onset (regular use <16 years old; n=19), late-onset (regular use ≥16 years old; n=15) heavy cannabis users and non-users (n=28) on the Stroop interference task (Gruber et al., 2012). No differences were found in task performance for the late-onsetters relative to the non-user controls, but the early-onsetters made more commission errors and had lower overall accuracy than the controls. Such results remained apparent when a continuous measure of age of onset was correlated with task performance. However, the early-onset users were currently using cannabis twice as many times per week, and three times the amount (in grams) of cannabis per week, compared to the late-onsetters; these measures also correlated with errors and accuracy. Adjusted regressions were not conducted due to limits of samples size (Sagar et al., 2015), so we cannot say whether age of onset was a predictor of response inhibition independent of frequency and heaviness of use. Meanwhile, in the largest study to date, Pope et al compared current or former daily cannabis users who first used <17 years old (n=69) to those who first used ≥17 years old (n=53), and to minimal user controls (reporting 1-50 lifetime uses of cannabis; n=87) (Pope et al., 2003). After 28 days monitored abstinence, and adjusting for gender, age, ethnicity and family of origin, there were no group differences on Stroop interference performance.

Given the inconsistent findings, it is unclear whether reported associations reflect reduced response inhibition resulting from young cannabis use; greater usage in general by early-onset users; or pre-morbid lower response inhibition which increases the likelihood of cannabis use from a young age (De Wit, 2009; Nigg et al., 2006; Norman et al., 2011). Longitudinal studies are needed to better delineate these potential explanations.

### 1.4.4 Summary of chapter 1

- Non-acute human findings tend to be similar in adults and adolescents (though in general fewer studies have been conducted with adolescents, especially for response inhibition)

- The animal literature (both acute and non-acute) is limited but may suggest increased vulnerability in adolescence
- A clear knowledge gap is the lack of acute studies with anyone under the age of 18

# 2 Chapter 2. Cannabis, psychosis and mood

## 2.1 Cannabis and psychosis

Alongside transient subjective and cognitive effects, cannabis can also acutely lead to psychotic-like experiences (Sherif, Radhakrishnan, D'Souza, & Ranganathan, 2016), and non-acutely cannabis use is associated with increased risk of psychosis and psychotic disorders such as schizophrenia (Gage, Hickman, & Zammit, 2016; T. H. Moore et al., 2007).

Psychosis refers to a mental disturbance characterized by aberrant perceptions (hallucinations) and thoughts (delusions) that causes an individual to lose touch with external reality (Curran et al., 2016). Psychotic experiences are fairly common in the general population, for instance up to 10% of people report experiencing hallucinations, and they are not necessarily negative experiences (for instance, religious visions). However, psychosis can be problematic if the experiences are distressing for the individual, or if they onset acutely, as can be the case with drug-induced psychosis. Psychotic symptoms may be transient, as is typically the case with drug-induced psychoses which typically resolve once the drug is eliminated from the system, or may be more long-lasting in which case they may form part of a diagnosis for more long-lasting difficulties such as schizophrenia, schizoaffective disorder or bipolar disorder. However, psychotic symptoms (often clinically termed positive symptoms) are often only one aspect of a clinical diagnosis, for instance a diagnosis of schizophrenia may also recognise negative symptoms (such as anhedonia and difficulties with initiation), and cognitive impairments (such as working memory problems).

This leads me to a quick note on terminology. In this chapter I will use the terms psychosis and psychotic experiences interchangeably to refer to experiences described above including hallucinations and delusions, that do not necessarily represent a clinical problem for the individual. When specifically referring to acute drug effects I will interchangeably use the terms psychotic-like experiences or psychotomimetic effects. When studies refer to patient populations who have received a clinical diagnosis relating to these experiences, I will either

use the term psychotic disorder or else I will refer to the specific diagnoses the study population refers to, for instance schizophrenia or schizoaffective disorder.

Cannabis and psychosis are implicitly linked, in that many of the effects of cannabis intoxication, including those desired by users, are psychotic-like in nature. Describing the experience of cannabis intoxication, Tyler (1986) states that users report heightened perceptual awareness of music, sounds and colours, alongside insights into new meanings. This has been recognised for millennia, with ancient medical texts from China and India describing such effects, for instance the oldest known pharmacopoeia, from China 2727 BC, described psychiatric effects of cannabis (Murray, Morrison, Henquet, & Di Forti, 2007). However, concerns about potentially longer-lasting psychotic effects of cannabis have only become prominent in the scientific community since the 1970's, with case reports emerging of cannabis use leading to or exacerbating severe psychotic symptoms (Davison & Wilson, 1972; Spencer, 1970; Treffert, 1978), followed by the first longitudinal population cohort study to demonstrate associations between cannabis use and schizophrenia (Andréasson, Engström, Allebeck, & Rydberg, 1987).

In this chapter I will first describe the evidence of associations between cannabis use and psychotic disorder, before focusing on a number of cannabis use behaviours that may increase the risk of psychotic disorder. Next I will discuss the problems with determining causality from such studies, before discussing why adolescent cannabis users may be at heightened risk. Next I will describe past research investigating the acute effects of cannabis on psychotic-like symptoms and refer to any evidence relating to potential differences in the acute effects for adolescents. Finally, I will briefly discuss the more limited field of research linking cannabis use to mood problems including depression.

### 2.1.1 Associations between cannabis use and psychotic disorder

Following these early studies, decades of preclinical, epidemiological and experimental evidence now converge to support an association, and possibly a causal link, between cannabis

use and psychotic disorders (Gage et al., 2016; Murray & Di Forti, 2016; Ranganathan, Skosnik, & D'Souza, 2016). Moore and colleagues' meta-analysis of longitudinal population cohort studies estimated an odds ratio (OR) of 1.4 for any psychotic outcome (including schizophrenia, psychotic disorders, and psychotic experiences) in cannabis users compared with never users (T. H. Moore et al., 2007). This meta-analysis was recently updated to include cohort studies published since 2007, producing a similar OR of 1.5 for any psychotic outcome in cannabis users compared with never users (Gage et al., 2016).

However, given the high prevalence of cannabis use in the general population (the European Drug Report 2016 estimated that 24.8% of Europeans aged 15-64 years had used cannabis at least once in their lifetime, while around 1.0% were estimated to near-daily or daily users (*European Drug Report 2016: Trends and Developments.*, 2016; Thanki et al., 2013)), and the very low prevalence of psychotic disorder (a meta-analysis of prevalence estimates from 20 population based cohorts estimated the global lifetime morbid risk to be 0.7% (Saha, Chant, Welham, & McGrath, 2005)), it is clear that not everyone who uses cannabis develops psychosis. Indeed, only a small percentage of the cannabis using population will ever be diagnosed with a psychotic disorder, and furthermore many people who experience a psychotic disorder have never used cannabis.

Assuming a causal explanation between cannabis and psychosis (which, as I will discuss shortly, is not necessarily a sound assumption), this implies a number of things about the relationship. Firstly, this implies that there is variation in the susceptibility to cannabis-related psychosis, such that there may be specific vulnerability and resilience factors that moderate an individual's risk. For instance, genetic polymorphisms of AKT1 have been identified that are only predictive of psychosis risk in cannabis users (Di Forti et al., 2012; Morgan et al., 2016; van Winkel, van Beveren, & Simons, 2011), and there is evidence that the influence of early cannabis use on psychosis may be dependent upon having experienced childhood trauma (Harley et al., 2010; Houston, Murphy, Adamson, Stringer, & Shevlin, 2008). Secondly, this implies that there may be risky and less risky cannabis use behaviours, some of which increase

likelihood of psychotic disorder more than others. Indeed, a growing literature suggests that certain cannabis use behaviours, including the type of cannabis consumed (Di Forti et al., 2009; Morgan et al., 2012), how often you use cannabis (Di Forti et al., 2014; T. H. Moore et al., 2007) and, most importantly for this thesis, the age at which you start using cannabis (Arseneault et al., 2002; Di Forti et al., 2014), may affect risk of psychosis.

### 2.1.2 Cannabis use behaviours and psychosis

#### *2.1.2.1 Frequency and duration of use*

As described above, the risk of psychosis is estimated to be 40-50% higher in cannabis users relative to never users (Gage et al., 2016; T. H. Moore et al., 2007); however this association strengthened to approximately an 110% increased risk for heavier or more regular cannabis user groups (T. H. Moore et al., 2007).

A case-control study of patients with first-episode psychosis from South London found that the patient group were approximately six times more likely to be current daily users (OR= 6.4; adjusted for age, gender, ethnicity, level of education achieved, and employment status) relative to controls, despite finding no case-control difference in likelihood of ever having tried cannabis (Di Forti et al., 2009). Further, patients were more likely to been cannabis users for more than 5 years (versus less than 5 years; OR= 2.1; adjusted as above) than controls. Recently an additional study by Di Forti and colleagues, of psychosis patients, estimated that daily cannabis users had an earlier age of psychosis onset by 2.7 years relative to less regular users (Di Forti et al., 2014). McGrath and colleagues (2010) conducted a sibling pair analysis with data from a prospective birth cohort from Brisbane, reporting that longer duration since first cannabis use was associated with greater psychotic symptoms at age 21 (McGrath et al., 2010). Even relative to matched siblings, those with a longer duration of cannabis use had greater psychotic symptoms, increasing confidence that the association was not as a result of unmeasured confounding. It is apparent therefore that both frequency and duration of use potentially represent risk factors for both the onset of, and earlier onset of, cannabis-related psychosis.

#### *2.1.2.2 Cannabis type*

Of interest, Di Forti et al's 2009 case-control study also found that within the cannabis users, those with psychosis were considerably more likely to report being skunk-users than the non-psychotic controls (OR= 6.8), while in their 2014 study they reported that preferential skunk-users had an age of psychosis onset 3.4 years earlier than hash-users, even after adjusting for frequency of use (Di Forti et al., 2014). Furthermore, in 2015 Di Forti and colleagues reported that those who preferentially used skunk had 3 times the risk of psychosis relative to never-users, increasing to 5 times for daily skunk-use, while those who predominantly used hash-type cannabis had no increased risk relative to never-users (Di Forti et al., 2015). Perhaps therefore cannabis type affects associated risk of psychosis.

One proposed explanation for the difference in risk according to cannabis type is variation in CBD content. Skunk cannabis has a very low CBD content, while hash cannabis typically contains higher levels of CBD. Indeed CBD has been shown to have anti-psychotic properties equivalent to the licensed drug for psychosis, amisulpride, in an RCT of schizophrenia patients (Leweke et al., 2012), and has been shown to buffer against the acute psychotic-like effects of cannabis (Englund et al., 2013). Further, Morgan and colleagues (Morgan & Curran, 2008) found evidence that cannabis users with traces of CBD in their hair (indicating use of cannabis containing CBD) had lower off drug psychotic symptoms relative to those without CBD traces, a finding they later replicated in recreational but not daily cannabis users (Morgan et al., 2012).

Perhaps therefore CBD's putative anti-psychotic effects are protective against cannabis-related psychosis. However, one major confound of the non-controlled studies is that cannabis lacking CBD also typically has a lower THC content. As described previously, THC is responsible for the acute psychotomimetic effects of cannabis, and as I will discuss below, these acute effects of THC are dose-dependent. Findings linking skunk but not hash cannabis to psychotic outcomes may therefore be explained not by the CBD-content but by the higher THC potency of skunk.

#### *2.1.2.3 Tobacco and routes of administration*

Recently, focus has fallen on associations between tobacco use and psychosis, and whether this may reflect a causal relationship (Gage et al., 2014; Gurillo, Jauhar, Murray, & MacCabe, 2015). A recent meta-analysis of epidemiological studies demonstrated that daily tobacco smoking versus non-smoking was associated with a 2-fold (based only on prospective cohort studies) or 3-fold (based only on case-control studies) increase in risk of psychotic disorder (Gurillo et al., 2015). Further they found that daily tobacco smokers diagnosed with a psychotic disorder experienced their first episode approximately 1 year earlier than non-smokers, and that those with psychotic disorder started tobacco smoking at a younger age than controls.

Tobacco and cannabis use are highly correlated, both in terms of independent use and co-administration. Indeed, cannabis administration routes including tobacco were reported by the majority (65.6%) of users in the 2014 Global Drugs Survey (Hindocha, Freeman, Ferris, Lynskey, & Winstock, 2016). It has therefore been suggested that associations between cannabis and psychosis may in fact be driven by the co-morbidity of cannabis and tobacco use. Regardless of whether the association between tobacco use and psychosis is causal or not (for instance, a self-medicating hypothesis has been suggested to explain the associations between cigarette smoking and schizophrenia, given the acute cognitive enhancing effects of nicotine (Heishman et al., 2010)), this relationship may be confounding the apparent associations between cannabis and psychosis. However, tobacco use is less likely to be confounding the associations between cannabis type and psychosis, assuming that tobacco is used similarly for different types of cannabis.

While population cohorts from the UK and much of Europe are inherently problematic in that cannabis and tobacco are commonly co-administered, cannabis users in the USA are much less likely to use routes of cannabis administration including tobacco (Hindocha et al., 2016). This results in much lower co-linearity between cannabis and tobacco use in the USA. In the coming years data from cohorts from countries such as the USA will hopefully be able to better answer the question of whether cannabis-psychosis links are being driven by tobacco use. Relatedly, in chapter 3 I will report evidence of a potentially similar confounding effect of tobacco use on the

associations between cannabis use and IQ and educational outcomes in the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort.

#### *2.1.2.4 Age*

In the section above we have therefore seen that the risk of psychosis varies according to different cannabis use behaviours; but most relevant to this thesis, does the age of cannabis use influence risk of psychosis?

Despite the common concern that starting cannabis use from a younger age may result in an increased risk of psychotic disorder, few studies have directly addressed this question (Murray et al., 2007). Relatedly however, there is evidence to suggest that, amongst psychosis patients, cannabis users typically have an earlier age of psychosis onset than non-users. Indeed, a meta-analysis of 131 studies, comprising 22,519 psychosis patients, found that substance using patients (but not alcohol drinkers) had a younger age of psychosis onset than non-users (2.0 years earlier), with the largest effect size for cannabis-specific samples (2.7 years earlier) (Large, Sharma, Compton, Slade, & Nielssen, 2011).

Meanwhile, whether younger age of cannabis use increases risk of psychosis is less known, with few studies reporting such analyses. A number of studies have demonstrated that cannabis use is associated with psychosis outcomes measured in adolescence (for instance, at age 15-16 years (Miettunen et al., 2008), and age 18 years (Fergusson, Horwood, & Swain-Campbell, 2003; Gage et al., 2014)), however few have directly compared the risk of psychosis associated with younger versus older age of cannabis use. Indeed, while there have been 10 longitudinal population cohort studies assessing the association between cannabis use and psychosis (Gage et al., 2016), only two of these have stratified risk of psychotic disorder by age of first cannabis use. In the Swedish conscripts study (Zammit, Allebeck, Andreasson, Lundberg, & Lewis, 2002), there was no increased schizophrenia risk in those who first used cannabis before age 16 years, compared to those who started after reaching age 16 ((Zammit, 2004) as described in (T. H. Moore et al., 2007)). Meanwhile in the Dunedin cohort, those who reported having used

cannabis at least 3 times by age 15, as opposed to by age 18, had a greater likelihood of psychotic symptoms and schizophreniform disorder in their mid-20's (Arseneault et al., 2002). However, those who started using at a younger age also had a longer duration of use. As described above, longer duration of cannabis use is also associated with an increased risk of psychosis, so this finding may be driven by the longer duration of use in those who started using from a younger age.

In the 2009 Di Forti study discussed above, after adjustment for age, gender, ethnicity, other stimulant use, level of education achieved and employment status, using cannabis for the first time before, as opposed to after, 17 years old did not increase likelihood of developing psychosis (Di Forti et al., 2009). However, their 2014 study (again described above) found that having tried cannabis before, as opposed to after, age 15 years predicted an earlier onset of psychosis (median onset age= 26.9 years vs. 27.8 years), even after adjusting for gender and importantly duration of use (hazard ratio (HR)= 1.36; 95% CIs: 1.04, 1.80) (Di Forti et al., 2014). Although, those who had used cannabis before age 15 also reported more frequent use and greater preference for skunk cannabis, and after further adjusting for type of cannabis used and frequency of use, the association between age of cannabis onset and age of psychosis onset was attenuated and became non-significant (HR= 1.18; 95% CIs: 0.81, 1.73). This potentially suggests that the link between age of cannabis onset and psychosis onset may be confounded by other factors, including frequency of cannabis use and type of cannabis used.

Further evidence of the importance of cannabis age of onset comes from a cross-sectional survey of a representative population sample, investigating the association between adolescent cannabis use and psychotic experiences (Stefanis et al., 2004). The authors reported greater psychotic experiences (and negative symptoms) for those who had used cannabis relative to never-users, with evidence of a dose-response relationship in that greater previous cannabis use predicted greater psychotic experiences. Importantly, analyses were then split by age of cannabis use onset. Those who had started using cannabis before, as opposed to after, reaching age 16 years, reported greater psychotic experiences at age 19 years, even after adjustment for

previous cannabis use. However, the categorisation of past cannabis use is difficult to interpret, as lower usage was indexed by cumulative uses (never, once, 2-4 times, 5 times or more) while the top-category was indexed by frequency (daily or almost daily use). Given the likely huge variation in the number of past cumulative uses of those who answered 5 times or more, adjustment for this variable when assessing the impact of age of cannabis onset is not convincing evidence against the suggestion that, as discussed above, greater frequency of use or longer duration of use may account for such age-related findings.

There is, therefore, limited evidence to date linking younger age of cannabis use onset with an increased risk of psychosis. The evidence is far from comprehensive and not without limitations, precluding conclusions in either direction. Importantly, a lack of relevant studies does not necessarily mean a lack of effect.

### 2.1.3 Problems with determining causality

The majority of studies I have described so far in this chapter cannot provide evidence of causation. Observational studies, including population cohort and case-control designs are difficult to interpret causally since groups are self-selecting samples; there are likely to be many unmeasured confounds that differ between those who choose to be daily or weekly cannabis users, or those who choose to be skunk or hash users. For instance, the finding that preference for skunk cannabis and greater frequency of use were both associated with younger age of onset (Di Forti et al., 2014) suggests that using from a younger age does not occur independently of other putatively riskier cannabis use behaviours. It is therefore important to recognise that often different indicators of 'heavier' cannabis use behaviours will be correlated, making it difficult to separate the individual contribution of one behaviour over another in terms of associated risk, even when using stratification or statistical covariate adjustment. Randomised controlled trials are the only method of truly identifying a causal relationship, however clear ethical considerations preclude the use of human RCTs to assess the effects of repeated cannabinoid

exposure. In the next section however I will discuss the use of RCTs using acute cannabinoid administration to investigate the links between cannabis and psychosis.

### 2.1.4 Cannabis, psychosis, and adolescent brain development

As described in chapter 1 section 1.3, it is likely that the eCB system plays a role in adolescent maturational development, given the system's role in gestational and early life neurodevelopment, alongside evidence of functional development of the system during the adolescent period. As described in chapter 1, cannabis use is associated with eCB system alterations, including altered eCB levels in cerebrospinal fluid (Morgan et al., 2013) and downregulated cortical $CB_1Rs$ (D'Souza et al., 2016; Hirvonen et al., 2012) suggesting that cannabis consumption during adolescence may impact on eCB functioning and in turn on typical maturational processes. Importantly, psychotic disorders such as schizophrenia are increasingly thought of as complex neurodevelopmental disorders resulting in disrupted brain connectivity and altered circuitry, potentially implicating a role of adolescent cannabis exposure in its aetiology.

As discussed earlier however, very few cannabis users, even those with adolescent onset of cannabis use, go on to develop psychotic disorder. As such it is likely that these potential mechanisms only lead to considerable maturational disruption in certain vulnerable individuals. As discussed previously, genetic x environment interactions are likely to increase vulnerability to psychosis (Henquet, Di Forti, Morrison, Kuepper, & Murray, 2008). The two-hit hypothesis of schizophrenia argues that an early life "first hit" (for instance, specific genetic polymorphisms or an early developmental insult) results in vulnerability to psychosis, however does not lead to symptoms unless a later life "second hit" (for instance, a serious adverse event or cannabis exposure) occurs, thus leading to psychotic disorder (Bayer, Falkai, & Maier, 1999; Maynard, Sikich, Lieberman, & LaMantia, 2001).

Whether there are genetic vulnerabilities that specifically interact with adolescent onset of cannabis use (as opposed to cannabis use at all ages) is not clear. Caspi and colleagues found

evidence that a functional polymorphism at Val158Met in the catechol-O-methyltransferase (COMT) gene moderated the influence of adolescent- but not adult-onset cannabis use on risk of developing psychosis in adulthood (Caspi et al., 2005). Carriers of the Val allele, but not carriers of two Met alleles, were more likely to develop psychotic symptoms and schizophreniform disorder in adulthood if they were adolescent-onset cannabis users. However, a subsequent study failed to replicate this exciting finding in patients with schizophrenia, finding no association between the Val158Met COMT polymorphism and cannabis use, even when considering only those who started using cannabis before the age of 18 (Zammit et al., 2007).

### 2.1.5 Acute psychotomimetic effects of cannabis

#### *2.1.5.1 Experimental psychopharmacology*

Given the difficulties of addressing causal hypotheses with the epidemiological (and naturalistic (Morgan et al., 2010)) work discussed above, placebo-controlled experimental psychopharmacology studies- directly assessing the acute psychotomimetic effects of the drug in humans- can make a major contribution to the field (Sherif et al., 2016).

The transient acute effects of a drug and any longer lasting non-acute effects of repeated use do not necessarily equate. Indeed, there are many instances in psychopharmacology of the acute effects of a substance directly opposing the non-acute effects seen as a result of repeated use, for instance as described above, repeated cannabinoid administration in rats reduces presynaptic dopaminergic function (Ginovart et al., 2012), however acute administration increases dopamine release (French, Dillon, & Wu, 1997). Nevertheless, there are a number of strengths of the experimental method in the case of cannabis and psychosis (Sherif et al., 2016), as I will discuss below, followed by some limitations.

Acute administration studies are able to directly address causality as drug or placebo are administered and effects compared. When such studies are repeated measures, such that the

same participant receives placebo and drug on separate occasions, we can make strong conclusions about the acute effects of the drug. Furthermore, such protocols are assessing drug effects in the moment and are therefore are not subject to memory problems and biases that can influence retrospective survey responses. Acute administration studies also allow dose-response relationships to be quantified and defined with much greater degree of accuracy than epidemiological studies, since dosing, cannabinoids and route of administration are controlled. Importantly, controlled administration studies can also be designed reactively to promptly answer new questions that arise in the field, and thus allow testing of very specific hypotheses. This is in contrast to many epidemiological studies, in particular birth cohort studies, which can take decades from the point of planning to being able to assess outcomes.

Nevertheless, acute administration studies to explore links between cannabis and psychosis have limitations. While the acute psychotomimetic effects of cannabis indeed appear to reflect some aspects of psychosis, psychotic disorders such as schizophrenia are complex; as described above they are thought to be influenced by a complicated interplay of genetic vulnerability, atypical early and adolescent neurodevelopment, and negative environmental exposures. It is therefore simplistic to assume that a single dose of a partial or full cannabinoid agonist can fully replicate such a disorder, however drug models can aid in the understanding of the aetiology of psychosis and help to guide treatment innovation. Indeed, the National Institute of Mental Health (NIMH, USA) has recently implemented the Research Domain Criteria (RDoC) initiative. The RDoC aims to shift the focus of mental health research away from diagnostic categories (for which aetiological explanations are likely to be complex, varied, and resulting from many inter-related mechanisms) and more towards understanding the basic symptoms and mechanisms that underlie the full range of human behaviour. Previous research has attempted to identify the mechanisms by which cannabis induces specific psychotic-like symptoms, for instance for paranoia (D. Freeman et al., 2014) and delusion-formation (Corlett, Honey, & Fletcher, 2007). Such findings can clearly contribute to our understanding of how such symptom form in clinical populations. In chapters 5 and 6 I will return to this, describing two studies in which I attempt to

better describe the specific psychotic-like and mood-related symptoms induced by cannabis, assessing whether acute cannabis may provide a pharmacological model of auditory-verbal hallucinations (AVH; chapter 5) and anhedonia (chapter 6).

Further, acute administration studies often intentionally exclude participants with a history or risk of psychotic disorder, to reduce risk of participation. This has the effect of biasing samples away from those who may be more vulnerable to psychotic effects of cannabis and thus may underestimate any negative effects. On the other hand, this is also an interesting population to study, given that the majority of people who use cannabis do not experience non-acute psychotic experiences. These samples can thus contribute to addressing questions about whether cannabis alone can contribute to psychosis in the absence of other known risk factors for psychosis. Relatedly, given that the acute psychotomimetic effects of cannabis have been shown to be magnified in psychosis patients and those at risk of psychosis (D'Souza et al., 2005), suggesting that the degree of acute psychotomimetic effects of cannabis are related to psychosis vulnerability, such studies can also aid in the identification of potential risk factors and directly address questions about whether certain cannabis use behaviours (for instance, using CBD-rich versus CBD-lacking cannabis) are more prone to lead to psychotomimetic effects than others.

#### *2.1.5.2 Previous findings*

A large number of human experimental psychopharmacological studies have assessed the psychotomimetic effects of cannabis, with a number of key findings. Firstly, cannabis, or isolated or synthetic cannabinoids with $CB_1R$ agonist properties (for instance THC, nabilone), administered orally, intravenously, and via inhalation, reliably increase psychotic-like symptoms, as indexed by both clinician-rated (for instance, the Brief Psychiatric Rating Scale (Tool, 1988) and Clinician Administered Dissociative States Scale (Bremner et al., 1998)) and self-rated (for instance, the Psychotomimetic States Inventory (Mason, Morgan, Stefanovic, & Curran, 2008) and visual analogue scales) measures (e.g. (D'Souza et al., 2004; Mason et al., 2008; Wesnes et al., 2009)). Secondly, induced psychotomimetic symptoms are dose-

dependent; higher doses lead to greater effects (Sherif et al., 2016). Thirdly, induced psychotomimetic symptoms are transient, typically fading to baseline levels within hours of administration, likely dependent on route (Chait & Zacny, 1992; Ohlsson et al., 1981). Fourthly, $CB_1R$ agonists transiently exacerbate psychotic symptoms in schizophrenia patients, and such patients are more sensitive to many of the cognitive, behavioural and mood effects of cannabis (D'Souza et al., 2005; Henquet et al., 2010).

There is also some evidence to suggest that participants with higher baseline psychosis-related traits, as indexed by high scores on measures of schizotypy, have heightened psychotic-like reactions to cannabis (Barkus & Lewis, 2008; Barkus, Stirling, Hopkins, & Lewis, 2006; Mason et al., 2009). Importantly this suggests a link between psychosis vulnerability and the acute effects of cannabis on transient psychotic-like symptoms.

Recently it was found that an oral dose of CBD prior to IV THC administration blunted the psychotomimetic effects of THC (Englund et al., 2013), suggesting that using cannabis containing CBD may reduce the psychotomimetic effects of cannabis. Subsequently Morgan et al found no effect of CBD-content on psychotomimetic experiences in cannabis users smoking their own cannabis (Morgan et al., 2010), however to date no controlled study has compared the effects CBD-rich and CBD-lacking cannabis.

In summary, acutely, cannabis transiently and dose-dependently induces psychotic-like symptoms in both healthy controls and psychosis patients, thus providing an experimental model of cannabis-induced psychosis that can help to answer specific causal questions regarding the link between cannabis and psychotic experiences.

#### *2.1.5.3 Age-related findings: acute*

Despite the large number of studies assessing the acute psychotomimetic effects of cannabinoids in humans, none have reported whether age of the participant affects such experiences. As described in chapter 1, there is a mixed and limited preclinical body of work demonstrating differences between adolescent and adult rodent behavioural responses to

cannabinoids. A number of animal models of putative psychotic mechanisms have been developed, though I have not referred to them here as to my knowledge there have been no comparisons of the acute effects of cannabinoids between adolescent and adult animals using these models. As covered above, there is some human observational evidence to suggest adolescent cannabis use increases risk of psychosis and/or reduces the age of psychosis onset, however these findings have yet to be explored with an acute administration model.

## 2.2 Cannabis, mood and anxiety

In this chapter I have focused primarily on the links between cannabis and psychosis, reflecting the large amount of research in this area. Nevertheless, cannabis use has also been linked to a number of other mental health issues, including depression and anxiety.

### 2.2.1 Associations between cannabis use and depression and anxiety

Epidemiological evidence indicates a possible association between regular cannabis use and the development of anxiety and depression. However, the evidence is more mixed and less consistent than that for an association between cannabis use and psychosis (T. H. Moore et al., 2007). One recent study compared the mental health of individuals who were addicted to cannabis (according to the DSM-IV) with that of non-addicted cannabis users who had similar patterns of cannabis use, finding that only the addicted users had depression and anxiety problems (Pol et al., 2013). Compared with the general population, non-addicted frequent users were more likely to show externalizing disorders (such as attention-deficit hyperactivity disorder), which were likely to have predated their cannabis use. Otherwise, these individuals were similar in terms of mental health to the general population, suggesting that cannabis contributes to mental health problems only in those who are vulnerable for other reasons. Depression and anxiety disorders not only are associated with cannabis addiction (Degenhardt, Hall, & Lynskey, 2003b) but also are predictive of whether individuals transition from use to addiction (Flórez-Salamanca et al., 2013).

### 2.2.1 Does adolescent cannabis use lead to increased risk of depression and anxiety?

In the ALSPAC birth cohort, Gage et al demonstrated that self-reported cannabis use by age 16 was associated with an increased risk of depression (but not anxiety disorder), that persisted after adjusting pre-birth and childhood confounders (Gage et al., 2015). In a sample of young (16–24-year-old) daily cannabis users, Morgan et al found that levels of THC in hair were significantly associated with self-reported levels of both depression and anxiety (Morgan et al., 2012). Others however have found no consistent associations between adolescent cannabis use and depression at the age of 29 years (Degenhardt et al., 2003b), suggesting that increases in self-reported depression in young cannabis users may not be long-lasting. By contrast, the same study showed that daily cannabis use and cannabis addiction in early adulthood were associated with more than double the non-user control rate of anxiety disorders at 29 years of age.

Of particular relevance to this thesis, in an Australian birth cohort, Hayatbakhsh et al assessed the associations between early- and late-onset cannabis use and depression and anxiety symptoms at age 21 (Hayatbakhsh et al., 2007). Following adjustment for many potential confounders (including cigarette use, alcohol use, adolescent depression and anxiety symptoms) both early- (<15 years) and late-onset (≥15 years) cannabis use predicted future symptomology in frequent cannabis users (but not occasional; at age 21), and the effect size was of greater magnitude for the early-onset cannabis users. Such findings suggest that younger age of cannabis use may confer heightened risk of depression and anxiety, however since this effect was found only in currently frequent users, whether depression and anxiety symptoms would persist following abstinence is not known.

### 2.2.2 Role of the endocannabinoid system in depression and anxiety

Strikingly, a high number of cases of depression and anxiety disorders were reported among obese individuals who were treated with the anti-obesity drug rimonabant, a $CB_1R$ antagonist. Many of these individuals had no prior history of these disorders (Christensen, Kristensen, Bartels, Bliddal, & Astrup, 2007; Nissen et al., 2008), and so this led to the withdrawal of

rimonabant from therapeutic use. These findings suggest that $CB_1R$ antagonists increase the risk of depression and/or anxiety. Moreover, preclinical studies have shown that mice that genetically lack $CB_1Rs$ show increased depressive-like symptoms (Valverde & Torrens, 2012) and, in wild-type mice, CBD has antidepressant effects (Zanelati, Biojone, Moreira, Guimaraes, & Joca, 2010). Rodent studies have implicated the eCB system in the regulation of emotion (Moreira & Wotjak, 2010). Similarly, there are also data from rodent studies suggesting that impaired $CB_1R$ signalling leads to depression-like symptoms, and that enhancement of $CB_1R$ signalling produces antidepressant-like behavioural effects in rodents (Sidhpura & Parsons, 2011). Interestingly, such findings potentially lead to the hypothesis that THC, a $CB_1R$ agonist, would not lead to increased depressive symptoms and may even have anti-depressant effects.

### 2.2.3 Acute effects of cannabis on depression and anxiety symptoms

#### *2.2.3.1 Anxiety*

Controlled administration studies of the acute effects in of THC in humans have shown that THC increases anxiety (D'Souza et al., 2004), whereas CBD decreases it (Bergamaschi et al., 2011). This is interesting, because there is also evidence that cannabis is often 'used' to self-medicate social anxiety in vulnerable individuals (Buckner & Carroll, 2010; Van Dam, Bedi, & Earleywine, 2012), and in a longitudinal study Buckner et al found that social anxiety disorder at baseline predicted future cannabis dependence (Buckner et al., 2008). While $CB_1R$ agonists lead to anxiety-related behaviours in adult rats, intriguingly there is some evidence to suggest that THC has less anxiogenic (Schramm-Sapyta et al., 2007) or even anxiolytic (Acheson et al., 2011) effects in adolescent rats.

#### *2.2.3.2 Depression*

The acute effect of cannabis on general depressive symptoms have been little explored, though a number of studies have demonstrated increased self-rated anhedonia following acute cannabis administration (Mason et al., 2008; Stokes, Mehta, Curran, Breen, & Grasby, 2009). Anhedonia

is a cross-diagnostic symptom, experienced by patients with schizophrenia, depression and substance use disorders. Non-acutely a number of studies have also reported associations between cannabis use and anhedonia, as indexed by self- and clinician-rated scales (Bovasso, 2001; Dawes, Sitharthan, Conigrave, Phung, & Weltman, 2011; Dorard, Berthoz, Phan, Corcos, & Bungener, 2008) (but also see: (Johnson, Bonn-Miller, Leyro, & Zvolensky, 2009)).

As discussed above regarding psychosis, given the potential utility of pharmacological models in determining the aetiology and development of novel treatments for specific mental health symptoms, better identifying the specific mood-related symptoms induced by cannabis is key. While some evidence demonstrates increased anhedonia following cannabis acutely, and non-acute associations between cannabis use and anhedonia and depression, other evidence suggests that THC may have anti-depressant effects. In chapter 6 I will therefore address this discrepancy by administering a number of measures of anhedonia following cannabis administration in both adolescents and adults.

## 2.3 Summary of chapter 2

- There is considerable epidemiological evidence linking repeated cannabis use to psychotic disorder, and it is clear that cannabis acutely causes psychotic-like symptoms in healthy controls, regular cannabis users and patients with schizophrenia
- There is limited evidence of an association between younger age of cannabis use and increased psychosis risk, though few have actually addressed this question
- While one promising genetic finding may have isolated an adolescent-onset specific relationship between COMT and risk of psychotic disorder, this finding was not replicated in a subsequent study
- Cannabis use is often found to be associated with depression and anxiety, though findings are mixed. Some evidence suggests that earlier onset of cannabis use may confer greater risk.
- A clear knowledge gap is the lack of acute studies with anyone under the age of 18

## 2.4 Summary of chapters 1 and 2

In the first two chapters of my thesis I have demonstrated the often limited evidence to suggest that adolescent relative to adult cannabis use may increase vulnerability to related harms. While such assertions are often made throughout the literature, the evidence to support a link between younger age of use and worse cognitive and psychosis outcomes is currently lacking.

There are many methodological issues which are similar in the adult and adolescent research literature. As I have reviewed, these include small sample sizes, failure to control for confounders, poor control groups (for instance, groups not being matched for alcohol and other drug use); lack of consistent reporting of tasks dependent variables; difficulties quantifying cannabis use; lack of consistency between studies regarding cannabis use groups (for instance, studies often define frequent and infrequent cannabis use differently); and a lack of biological verification of cannabis use to identify specific cannabinoids used and to confirm abstinence.

In summary, to better address the question of whether adolescent cannabis use indeed has greater potential for harm than adult use, direct comparisons between adolescent and adult cannabis users are clearly needed for studies assessing both the acute and non-acute effects of cannabis. Moreover, acute studies with adolescent participants are clearly required.

### 2.4.1 Research questions

Following on from the literature described in chapters 1 and 2, my thesis set out to address the following question:

Does adolescent cannabis use have greater potential for harm than adult use?

To answer this, I designed a series of five studies to address the following specific research questions:

1. Are IQ and educational outcomes in teenagers related to their cannabis use?

2. Are adolescents more vulnerable to the acute subjective, physiological, memory and inhibition effects of cannabis than adults?
3. Does cannabis increase the incidence of auditory-verbal hallucinations (AVH)? Are adolescents more vulnerable to the psychotomimetic effects, including AVH, of cannabis than adults?
4. Do higher levels of CBD in cannabis offset the psychotomimetic effects, including AVH, of cannabis in adults?
5. Does cannabis increase anhedonia, and are adolescents more vulnerable than adults to these effects?

In the final chapter I will then overview and integrate the evidence gathered in these empirical studies.

# 3 Chapter 3. Are IQ and educational outcomes in teenagers related to their cannabis use? A prospective cohort study

## 3.1 Introduction

As described in chapter 1, acutely cannabis induces robust and dose-dependent episodic memory impairments (Ranganathan & D'souza, 2006), with more mixed reports of impaired working memory, response inhibition, attention, psychomotor control and abstract reasoning (Crane et al., 2013; Crean, Crane, & Mason, 2011; Gonzalez, 2007). More debated is whether there are non-acute cognitive effects of using cannabis. Although, as described in chapter 1, associations between cannabis use and non-acute cognitive impairments have been reported across many domains including memory and response inhibition, many studies have been cross-sectional and therefore cannot exclude the possibility of pre-existing group differences in cognitive ability. However, case–control and prospective cohort studies have found associations between cannabis use and both lower IQ and lower educational attainment, suggesting more global impairments to intellectual and educational functioning.

### 3.1.1 Does cannabis use affect IQ?

To date, there have been three longitudinal investigations that have assessed the relationship between cannabis use and IQ. One small-scale study of young adults (N=113, including 59 never-users) (Fried, Watkinson, & Gray, 2005) found evidence of lower IQ in current but not former cannabis users, similar to the findings of Schreiner and Dunn's meta-analysis described in chapter 1 (Schreiner & Dunn, 2012). The meta-analysis found robust evidence of lower cognitive performance across many domains when they included all eligible studies, but following subsequent exclusion of all samples with fewer than 25 days of abstinence, they found no evidence of impairments in any domain.

In a New Zealand birth cohort study of 1,037 38-year-old individuals born in 1972 or 1973, persistent cannabis dependence was associated with a decline of up to 6 IQ points from that

measured at the age of 7–13 years (Meier et al., 2012). Of particular interest for this thesis, the decline was particularly evident for those who developed cannabis dependence in adolescence, and remained apparent even for those who, by the age of 38, used cannabis less than once a week. Indeed, amongst those cannabis users who were diagnosed with cannabis dependence on at least two of the five waves in adulthood, there was no evidence of IQ decline in the adult-onset (>17 years of age) users but an apparent drop of up to 8 IQ points in those with adolescent-onset (≤17 years of age). To exclude possible confounders, the authors ran a series of stratification analyses, in which they serially excluded participants with past-week cannabis use, a lifetime diagnosis of schizophrenia, and persistent diagnoses of tobacco, alcohol, or other illicit drug dependence throughout adulthood, finding that the associations with cannabis dependence remained. However, no adjustment was made for adolescent use of other substances or other highly relevant factors such as depression or socioeconomic status (Rogeberg, 2013). Furthermore, their findings do not allow separation of a potentially negative effect of cannabis dependence, rather than cannabis use per se.

By contrast, a recent US prospective cohort study of 3,066 17–20-year-old individuals found no difference in IQ from that measured at the age of 9–12 years between monozygotic (MZ) and dizygotic (DZ) twins discordant for cannabis use (Jackson et al., 2016). Twin studies are theoretically able isolate the role of substance use in predicting outcomes, by controlling for familial factors (both genetic & environmental) shared by twins discordant for substance use. Twins (both MZ and DZ) are assumed to share 100% of their familial environmental and either 50% (DZ) or 100% (MZ) of their genes. If cannabis use was causally linked to IQ decline, we would expect lower IQ in a twin that uses cannabis relative to their twin pair who does not. Meanwhile if cannabis use is not causally linked to IQ decline, but is associated with lower IQ due to common risk factors that contribute to both cannabis use and poorer intellectual functioning, we would expect no difference between twin pairs discordant for cannabis use, as indeed Jackson et al found. Such findings therefore suggest that lower IQ in cannabis users may result from overlapping familial risk factors rather than as a direct result of cannabis exposure.

This study therefore suggests that confounding by overlapping risk factors may explain the findings of Meier et al (2012), however the younger age of IQ measurement and moderate degree of cannabis exposure in Jackson et al must be noted. Moreover, the study included only 47 discordant twin pairs in which the cannabis-using twin had used cannabis frequently (more than 30 cumulative uses, and/or daily use), warranting caution against making strong conclusions.

### 3.1.2 Does cannabis use affect educational attainment?

Relatedly there is a wide evidence base linking adolescent cannabis use to early school leaving and poorer educational performance (Fergusson, Horwood, & Beautrais, 2003; Lynskey, Coffey, Degenhardt, Carlin, & Patton, 2003; Lynskey & Hall, 2000; McCaffrey, Liccardo Pacula, Han, & Ellickson, 2010; Silins et al., 2014; Stiby et al., 2014). Typically, these associations are robust to adjustment for potential confounds. However, the mechanisms leading to these associations remain hotly debated.

Causal explanations have posited that heavy cannabis use results in cognitive and/or motivational deficits, which in turn result in poorer educational attainment. Indeed, there are many anecdotes about an 'amotivational syndrome' resulting from heavy cannabis use, and a recent positron emission tomography study demonstrated that cannabis users had reductions in striatal dopamine synthesis that correlated with a measure of amotivation (M. A. Bloomfield, Morgan, Kapur, Curran, & Howes, 2014). A recent study further demonstrated that acutely cannabis produced motivational deficits, such that following cannabis exposure participants were less willing to exert effort for a monetary reward (Lawn, Freeman, Pope, Joye, Harvey, Hindocha, Mokrysz, Moss, Wall, Bloomfield, et al., 2016). However, the same paper also found no evidence of reduced reward sensitivity in dependent cannabis users relative to healthy controls, in conflict to the notion of an 'amotivational syndrome' attributable to cannabis.

Alternatively, reverse causality has been also suggested; that is, perhaps poorer educational attainment in fact leads to cannabis use (Fergusson, Horwood, & Beautrais, 2003; Lynskey &

Hall, 2000). While compelling as an argument, the one study to my knowledge that addressed this hypothesis showed that the association between early school leaving and later cannabis use could be accounted for by use of cannabis before leaving school (Fergusson, Horwood, & Beautrais, 2003). Future studies should test this hypothesis further, as reverse causality cannot be fully discounted on the evidence of this one study alone.

The other alternative is that educational attainment and cannabis use may not be causally related but instead share common risk factors (Lynskey & Hall, 2000; McCaffrey et al., 2010; Verweij, Huizink, Agrawal, Martin, & Lynskey, 2013). Reported associations between cannabis use and lower educational attainment have typically been robust to adjustment for some potential confounders such as early-life factors, baseline school performance or cognitive ability, social disadvantage and parental educational achievement (Silins et al., 2014; Townsend, Flisher, & King, 2007). However, the potential role of teenage behaviours that typically occur alongside cannabis use – including use of other substances and other 'risky' behaviours such as truancy – remain relatively unexplored (Mokrysz et al., 2016; Verweij et al., 2013). Indeed, recent analyses showed that adjusting for teenage use of other substances attenuated the association between cannabis use and school attainment (Hooper, Woolley, & De Bellis, 2014; Stiby et al., 2014). Furthermore, the common risk factors explanation is strongly supported by recent genetic studies that found no difference in early school leaving (Verweij et al., 2013) or years of education (J. D. Grant et al., 2012) between both MZ and DZ twin pairs discordant for cannabis use (J. D. Grant et al., 2012; Verweij et al., 2013).

### 3.1.3 Strengths and limitations of the literature to date

Many of the studies described above utilised large prospective cohort samples. These have a number of strengths, including the ability to ascertain temporal sequence of exposure and outcome and to adjust for functioning prior to cannabis exposure. The use of general population representative samples also reduces concerns regarding sampling bias, for instance the recruitment of undergraduate samples for a control group likely results in particularly high

functioning participants to compare cannabis users to. Nevertheless, determining causality is still challenging in prospective cohort studies since we cannot assume that cannabis users and non-users would have developed along similar trajectories if cannabis use had not occurred (Rogeberg, 2013). Adolescents who use cannabis regularly also tend to have higher rates of social adversity (von Sydow et al., 2002), early-onset behavioural problems (Heron et al., 2013) and other adolescent substance use (Hibell et al., 2012), all of which may confound the relationships between cannabis use and poorer intellectual and educational outcomes.

### 3.1.4 Study aims and hypotheses

In this chapter I examined the associations between adolescent cannabis use and both IQ and educational attainment within a large adolescent cohort sample. Assessing intellectual and educational outcomes in the same longitudinal cohort, with similar confounder adjustment, enables better integration of findings across both domains. I considered several factors commonly associated with teenage cannabis use that may account for previously reported associations with IQ and educational performance. In particular, I addressed the role of other drug use, using detailed measures of cigarette, alcohol and other recreational drug use. In accordance with previous research, I hypothesised that cannabis use would be associated with both IQ and educational performance (Meier et al., 2012; Silins et al., 2014), but that these associations would be attenuated after adjusting for potential confounders (Lynskey & Hall, 2000; Rogeberg, 2013; Verweij et al., 2013).

## 3.2 Methods

### 3.2.1 Design and Participants

Participants were members of the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort, a prospective study in Bristol (UK) following women and their children since pregnancy. ALSPAC recruited pregnant mothers from the former Avon Health Authority with an expected delivery date from April 1991 to December 1992. The core cohort comprised 14541

pregnancies, with 13988 babies alive at one year (Boyd et al., 2012). Following further recruitment of eligible cases, the cohort now comprises 15458 foetuses, with 14701 babies alive at one year. IQ scores at both 8 and 15 years of age were available for 4621 participants. Of these, and after exclusions (n= 41: mother reported child head injury resulting in unconsciousness, n= 38; child indicated use of imaginary drug 'spanglers' (a fictional drug included in the questionnaire to test veracity of participants' responses) since 15th birthday, n= 3), 2235 individuals had complete data for all key variables and confounders, and so were included in the main analyses (complete-case sample; Figure 3.1.). The study website contains details of all available data through a fully searchable data dictionary (http://www.bris.ac.uk/alspac/researchers/devata-access/data-dictionary). Ethical approval was obtained from ALSPAC Law and Ethics Committee.

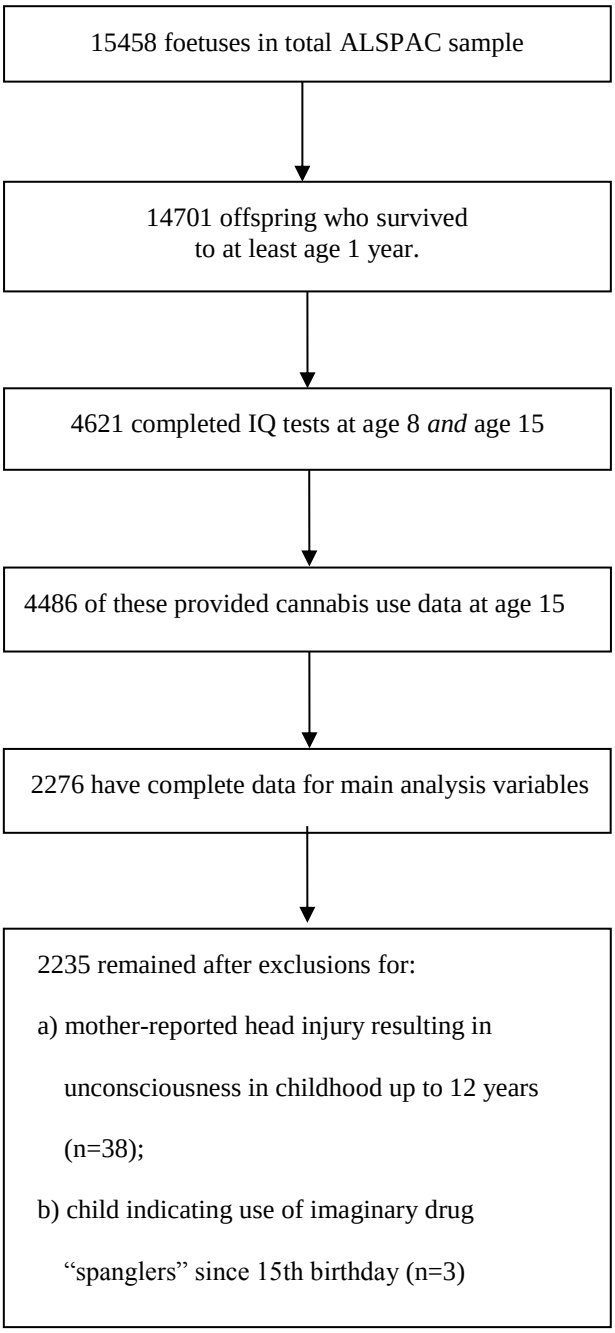


***Figure 3.1.*** *Study participant flow diagram.*

### 3.2.2 Measures

#### *3.2.2.1 Cannabis use*

Participants provided cumulative lifetime cannabis use data at the age of 15, via a self-report questionnaire administered during attendance at clinic sessions. Initial responses were categorical, with six levels: 'never', 'less than 5 times', '5–19 times', '20–49 times', '50–99 times' and '100 times or more'. For the present study, sample size considerations resulted in the two highest levels being combined into one response level of '50 times or more', creating a five-level categorical variable of cumulative cannabis use.

#### *3.2.2.2 IQ*

Participants were administered the Wechsler Intelligence Scale for Children 3rd Edition (WISC-III) (Wechsler, 1991) at an individual clinic session at the age of 8 years. Alternate items of the WISC-III were administered for all sections, apart from the coding subtest for which all items were included. IQ measurements were calculated for each individual, adjusting for age. At the age of 15 years participants were administered the Vocabulary and Matrix Reasoning subsections of the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999). IQ was again calculated for each individual, adjusting for age. To ease interpretation, IQ scores were rescaled around the complete-case sample included in the present analysis, to a mean of 100 and standard deviation of 15.

#### *3.2.2.3 Education*

In England, children attending state-maintained schools are educated in line with the National Curriculum, which defines what subjects must be taught and the standards children should reach at each stage. The Curriculum is split into a series of 'Key Stages', which are assessed by compulsory teacher assessments or national tests at the end of each stage (for further information, see www.gov.uk/national-curriculum/overview). Data linkage between ALSPAC and the National Pupil Database (a central repository for pupil-level educational data in

England) provided educational assessment data for participants who attended state-funded schools at Key Stages 2 (age 11) and 4 (age 16). Data linkage was performed by a third-party company and checked by the ALSPAC team (for further information, see www.adls.ac.uk/department-for-education/dcsf-npd/?detail). Raw scores at the age of 11, when children sit Key Stage 2 tests for Maths, English and Science, were converted to percentages and averaged across the three subjects. Educational performance at the age of 16, when pupils complete Key Stage 4 national testing, was quantified using a standard capped scoring method (see http://nationalpupildatabase.wikispaces.com/KS4) in which grades achieved at General Certificate of Secondary Education (GCSE) or equivalent for their best eight subjects are converted to a numerical score (e.g. A*=58 points … G=16 points) and summed. Capped scores, out of a maximum possible score of 464, were then converted to a percentage.

#### *3.2.2.4 Potential confounds*

Potential confounds were chosen to reflect variables associated with adolescent cannabis use and intellectual and educational outcomes in accordance with theoretical considerations and previous literature:

1. *maternal and early-life factors* (Fergusson & Horwood, 1997; Fergusson, Horwood, & Lawton, 1990; Heron et al., 2013; von Sydow et al., 2002): maternal education [None/ Certificate of Secondary Education, O-levels, A-levels, Degree]; child sex; maternal depressive symptoms during pregnancy and up to 8 months postnatal [mother completed depression items of the Crown–Crisp experiential index; (Crown & Crisp, 1979)]; maternal substance use during the first three months of pregnancy [alcohol use: None, Less than weekly, At least weekly; cigarette use: No, Yes; cannabis use: No, Yes];

2. *childhood behavioural factors* (Heron et al., 2013; Lynskey & Fergusson, 1995; Shedler & Block, 1990): hyperactivity and conduct problems at age 11 [mother-completed Strengths and Difficulties Questionnaire; (Goodman, 2001)]; mother suspected truancy at age 14 [No, Yes];

3. *childhood mental health* (Degenhardt, Hall, & Lynskey, 2003a; Patton et al., 2002): depressive symptoms at age 12 [child completed depression items of the Short Mood and Feelings Questionnaire; (Angold, Costello, Messer, & Pickles, 1995)]; psychotic-like symptoms at age 12 [child-completed semi-structured interview; (Horwood et al., 2008)];

4. *other adolescent drug use* (Chamberlain, Odlaug, Schreiber, & Grant, 2012; Fergusson, Boden, & Horwood, 2006; Fergusson & Horwood, 2000; Patton, Coffey, Carlin, Sawyer, & Lynskey, 2005; Rob, Reynolds, & Finlayson, 1990; Stiby et al., 2014): cumulative cigarette use self-reported at age 15 [Never, 1-4, 5-20, 21-60, 61-100, >100 times]; cumulative alcohol use self-reported at age 15 [Never, 1-5, 6-19, 20-39, 40-99, ≥100 times]; and other recreational drug use since 15th birthday, including ketamine, LSD, cocaine, ecstasy, amphetamine and inhalants, self-reported at age 15 [None, Used one other drug, Used more than one other drug].

### 3.2.3 Statistical analyses

Analyses were conducted using Stata/SE version 13.1 (StataCorp LP, College Station, TX, USA).

A series of nested linear least-squares regression analyses was employed and adjusted by potential confounders to test the relationships between cumulative cannabis use and: a) IQ at the age of 15 and b) educational performance at the age of 16.

Unadjusted estimates of the relationship between cumulative cannabis use (dummy-coded from 1 to 5, representing the categories explained above) and IQ age 15 (Model IQ1) were compared to adjusted estimates derived from a series of nested models that additionally included: first, pre-exposure IQ at the age of 8 (Model IQ2); then, in addition to IQ2, maternal, early-life and childhood behavioural factors (Model IQ3); then, in addition to IQ3, adolescent mental-health factors (Model IQ4); then, in addition to IQ4, cigarette use (Model IQ5a), alcohol use (Model IQ5b), or other drug use (Model IQ5c); finally, a fully adjusted model (Model IQ6) which included all potential confounds.

Unadjusted estimates of the relationship between cumulative cannabis use (again dummy-coded from 1 to 5, representing the categories explained above) and educational performance at the age of 16 (Model Ed1) were compared to adjusted estimates derived from a series of nested models that initially included educational performance at age 11 (Model Ed2). Models Ed3–6 were then adjusted as for Models IQ3–6.

#### *3.2.3.1 Multiple imputation analyses*

For clarity I have focused primarily on the results of the complete-case analyses. However, to supplement these findings, I repeated planned analyses after implementing multiple imputation with chained equations to account for missing data (20 imputations, using the *ice* command in Stata). Multiple imputation was carried out for all participants alive at one year, resulting in a sample size of 14552 after multiple imputation and exclusions. This method assumes data are missing at random (i.e. that the probability of a data point being missing depends only on observed data). Previously described guidelines were followed when selecting variables for the imputation model from the many variables collected by ALSPAC (Van Buuren, Boshuizen, & Knook, 1999). Due to the large number of variables that met these criteria, different sets of variables were selected for the imputation of groups of variables. Data were imputed for all outcome, key predictor, and confounder variables.

#### *3.2.3.2 Post-hoc analyses*

Post hoc linear least-squares regression analyses were then employed and adjusted by potential confounders to test the relationships between cumulative cigarette use and (a) IQ at the age of 15 and (b) educational performance at the age of 16, after exclusion of cannabis users. Unadjusted estimates of the relationship between cumulative cigarette use (binary outcome due to a smaller sample: never used/ever used cigarettes) and (a) IQ age 15 (Model CigIQ1) were compared to fully adjusted estimates (Model CigIQ2), and (b) educational performance at the age of 16 (Model CigEd1) were compared to fully adjusted estimates (Model CigEd2).

## 3.3 Results

Of the complete-case data set (N= 2235), 23.5% (n= 526) reported having tried cannabis at least once, and 3.3% (n= 74) reported cumulative usage of at least 50 times. Table 3.1. shows the demographics of the sample according to reported cumulative cannabis use at an average age of 15.4 years. Unadjusted analyses demonstrate that cannabis use was associated with maternal cigarette and cannabis use during pregnancy, truancy from school, childhood hyperactivity, conduct problems and depressive symptoms, and adolescent cigarette, alcohol and other drug use.

***Table 3.1.*** *Demographic and baseline variables for each cannabis use group; p-values reflect omnibus test of cannabis use group differences.*

| | Never | Less than 5 | 5-19 | 20-49 | At least 50 | |
|---|---|---|---|---|---|---|
| | % (n) | % (n) | % (n) | % (n) | % (n) | p-value |
| **Sample (complete-cases)** | *76.5 (1709)* | *11.1 (248)* | *6.0 (133)* | *3.2 (71)* | *3.3 (74)* | |
| **Female** | *53.5 (914)* | *59.7 (148)* | *52.6 (70)* | *46.5 (33)* | *39.2 (29)* | *.060* |
| **Mother had no higher education** | *80.2 (1371)* | *85.1 (211)* | *77.4 (103)* | *70.4 (50)* | *77.0 (57)* | *.171* |
| **Cigarette use during first 3 months of pregnancy** | *10.5 (179)* | *18.6 (46)* | *22.6 (30)* | *23.9 (17)* | *33.8 (25)* | *≤001* |
| **Weekly alcohol use during first 3 months of pregnancy** | *13.5 (231)* | *14.9 (37)* | *20.3 (27)* | *16.9 (12)* | *16.2 (12)* | *.074* |
| **Cannabis use during first 3 months of pregnancy** | *0.9 (16)* | *2.0 (5)* | *5.3 (7)* | *4.2 (3)* | *8.1 (6)* | *≤001* |
| **Truancy from school, age 14** | *0.7 (12)* | *2.4 (6)* | *3.8 (5)* | *9.9 (7)* | *6.8 (5)* | *≤001* |
| **Lifetime cigarette use >20 times, age 15** | *4.5 (77)* | *34.3 (85)* | *52.6 (70)* | *71.8 (51)* | *83.8 (62)* | *≤001* |
| **Lifetime alcohol use >20 times, age 15** | *26.4 (452)* | *63.7 (158)* | *77.4 (103)* | *93.0 (66)* | *97.3 (72)* | *≤001* |
| **Other illicit drug use, since 15th birthday** | *5.7 (97)* | *28.6 (71)* | *43.6 (58)* | *54.9 (39)* | *67.6 (50)* | *≤001* |
| | **Mean (SE)** | **Mean (SE)** | **Mean (SE)** | **Mean (SE)** | **Mean (SE)** | **p-value** |
| **IQ score age 8** | *99.7 (0.4)* | *100.3 (1.0)* | *101.6 (1.2)* | *101.7 (1.9)* | *102.0 (1.7)* | *.335* |
| **Educational performance, age 10/11** | *73.2 (0.3)* | *73.4 (0.8)* | *73.3 (1.0)* | *70.0 (1.8)* | *72.3 (1.5)* | *.202* |
| **Maternal depressive symptoms** | *3.6 (0.1)* | *3.5 (0.1)* | *4.0 (0.2)* | *4.1 (0.3)* | *3.9 (0.2)* | *.050* |
| **Hyperactivity, age 11** | *2.4 (0.1)* | *2.5 (0.1)* | *2.5 (0.2)* | *3.5 (0.3)* | *3.4 (0.3)* | *≤001* |
| **Conduct problems, age 11** | *1.0 (0.0)* | *1.2 (0.1)* | *1.2 (0.1)* | *1.7 (0.2)* | *1.4 (0.2)* | *≤001* |
| **Childhood depressive symptoms, age 12** | *4.3 (0.0)* | *4.9 (0.3)* | *5.0 (0.4)* | *5.1 (0.5)* | *6.6 (0.7)* | *≤001* |
| **Childhood psychotic-like symptoms, age 12** | *0.3 (0.0)* | *0.2 (0.1)* | *0.3 (0.1)* | *0.4 (0.1)* | *0.3 (0.1)* | *.549* |

Table 3.2 shows patterns of cannabis use according to cumulative use groups. Within those who had tried cannabis at least once, greater exposure was associated with a younger age of first cannabis use (p< .001) and a longer time since first usage (p< .001). Those who had ⩾50 cannabis exposures had first used at a mean age of 13.1 years, and for a mean duration of 2.3 years. Of those with ⩾50 exposures, 98.7% had used in the past year, 60.8% were currently using at least weekly and 47.3% had used in the three days prior to the IQ test. The majority (91.0%) usually mixed tobacco with their cannabis.

***Table 3.2****. Cannabis use patterns between levels of cannabis use groups, % (n) unless otherwise noted; p-values reflect omnibus test of cannabis use group differences.*

| | **<5** | **5-19** | **20-49** | **≥50** | **p-value** |
|---|---|---|---|---|---|
| **Sample (complete-cases; cannabis users)** | ***47.1 (248)*** | ***25.3 (133)*** | ***13.5 (71)*** | ***14.1 (74)*** | |
| **Age first tried cannabis, years, mean (SE)** | *14.3 (0.1)* | *14.0 (0.1)* | *13.4 (0.1)* | *13.1 (0.1)* | *≤.001* |
| **Time since first cannabis use at time of IQ test, years, mean (SE)** | *1.1 (0.1)* | *1.4 (0.1)* | *2.0 (0.1)* | *2.3 (0.1)* | *≤.001* |
| **Currently uses cannabis at least weekly** | *0.0 (0)* | *3.8 (5)* | *23.9 (17)* | *60.8 (45)* | *≤.001* |
| **Has used cannabis in the past year** | *62.0 (153)* | *94.0 (125)* | *90.1 (64)* | *98.7 (73)* | *≤.001* |
| **Had used cannabis in the previous 3 days at time of IQ test** | *2.4 (6)* | *6.8 (9)* | *23.9 (17)* | *47.3 (35)* | *≤.001* |
| **Usually smokes cannabis mixed with tobacco** | *90.7 (127)* | *87.7 (107)* | *93.0 (66)* | *94.5 (69)* | *.272* |

### 3.3.1 IQ

Unadjusted IQ data for the cannabis use groups can be found in Table 3.3. Model estimates are displayed in Figure 3.2 and Table 3.4. In the unadjusted analyses, no difference in IQ between the cannabis use groups was apparent (p= .237). However, after adjusting for IQ measured at the age of 8, cumulative cannabis use was negatively associated with IQ measured at the age of

15 (Model IQ2; p< .001). Those who had used cannabis ⩾50 times were estimated to have an IQ at the age of 15 that was 2.9 points lower than never-users in this model. Adjustment by maternal, early-life and childhood behavioural factors (Model IQ3) and by mental-health factors (Model IQ4) had little effect on point estimates. Adjustment for cigarette (Model IQ5a), alcohol (Model IQ5b) or other substance use (Model IQ5c) attenuated the association between cannabis use and IQ at the age of 15, with cigarette use having the most marked influence. Model IQ6 fully attenuated the association between cannabis use and IQ at the age of 15 (p=0.959), with cumulative use of ⩾50 times now predicting an adjusted IQ score of 0.1 points lower (p= .941) relative to never-users.

***Table 3.3****. Mean and 95% confidence intervals (CIs) of Wechsler Abbreviated Scale of Intelligence (WASI) IQ at the age of 15 and educational performance (% GCSE points) at the age of 16 for each of the cannabis use groups.*

| | | **WASI IQ (age 15)** | | | **Educational performance % (age 16)** | | |
|---|---|---|---|---|---|---|---|
| | | | **95% CIs** | | | **95% CIs** | |
| **Cumulative cannabis use** | **% (N)** | **Mean** | **Lower** | **Upper** | **Mean** | **Lower** | **Upper** |
| Never | *76.5 (1709)* | *100.4* | *99.7* | *101.1* | *80.8* | *80.2* | *81.4* |
| <5 | *11.1 (248)* | *98.6* | *96.8* | *100.5* | *77.8* | *76.2* | *79.4* |
| 5-19 | *6.0 (133)* | *98.8* | *96.2* | *101.4* | *76.5* | *73.9* | *79.1* |
| 20-49 | *3.2 (71)* | *98.3* | *94.6* | *101.9* | *72.8* | *68.8* | *76.8* |
| ≥50 | *3.3 (74)* | *98.9* | *95.6* | *102.2* | *69.2* | *65.0* | *73.3* |
| Overall | *100.0 (2235)* | *100.0* | *99.4* | *100.6* | *79.6* | *79.0* | *80.1* |

***Table 3.4.*** *Linear regression nested models for complete-cases dataset displaying difference in IQ at the age of 15 (SE), estimated between each cannabis use group compared to never-users.*

| **Cumulative cannabis use** | **IQ1** | **IQ2** | **IQ3** | **IQ4** | **IQ5a** | **IQ5b** | **IQ5c** | **IQ6** |
|---|---|---|---|---|---|---|---|---|
| <5 times | *-1.8 (1.0)** | *-2.1 (0.8)*** | *-1.7 (0.8)** | *-1.7 (0.8)** | *-0.5 (0.9)* | *-1.2 (0.8)* | *-1.6 (0.8)** | *-0.2 (0.9)* |
| 5-19 times | *-1.6 (1.3)* | *-2.7 (1.1)** | *-2.8 (1.1)**** | *-2.9 (1.1)**** | *-1.0 (1.2)* | *-2.4 (1.1)** | *-2.6 (1.1)** | *-0.9 (1.2)* |
| 20-49 times | *-2.1 (1.8)* | *-3.3 (1.5)** | *-3.1 (1.5)** | *-3.3 (1.5)** | *-1.0 (1.6)* | *-2.8 (1.5)** | *-2.8 (1.5)** | *-0.8 (1.7)* |
| ≥50 times | *-1.6 (1.8)* | *-2.9 (1.4)** | *-2.5 (1.4)** | *-2.7 (1.4)** | *- 0.2(1.7)* | *-1.4 (1.6)* | *-2.1 (1.6)* | *-0.1 (1.8)* |

* two-tailed t-tests, compared to never-users (p<.100)
** two-tailed t-tests, compared to never-users (p<.050)
*** two-tailed t-tests, compared to never-users (p<.010)

Model IQ1: Adjusted only by cumulative cannabis use at age 15

Model IQ2: As model 1 plus adjustment for full-scale IQ age 8

Model IQ3: As model 2 plus adjustment for maternal, early-life, and behavioural factors

Model IQ4: As model 3 plus adjustment for depressive symptoms and psychotic-like experiences

Model IQ5a: As model 4 plus adjustment for cumulative cigarette use at age 15

Model IQ5b: As model 4 plus adjustment for cumulative alcohol use at age 15

Model IQ5c: As model 4 plus adjustment for other recreational drug use at age 15

Model IQ6: As model 4 plus adjustment for cumulative cigarette use, cumulative alcohol use, and other recreational drug use at age 15

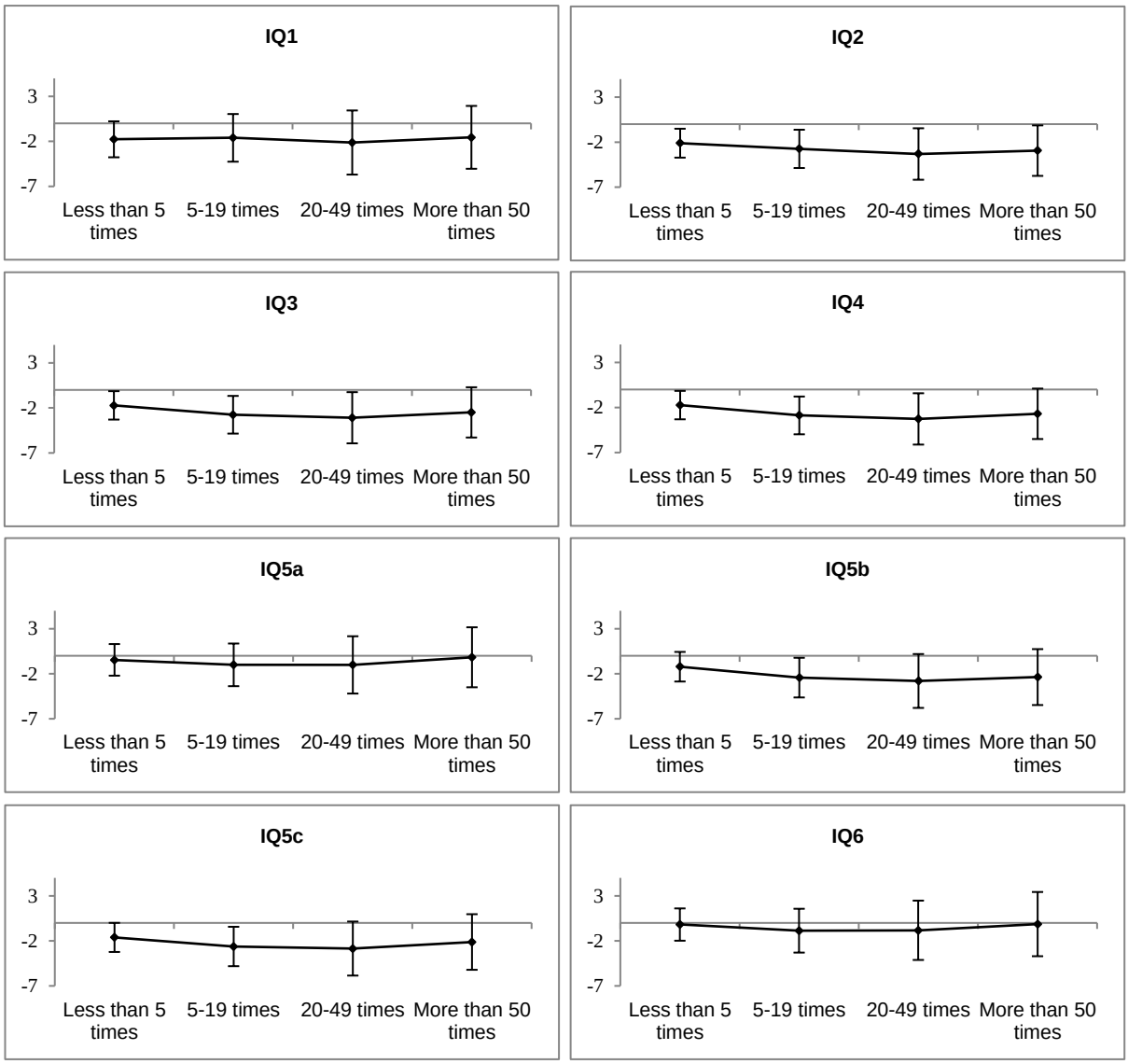


***Figure 3.2.*** *Linear regression nested models for complete-cases dataset displaying difference in IQ at the age of 15, estimated between each cannabis use group compared to never-users. Error bars represent 95% confidence intervals.*

### 3.3.2 Educational performance

Unadjusted educational performance data for the cannabis use groups are shown in Table 3.3. Model estimates are displayed in Figure 3.3. and Table 3.5. Increasing cumulative cannabis use correlated with poorer educational performance at the age of 16 ($p < .001$). Cannabis use of ⩾50 times predicted an average score of 11.6 percentage points lower than never-users ($p < .001$). After adjusting for educational performance at the age of 11, cannabis use remained associated with educational performance at the age of 16 (Model Ed2; $p < .001$), with those who had used cannabis ⩾50 times estimated to have scored 11.0 percentage points lower than never-users ($p < .001$). Adjustment by maternal, early-life and childhood behavioural factors (Model Ed3) and mental health factors (Model Ed4) had little effect on point estimates. Adjustment by alcohol (Model Ed5a), cigarette (Model Ed5b) or other substance use (Model Ed5c) attenuated the association between cannabis use and educational performance at the age of 16, with cigarette use again having the most marked influence. Model Ed6 attenuated the association between cannabis use and educational performance at the age of 16 ($p = .184$), with cumulative use ⩾50 times now predicting an adjusted score of 2.2 ($p = .083$) percentage points lower than never-users.

***Table 3.5.*** *Linear regression nested models for complete-cases dataset displaying difference in educational performance at the age of 16 (SE), estimated between each cannabis use group compared to never-users.*

| **Cumulative cannabis use** | **Ed1** | **Ed2** | **Ed3** | **Ed4** | **Ed5a** | **Ed5b** | **Ed5c** | **Ed6** |
|---|---|---|---|---|---|---|---|---|
| <5 times | *-2.9 (0.9)**** | *-3.1 (0.6)**** | *-2.7 (0.6)**** | *-2.7 (0.6)**** | *-0.7 (0.6)* | *-2.0 (0.6)**** | *-2.4 (0.6)**** | *-0.4 (0.6)* |
| 5-19 times | *-4.3 (1.2)**** | *-4.4 (0.8)**** | *-4.0 (0.8)**** | *-4.0 (0.8)**** | *-0.7 (0.9)* | *-3.1 (0.8)**** | *-3.1 (0.8)**** | *-0.2 (0.9)* |
| 20-49 times | *-8.0 (1.6)**** | *-5.6 (1.1)**** | *-4.3 (1.0)**** | *-4.3 (1.0)**** | *0.2 (1.1)* | *-3.2 (1.1)**** | *-2.6 (1.1)*** | *1.2 (1.2)* |
| ≥50 times | *-11.6 (1.5)**** | *-11.0 (1.1)**** | *-9.4 (1.0)**** | *-9.3 (1.0)**** | *-3.4 (1.2)***** | *-7.9 (1.1)**** | *-6.9 (1.1)**** | *-2.2 (1.3)** |

* two-tailed t-tests, compared to never-users (p<.100)
** two-tailed t-tests, compared to never-users (p<.050)
*** two-tailed t-tests, compared to never-users (p<.010)

Model IQ1: Adjusted only by cumulative cannabis use at age 15

Model IQ2: As model 1 plus adjustment for full-scale IQ age 8

Model IQ3: As model 2 plus adjustment for maternal, early-life, and behavioural factors

Model IQ4: As model 3 plus adjustment for depressive symptoms and psychotic-like experiences

Model IQ5a: As model 4 plus adjustment for cumulative cigarette use at age 15

Model IQ5b: As model 4 plus adjustment for cumulative alcohol use at age 15

Model IQ5c: As model 4 plus adjustment for other recreational drug use at age 15

Model IQ6: As model 4 plus adjustment for cumulative cigarette use, cumulative alcohol use, and other recreational drug use at age 15

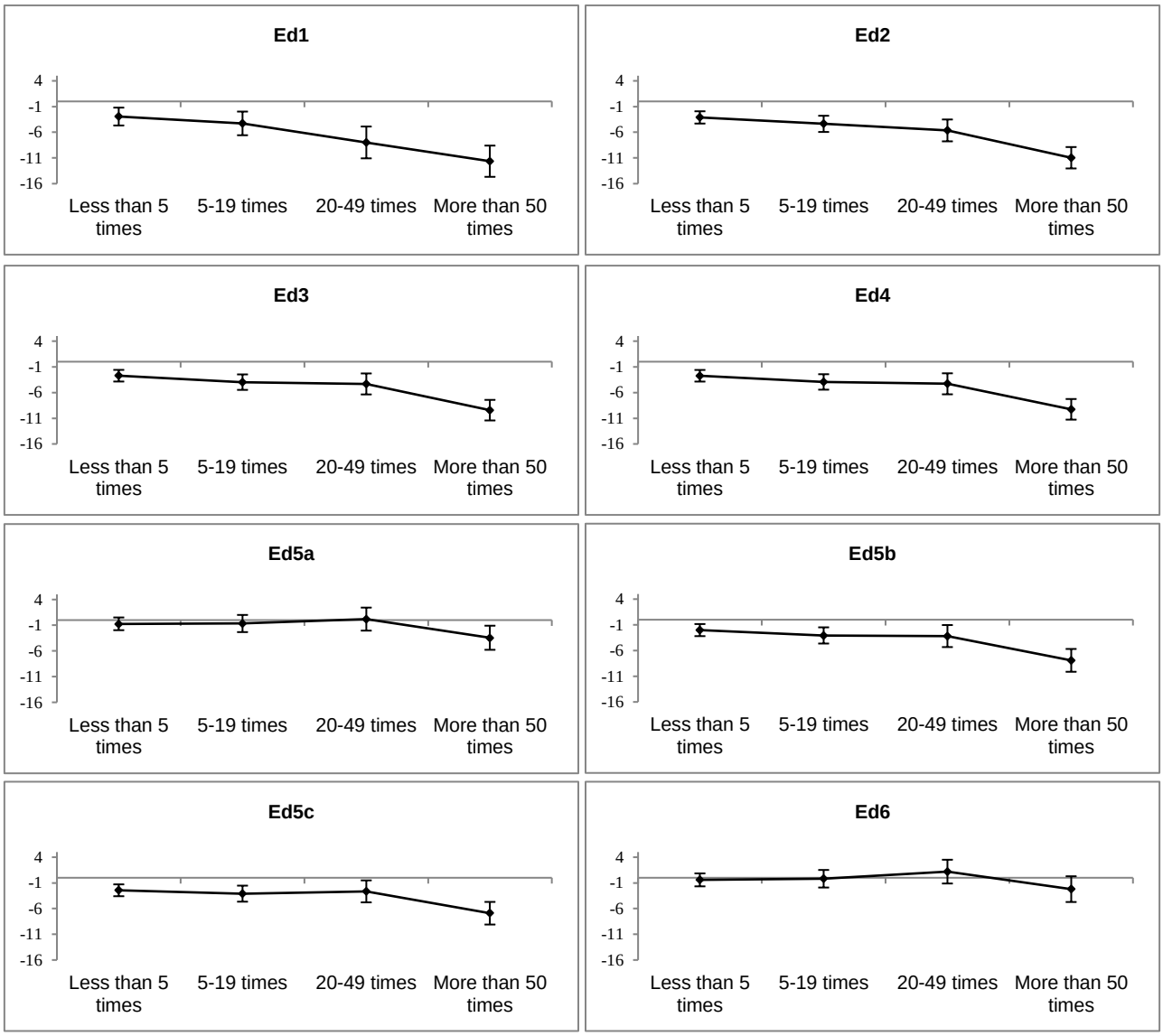


***Figure 3.3.*** *Linear regression nested models for complete-cases dataset displaying difference in educational performance at the age of 16, estimated between each cannabis use group compared to never-users. Error bars represent 95% confidence intervals.*

### 3.3.3 Cigarettes

In the above analyses, cumulative cigarette use was the key attenuator of the association between cumulative cannabis use and both IQ (Models IQ5a and IQ6) and educational performance (see Models Ed5a and Ed6). Furthermore, cumulative cigarette use remained negatively associated with both outcomes in the fully adjusted models. Those who had used cigarettes >100 times were estimated to have an age 15 adjusted IQ 3.2 points lower (p= .018) and an adjusted educational score 7.4 percentage points lower (p< .001) than never-users of cigarettes (see Tables 3.6 and 3.7).

***Table 3.6.*** *Fully adjusted model for complete-cases dataset displaying difference in WASI IQ at the age of 15, estimated between each cigarette use group compared to never-users.*

| | | | | | 95% Cis | |
|---|---|---|---|---|---|---|
| **Cumulative cigarette use** | **Adj. coef.** | **SE** | **t** | **p-value** | **Lower** | **Upper** |
| 1-4 times | *-0.85* | *1.06* | *-0.80* | *.425* | *-2.93* | *1.23* |
| 5-20 times | *-1.81* | *1.10* | *-1.64* | *.102* | *-3.97* | *0.36* |
| 21-60 times | *-2.55** | *1.23* | *-2.07* | *.039* | *-4.97* | *-0.13* |
| 61-100 times | *-3.79** | *1.75* | *-2.16* | *.031* | *-7.23* | *-0.35* |
| >100 times | *-3.22** | *1.36* | *-2.36* | *.018* | *-5.89* | *-0.55* |

***Table 3.7.*** *Fully adjusted model for complete-cases dataset displaying difference in educational performance at the age of 16, estimated between each cigarette use group compared to never-users.*

| Cumulative cigarette use | Adj. coef. | SE | t | p-value | 95% Cis | |
|---|---|---|---|---|---|---|
| | | | | | Lower | Upper |
| 1-4 times | *-0.69* | *0.75* | *-0.93* | *0.351* | *-2.16* | *0.77* |
| 5-20 times | *-1.39* | *0.78* | *-1.80* | *0.072* | *-2.92* | *0.13* |
| 21-60 times | *-3.29* | *0.87* | *-3.79* | *<.001* | *-4.99* | *-1.59* |
| 61-100 times | *-5.32* | *1.23* | *-4.32* | *<.001* | *-7.73* | *-2.90* |
| >100 times | *-7.35* | *0.95* | *-7.72* | *<.001* | *-9.22* | *-5.49* |

To explore these relationships further, I investigated associations with cigarette use in those who had never used cannabis (Table 3.8a). Of the complete-case sample, 76.4% (n= 1709) had never tried cannabis, of which 13.9% (n= 237) reported having tried cigarettes at least once. Analyses were repeated on this restricted sample with ever-use of cigarettes as the primary predictor (Table 3.8b). With adjustment for only pre-exposure IQ or educational performance, respectively, ever-use of cigarettes was associated with an age 15 IQ 6.2 points lower (p< .001; Model CigIQ1), and educational performance 7.8 percentage points lower (p< .001; Model CigEd1), relative to never-users of cigarettes. After full adjustment, these relationships were somewhat attenuated, with ever-use of cigarettes now predicting an age 15 adjusted IQ 1.5 points lower (p= .083; Model CigIQ2), and educational performance 2.9 percentage points lower (p< .001; Model CigEd2), relative to never-users of cigarettes.

***Table 3.8.***

***a)*** *Mean and 95% confidence intervals (CIs) of Wechsler Abbreviated Scale of Intelligence (WASI) IQ at the age of 15 and educational performance (% GCSE points) at the age of 16 for ever-users of cigarettes (n=237) compared to never-users of cigarettes (n=1472). Cannabis users were excluded from this analysis.*

| | | **WASI IQ (age 15)** | | | **Educational performance % (age 16)** | | |
|---|---|---|---|---|---|---|---|
| | | | **95% CIs** | | | **95% CIs** | |
| **Ever-use of cigarettes** | **% (N)** | **Mean** | **Lower** | **Upper** | **Mean** | **Lower** | **Upper** |
| Non-user | *86.1 (1472)* | *101.3* | *100.5* | *102.0* | *81.9* | *81.2* | *82.5* |
| Tried cigarettes ≥once | *13.9 (237)* | *95.1* | *93.4* | *96.8* | *74.1* | *72.5* | *75.7* |

***b)*** *Linear regression nested models displaying difference in IQ at the age of 15 and educational performance at the age of 16 estimated for ever-users of cigarettes (n=237) compared to never-users of cigarettes (n=1472). Cannabis users were excluded from this analysis.*

| | **WASI IQ (age 15)** | | **Educational performance (age 16)** | |
|---|---|---|---|---|
| | **Model CigIQ1** | **Model CigIQ2** | **Model CigEd1** | **Model CigEd2** |
| Tried cigarettes ≥once | *-6.2 (1.0)*** | *-1.5 (0.9)** | *-7.8 (0.8)*** | *-2.9 (0.6)*** |

* two-tailed t-tests, compared to never-users (p=.083)
** two-tailed t-tests, compared to never-users (p<.010)

### 3.3.4 Multiple imputation analyses

Key variables were compared for participants who were included in the complete-case analysis and the available data for the participants who had missing data but were alive at one year (Table 3.9.). Drop-out was related to a range of variables, including pre-cannabis exposure IQ score at the age of 8. To investigate whether drop-out may have influenced the results, my

colleague (Dr Rebecca Landy) conducted multiple imputation to supplement the main analyses, resulting in a large imputed data set (n= 14552).

***Table 3.9.*** *Analyses comparing participants who were included in the complete-cases analysis to those with missing data. Total number of cases with missing data varies by variable due to varying degrees of missingness.*

| | **Complete-cases** | | **Cases with missing data** | | |
|---|---|---|---|---|---|
| | **N** | **%** | **N** | **%** | **p-value** |
| Female | *1194* | *53.4* | *5955* | *47.8* | *<.001* |
| Mother has higher education | *1113* | *49.8* | *3279* | *32.2* | *<.001* |
| Cannabis use during first 3 months of pregnancy | *37* | *1.7* | *280* | *2.7* | *.003* |
| Mother in trouble with the law in 8 months following birth | *4* | *0.2* | *48* | *0.5* | *.029* |
| Moved house after the child was born | *215* | *9.8* | *1326* | *15.2* | *<.001* |
| Teacher reports child has played truant at age 10-11 years-old | *0* | *0.0* | *124* | *2.0* | *<.001* |
| | **Mean** | **SE** | **Mean** | **SE** | **p-value** |
| IQ score age 8 | *100.0* | *0.3* | *93.3* | *0.2* | *<.001* |
| Maternal depressive symptoms | *3.6* | *0.0* | *4.1* | *0.0* | *<.001* |

Point estimates and the patterns of attenuation observed after adjusting hierarchically for potential confounds were similar for the complete and imputed case analyses. However, while for the unadjusted complete-case IQ analysis (Model IQ1) there was no difference in IQ between cannabis use groups, for the unadjusted imputed analysis lower scores were associated with greater cannabis use (Table 3.10., Model IQ1i). Additionally, for the complete-case education analyses, adjustment for cigarette use (Models Ed5a) did not fully attenuate the association between cannabis use and educational performance. However, in the imputed analyses, this association was fully attenuated (Table 3.11., Models Ed5ai).

***Table 3.10.*** *Linear regression nested models for imputed dataset displaying difference in IQ at the age of 15 (SE), estimated between each cannabis use group compared to never-users.*

| | **IQ1i** | **IQ2i** | **IQ3i** | **IQ4i** | **IQ5ai** | **IQ5bi** | **IQ5ci** | **IQ6i** |
|---|---|---|---|---|---|---|---|---|
| <5 times | *-2.1* | *-1.6* | *-1.3* | *-1.4* | *-0.4* | *-0.9* | *-1.3* | *-0.2* |
| | *(0.7)*** | *(0.5)*** | *(0.5)*** | *(0.5)*** | *(0.5)* | *(0.5)** | *(0.5)*** | *(0.5)* |
| 5-19 times | *-1.8* | *-2.0* | *-1.5* | *-1.6* | *-0.0* | *-0.9* | *-1.4* | *0.2* |
| | *(0.8)*** | *(0.7)*** | *(0.6)*** | *(0.6)*** | *(0.6)* | *(0.6)* | *(0.6)*** | *(0.6)* |
| 20-49 times | *-2.7* | *-2.5* | *-1.8* | *-1.9* | *-0.1* | *-1.1* | *-1.6* | *0.3* |
| | *(1.3)*** | *(0.9)*** | *(0.9)*** | *(0.9)*** | *(0.9)* | *(1.0)* | *(0.9)** | *(0.9)* |
| ≥50 times | *-3.1* | *-2.8* | *-1.9* | *-2.0* | *-0.4* | *-1.2* | *-1.5* | *0.6* |
| | *(1.2)*** | *(0.9)*** | *(0.9)*** | *(0.9)*** | *(1.0)* | *(0.9)* | *(0.9)* | *(0.9)* |

* two-tailed t-tests, compared to never-users (p<.100)
** two-tailed t-tests, compared to never-users (p<.050)
*** two-tailed t-tests, compared to never-users (p<.010)

Model IQ1i: Adjusted only by cumulative cannabis use at age 15

Model IQ2i: As model 1i plus adjustment for full-scale IQ age 8

Model IQ3i: As model 2i plus adjustment for maternal, early-life, and behavioural factors

Model IQ4i: As model 3i plus adjustment for depressive symptoms and psychotic-like experiences

Model IQ5ai: As model 4i plus adjustment for cumulative cigarette use at age 15

Model IQ5bi: As model 4i plus adjustment for cumulative alcohol use at age 15

Model IQ5ci: As model 4i plus adjustment for other recreational drug use at age 15

Model IQ6i: As model 4i plus adjustment for cumulative cigarette use, cumulative alcohol use, and other recreational drug use at age 15

***Table 3.11.*** *Linear regression nested models for imputed dataset displaying difference in educational performance at the age of 16 (SE), estimated between each cannabis use group compared to never-users.*

| | **Ed1i** | **Ed2i** | **Ed3i** | **Ed4i** | **Ed5ai** | **Ed5bi** | **Ed5ci** | **Ed6i** |
|---|---|---|---|---|---|---|---|---|
| <5 times | *-3.7* | *-2.2* | *-1.4* | *-1.4* | *-0.1* | *-1.3* | *-1.2* | *-0.2* |
| | *(0.9)*** | *(0.5)*** | *(0.5)*** | *(0.5)*** | *(0.5)* | *(0.5)*** | *(0.5)*** | *(0.5)* |
| 5-19 times | *-5.4* | *-4.3* | *-3.0* | *-3.0* | *-0.5* | *-2.8* | *-2.6* | *-0.6* |
| | *(1.2)*** | *(0.9)*** | *(0.9)*** | *(0.9)*** | *(1.0)* | *(0.9)*** | *(0.9)*** | *(1.0)* |
| 20-49 times | *-7.3* | *-4.7* | *-2.8* | *-2.8* | *0.4* | *-2.5* | *-2.1* | *0.2* |
| | *(1.5)*** | *(1.0)*** | *(0.9)*** | *(0.9)*** | *(1.0)* | *(1.0)*** | *(0.9)*** | *(1.0)* |
| ≥50 times | *-10.9* | *-8.3* | *-5.1* | *-5.1* | *-1.1* | *-4.7* | *-4.2* | *-1.3* |
| | *(1.4)*** | *(0.8)*** | *(0.8)*** | *(0.8)*** | *(1.0)* | *(1.0)*** | *(0.9)*** | *(1.1)* |

* two-tailed t-tests, compared to never-users (p<.100)
** two-tailed t-tests, compared to never-users (p<.050)
*** two-tailed t-tests, compared to never-users (p<.010)

Model Ed1i: Adjusted only by cumulative cannabis use at age 15

Model Ed2i: As model 1i plus adjustment for educational performance age 11

Model Ed3i: As model 2i plus adjustment for maternal, early-life, and behavioural factors

Model Ed4i: As model 3i plus adjustment for depressive symptoms and psychotic-like experiences

Model Ed5ai: As model 4i plus adjustment for cumulative cigarette use at age 15

Model Ed5bi: As model 4i plus adjustment for cumulative alcohol use at age 15

Model Ed5ci: As model 4i plus adjustment for other recreational drug use at age 15

Model Ed6i: As model 4i plus adjustment for cumulative cigarette use, cumulative alcohol use, and other recreational drug use at age 15

## 3.4 Discussion

In line with previous work I found that cannabis users had lower teenage IQ scores and poorer educational performance than teenagers who had never used cannabis. Cannabis users also had higher rates of childhood behavioural problems, childhood depressive symptoms, other substance use (including use of cigarettes and alcohol) and maternal use of cannabis during pregnancy. After adjustment to account for these group differences, cannabis use by the age of 15 did not predict either lower teenage IQ scores or poorer educational performance. These findings therefore suggest that cannabis use at the modest levels used by this sample of teenagers is not by itself causally related to cognitive impairment. Instead, my findings imply that previously reported associations between adolescent cannabis use and poorer intellectual and educational outcomes may be confounded to a significant degree by related factors.

While I found no evidence of a robust link between adolescent cannabis use and IQ, previous work has indeed shown that persistent cannabis dependence starting with regular cannabis use in adolescence is associated with IQ decline by middle age (Meier et al., 2012). Together, these findings suggest that while persistent cannabis dependence may be linked to declining IQ across a person's lifetime, teenage cannabis use alone does not appear to predict worse IQ outcomes in adolescents. The present findings also highlight the importance of considering other adolescent substance use alongside cannabis, in particular cigarette use, when assessing links between cannabis and intellectual outcomes. This confound may contribute to previously reported associations between cannabis dependence and IQ decline, and associations reported in chapter 1 between adolescent cannabis use and poorer verbal and working memory. However, the young age at which my outcomes were measured, and the relatively modest levels of cannabis use in the present sample, do not allow me to rule out the possibility of future difficulties, perhaps following further cannabis exposure. Assessing outcomes at this young age, before the end of compulsory education, does, however, have the benefit of reducing the potentially confounding influence of selection into or out of cognitively demanding environments throughout a person's life on IQ performance (Rogeberg, 2013).

Attenuation of the association between cannabis use and educational performance contrasts with previous work demonstrating a robust relationship even after adjustment for confounders (Lynskey et al., 2003; Lynskey & Hall, 2000; Silins et al., 2014; Stiby et al., 2014). Notably, however, previous work reporting associations between cannabis use and poorer educational outcomes has not consistently addressed the possibility of group differences in pre-exposure educational performance or in rates of other substance use including adolescent cigarette use, which may explain differences between my findings and previous work. Indeed, my findings are in accordance with recent genetic studies that found no difference in early school leaving between both MZ and DZ twin pairs discordant for cannabis use (J. D. Grant et al., 2012; Verweij et al., 2013).

Compared with those in my sample who had never tried cannabis, teenagers who had used cannabis at least 50 times were 17 times more likely (84% vs. 5%) to have smoked cigarettes more than 20 times in their lifetime. Accounting for group differences in cigarette smoking dramatically attenuated the associations between cannabis use and both IQ and educational performance. Furthermore, even after excluding those who had never tried cannabis, cigarette users were found to have lower educational performance (adjusted performance 2.9 percentage points lower, approximately equivalent to dropping two grades on one subject taken at GCSE) relative to those who had never tried cigarettes. A relationship between cigarette use and poorer cognitive (Chamberlain et al., 2012; Hooper et al., 2014; Weiser, Zarka, Werbeloff, Kravitz, & Lubin, 2010; Whalley, Fox, Deary, & Starr, 2005) and educational (McCaffrey et al., 2010; Silins et al., 2014; Stiby et al., 2014) outcomes has been noted previously, and may have a number of explanations. Cigarette use may have a negative impact on cognitive ability. This is not supported by the experimental psychopharmacology literature, which robustly shows that acute nicotine administration results in transient cognitive enhancement (Heishman et al., 2010), although non-acute effects of nicotine are less well-known and studies comparing cognition in cigarette users to non-users are typically confounded by the potentially negative effects of smoking itself. Alternatively, reverse causality may contribute to this relationship, in that

performing poorly at school may lead to increased engagement in risky behaviours such as cigarette smoking. Residual confounding factors may also contribute to this link, as cigarette smoking may be a marker of unmeasured factors, such as social adversity during adolescence, that influence both IQ and educational attainment.

Overwhelmingly, the most common method of cannabis administration by participants in the study was smoking it combined with tobacco (as is typical in the UK (Hindocha et al., 2016)), potentially making it difficult to separate the independent contributions of cannabis and tobacco use on the outcomes. However, as noted above, lower educational performance remained apparent for cigarette smokers who had never used cannabis, even following adjustment for potential confounders. This suggests that it may be cigarette use, rather than tobacco consumption per se, that predicts poorer educational outcomes, potentially lending support to a non-pharmacological mechanism to explain links between cigarette use and poorer outcomes. Cigarette use has recently been highlighted as an important factor when exploring links between cannabis use and various outcomes, including psychosis (as described in chapter 2 (Gage et al., 2014)), educational outcomes (Stiby et al., 2014) and cannabis dependence (Hindocha, Shaban, et al., 2015). The relationship between cannabis and tobacco and/or cigarette use is complex, and there is a need to delineate the contribution of these substances when used alone and in combination (Randi M Schuster, Crane, Mermelstein, & Gonzalez, 2015). This would be helped by improved measures of tobacco and cannabis consumption, for example asking participants to estimate the ratio of tobacco to cannabis they use when rolling a joint, and by comparing findings from cohorts with differing degrees of combined cannabis/tobacco administration (for instance, rates of tobacco/cannabis co-administration are much lower in the USA than in the UK (Hindocha et al., 2016)).

A number of measurement limitations of the present study should be noted. Firstly, classification of cannabis users into groups based on self-reported cumulative use occasions does not provide information on actual dose (of THC), which varies according to cannabis weight and particular strain, as well as how the user titrates the dose (T. P. Freeman et al., 2014;

Pol et al., 2014; Temple, Brown, & Hine, 2011). This is a limitation of all cohort-based research to date, since objective biological markers (e.g. of cannabinoids in hair) are not typically collected. It is noteworthy that the ALSPAC cohort, born in the early nineties, may have been exposed to higher THC potency varieties than earlier cohorts, which may be expected to have made cognitive impairment more rather than less likely. Secondly, an abbreviated WASI was used for IQ assessment at the age of 15, which provides a less reliable estimate of IQ than full-scale tests (Axelrod, 2002). However, as all the participants completed the same assessments, my comparisons remain valid.

Further, nearly half of those who had used cannabis at least 50 times reported having used it in the three days prior to their age 15 IQ assessments. As described in chapter 1, cannabinoids can often be detected in blood and urine for one month or more following cannabis consumption, particularly in heavier users. Indeed, Schreiner and Dunn's (2012) meta-analysis demonstrates that residual cognitive effects of chronic cannabis exposure may last approximately one month following abstinence from the drug. Furthermore, as described in chapter 1, while studies assessing adolescent cannabis users after minimal abstinence periods reliably detect verbal learning and memory impairments relative to non-users (Dougherty et al., 2013), alcohol users (Solowij, Jones, et al., 2011), and less frequent cannabis users (Harvey et al., 2007), no or fewer impairments tend to be found amongst users abstinent for two or more weeks (Hanson et al., 2010; Mahmood et al., 2010). Sample-size considerations meant that I could not assess the impact of excluding recent cannabis smokers from analyses, but future work, perhaps with the ALSPAC sample at an older age, should address the possibility of residual effects directly. Nevertheless, despite high levels of recent cannabis usage, I found no robust association between cannabis use and poorer IQ performance.

In summary, the notion that cannabis use itself is causally related to lower IQ and poorer educational performance was not supported in this large teenage sample. However, this study indeed has limitations, in particular the young age of outcome assessment and the fairly moderate levels of cannabis use. While I have demonstrated that confounding may be an

explanation for links between cannabis use and poorer outcomes, large prospective cohorts tracking young people prior to, during and after stopping cannabis use, and using more objective measures of drug use (e.g. the new NIH-funded "ABCD study" in the USA), are required before strong conclusions can be made. To date, all prospective cohort studies, including my own reported here, have relied on retrospective self-report of cannabis use, have not been able to rule-out possible residual effects of the drug on IQ and educational test performance and have not addressed whether the potency or variety of cannabis used influences findings. Cigarette smoking in particular has once again (Hooper et al., 2014; McCaffrey et al., 2010; Silins et al., 2014; Stiby et al., 2014) been highlighted as an important factor in adolescent outcomes, as well as a robust independent predictor of educational performance, and the reasons for this need to be elucidated.

# 4 Chapter 4. The acute effects of cannabis on subjective ratings, memory and inhibition in adolescents and adults

## 4.1 Introduction

In Chapter 3, I demonstrated that associations between adolescent cannabis use and intellectual functioning and educational attainment may not be driven causally by cannabis use. Following this study, and given that I am based in a psychopharmacology lab, I became interested in the acute effects of cannabis in adolescents, and wondered whether they would be similar to the effects in adults. Given that approximately 50% of all cannabis users started before the age of 18-19 years old, it is surprising that, to my knowledge, no controlled study has administered cannabis (or indeed, other recreational substances) to anyone under the age of 18 years.

I was particularly intrigued by preclinical findings from the alcohol literature demonstrating that adolescent rodents experience blunted negative effects (for instance, motor and sedative effects) and heightened positive effects (for instance, social facilitatory and rewarding effects) acutely from alcohol (Spear, 2016). While similar preclinical research with cannabis remains in its infancy, as discussed in chapter 1 and below, there is emerging evidence of differential acute effects of cannabinoids in adolescent and adult rodents. These findings led to the series of experiments as detailed here and in chapters 5 and 6, in which I ask whether such findings translate to human adolescents.

### 4.1.1 Human findings

As described in chapter 1, a number of studies have compared cognitive function in adolescent cannabis users and non-users, as an indicator of non-acute effects of cannabis in adolescence. Compared to non-users, adolescent cannabis users are inconsistently found to have non-intoxicated poorer verbal and working memory, though there is scarce evidence of impairments following 2 weeks of abstinence. Meanwhile few studies, have assessed response inhibition in adolescent cannabis users, though the one study with an extended period of abstinence prior to

testing (28 days) found no evidence of impairment relative to non-users. Differences between adolescent cannabis users and non-users have also been reported for task-related neural responses (e.g. greater BOLD response during response inhibition (Tapert et al., 2007) or spatial working memory tasks (Alecia D Schweinsburg et al., 2008)), morphological differences in medial temporal and frontal cortices (Batalla et al., 2013) and white matter integrity (Epstein & Kumra, 2015; Gruber, Dahlgren, Sagar, Gönenç, & Lukas, 2014). However, findings are often mixed, limited by cross-sectional designs and small samples, and necessarily correlational in nature (Curran et al., 2016).

Epidemiological findings further suggest that younger age of cannabis use onset may be associated with increased risk of addiction (Chen & Anthony, 2003; Chen et al., 2005; Hines et al., 2015; von Sydow et al., 2002), cognitive impairment (Curran et al., 2016; Gruber et al., 2012; Meier et al., 2012) and psychotic illness (Arseneault et al., 2002; Di Forti et al., 2014; T. H. Moore et al., 2007). Again such findings are limited since individuals starting use younger will also have more cannabis exposures over a longer period of time, making it hard to dissociate the specific effect of age.

To my knowledge no studies to date with humans have assessed the acute effects of cannabis in adolescents, so instead we need to turn to the animal research.

### 4.1.2 Animal findings

#### *4.1.2.1 Non-acute effects*

Repeated administration studies with rats further suggest greater vulnerability to cannabis-related harm in adolescents. Adolescent exposure leads to adulthood deficits in novel object recognition and spatial working memory, but not spatial learning (Rubino & Parolaro, 2015). In adolescent rhesus monkeys Verrico and colleagues (Verrico et al., 2014; Verrico et al., 2012) found that both acute and repeated doses of THC led to impaired spatial but not object working memory; further, repeated THC prevented the maturational improvement in spatial working

memory typically seen at that age, but did not affect the earlier developing object working memory. However direct comparisons of adolescent versus adult chronic exposure in animals are scarce and findings have been inconsistent (Cha et al., 2007; Cha et al., 2006; Fox et al., 2009; O'Shea, Singh, McGregor, & Mallet, 2004; Quinn et al., 2008; Schneider & Koch, 2003).

#### *4.1.2.2 Acute effects*

As described in chapter 1, preclinical evidence for increased adolescent vulnerability to acute effects of cannabis is also mixed, with some suggesting acute cannabinoid treatment has a greater impairing effect on spatial and non-spatial learning (THC) (Cha et al., 2007; Cha et al., 2006) and object recognition (WIN) (Schneider et al., 2008) in adolescent compared to adult rats. Others however report the opposite, with evidence of greater acute impairments in adult rodents - including impaired novel object recognition (WIN) (Fox et al., 2009), and spatial learning (WIN) (Acheson et al., 2011).

As well as differential cognitive effects, some have also reported differential general intoxication effects in adolescent and adult rats. Adult rats developed conditioned place (WIN) (Carvalho, Reyes, Ramalhosa, Sousa, & Van Bockstaele, 2016) and taste (THC) (Quinn et al., 2008) aversion to cannabinoid treatment while adolescents did not, and adults produced more vocalisations when handled while intoxicated, suggesting greater drug-induced aversion (Quinn et al., 2008). THC has also been found to have less anxiogenic (Schramm-Sapyta et al., 2007) or even anxiolytic (Acheson et al., 2011) effects, alongside reduced locomotor-suppression effects (Schramm-Sapyta et al., 2007), in adolescent rats compared to adults.

#### *4.1.2.3 Translation*

Translation of these findings to humans is limited by a number of factors, including the common use of potent synthetic cannabinoids with full $CB_1R$ agonism (for instance, WIN) rather than THC, and often high doses compared with typical human consumption. Further, as described in chapter 1, the most convincing findings for acute effects of cannabis in humans relate to verbal learning and memory, however direct translation of such tasks to an animal

model is clearly not possible. The use of non-human primates at doses of THC that correspond well to human self-administration (Verrico et al., 2012) is preferable to rat models, though the expense and ethical considerations of working with non-human primates restricts more frequent usage of these animals in cannabis research.

### 4.1.3 Summary

Despite mixed findings, cannabinoid administration studies in adolescent rodents and non-human primates predominantly suggest that the adolescent brain is differentially sensitive to the effects of cannabis. Should these findings translate to humans, these age-related sensitivities may contribute to an increased risk of cannabis-related harms in teenagers. Indeed, it has been suggested that if adolescents are less sensitive to the acute negative effects (e.g. increased anxiety) of cannabis (and other recreational substances, as has been suggested for alcohol (Spear, 2016)) then this may lead to greater drug consumption than adults (Schramm-Sapyta et al., 2007). However acute studies in humans have rarely explored the influence of age on drug effects. Indeed, we are aware of no controlled studies in which cannabis was administered to individuals under 18 years of age.

### 4.1.4 Research questions and hypotheses

The present study therefore aimed to compare the acute effects of cannabis in adolescent and adult users, asking whether adolescents are more vulnerable to the acute subjective, physiological, memory and inhibition effects of cannabis than adults.

As described in chapter 1, in adults, acute cannabis administration typically induces verbal memory impairments (Broyd et al., 2015; Ranganathan & D'souza, 2006) and may impair working memory and response inhibition (Broyd et al., 2015). Acutely cannabis also increases subjective drug-related experiences (e.g. feeling 'stoned') (Green et al., 2003). Based on the preclinical findings described above of differential sensitivities to cannabinoids in adolescent and adult animals, I developed the following hypotheses:

1. Firstly, I hypothesised that adolescents would be less sensitive to the intoxicating (Carvalho et al., 2016; Quinn et al., 2008; Schramm-Sapyta et al., 2007) and anxiogenic (Acheson et al., 2011; Schramm-Sapyta et al., 2007) effects of cannabis compared to adults.
2. Secondly, I hypothesised greater cognitive impairment following cannabis in adolescents than adults (Cha et al., 2007; Cha et al., 2006; Schneider et al., 2008), as indexed by spatial working memory, episodic memory and response inhibition.

## 4.2 Methods

### 4.2.1 Design and Participants

A mixed within- and between-subjects, double-blind, cross-over design was used to compare the acute effects of both active and placebo cannabis on adolescents and adults. Treatment order was counterbalanced for task version and randomised via random number generator within each age group.

We recruited 20 adolescent (aged 16-17 years) and 20 adult (24-28 years) male cannabis users, via local and online (social media) advertising and word-of-mouth. The following inclusion criteria were assessed at telephone screening: male gender (due to evidence of sex differences in onset of puberty and ontogeny of adolescent brain development); current cannabis use between 1-3 days per week; at least 6 months of regular (at least once per week) cannabis use; no extended period (>1 month) of daily use; score $\leq$3 on the Cannabis Severity of Dependence Scale (SDS) reflecting the validated adolescent cut-off for dependence (Martin, Copeland, Gates, & Gilmour, 2006); no other illicit drug used >twice/month; no current mental health problem or history (personal or immediate family) of psychosis-related disorders; healthy-range body-mass index (BMI) and blood pressure (BP). Participants were required to be current cannabis users for a number of reasons: firstly, I was interested in the effects of cannabis use on

recreational users so selection of a representative sample was important for generalisability; and secondly, to prevent the administration of a commonly abused substance to cannabis-naïve individuals (as is standard practice in such studies). Participants were asked to remain abstinent from all drugs including alcohol but not cigarettes for 24 hours before each testing session.

The study was approved by UCL Research Ethics Committee. All participants provided written informed consent (in the UK 16-17 year olds are able to provide informed consent without additional parental consent or assent). Participants were reimbursed for their time (£7.50 per hour) and travel expenses.

### 4.2.2 Drug administration

Medicinal-grade active (Bedrobinol®; THC 12.0%) and placebo (THC <0.3%) cannabis were imported under UK Home Office licence from Bedrocan® in The Netherlands. Dose was weight-adjusted as age differences in body weight were anticipated. Following previous protocols (Bossong et al., 2009; Hindocha et al., 2014; Lawn, Freeman, Pope, Joye, Harvey, Hindocha, Mokrysz, Moss, Wall, & Bloomfield, 2016) participants received 0.89 mg/kg of cannabis, corresponding to approximately 8.0mg THC for an individual weighing 75kg. This dose corresponds to that contained in about a third of a typical joint (van der Pol et al., 2014). Similar doses have previously been shown to produce robust subjective effects via the administration method used in this study (Bossong et al., 2009; Hindocha et al., 2014; Lawn, Freeman, Pope, Joye, Harvey, Hindocha, Mokrysz, Moss, Wall, & Bloomfield, 2016).

Drug was administered via a Volcano Medic vaporiser (Storz and Bickel GmbH & Co., Germany), operating at 210°C. This method has been shown to be safe, producing equivalent pulmonary and plasma cannabinoid levels to those from smoked cannabis, but with lower expired carbon monoxide levels (Abrams et al., 2007; Hazekamp, Ruhaak, Zuurman, van Gerven, & Verpoorte, 2006; Lanz, Mattsson, Soydaner, & Brenneisen, 2016). Vapour was collected in a ‘balloon’ with a non-return valve, and inhaled according to a previous timed breath-holding protocol (Lawn, Freeman, Pope, Joye, Harvey, Hindocha, Mokrysz, Moss, Wall,

& Bloomfield, 2016). Participants inhaled, held their breath for 8 seconds and repeated this at their own pace until the balloon was empty. Each dose was vaporized in two sequentially administered balloons to minimise residual cannabinoids.

### 4.2.3 Measures

#### *4.2.3.1 Baseline Assessments*

##### 4.2.3.1.1 Questionnaires

Premorbid verbal intelligence was assessed by the Wechsler Test of Adult Reading (WTAR) (Holdnack, 2001), and scores were adjusted for age. The test includes 50 words with atypical grapheme to phoneme translations and is designed to assess previous learning of the word. Participants are required to read each item out loud in turn. The test is validated for use in individuals aged 16-89 years.

Depression was assessed on the 21 item self-report Beck Depression Inventory-II (BDI-II) (Beck, Steer, & Brown, 1996), rated for the past week. Response options vary by item, each item being rated between 0 (no experience of symptom) and 3 (severe experience of symptom). Higher scores indicate greater severity of depression.

Anxiety was assessed on the 21 item self-report Beck Anxiety Inventory (BAI) (Beck, Epstein, Brown, & Steer, 1988), rated for the past week. Items are rated between 0 (not at all) and 3 (severely). Higher scores indicate greater severity of anxiety.

A validated short version of the self-report UPPS-P Impulsive Behaviour Scale (SUPPS-P) (Cyders, Littlefield, Coffey, & Karyadi, 2014; Lynam, 2013) indexed impulsivity. The short version has 20 items that are rated between 1 (strongly agree) and 4 (strongly disagree); 6 items are reverse-scored. Higher scores indicate greater impulsivity.

The 74 item self-report Schizotypal Personality Questionnaire (SPQ) (Raine, 1991) indexed schizotypy. Items are rated either 0 (no) or yes (1). Higher scores indicate greater schizotypy.

#### 4.2.3.1.2 Drug use

A structured interview recorded: lifetime use (yes/no); time since last use (days); duration of use (years); frequency (days/month); and amount per session (alcohol units (standard UK units of alcohol; equivalent to 8g of pure alcohol or approximately 3/5ths of a NIAAA standardized drink) per typical drinking session; cigarettes/day; other illicit drugs grams/ pills/ tabs). Instant urine drug screens at the start of every session assessed recent use of illicit drugs (amphetamine, barbiturates, benzodiazepines, cocaine, MDMA, methamphetamine, methadone, opiates, oxycodone, phencyclidine, THC). Problematic drug use was assessed using the Cannabis Abuse Screening Test (CAST) (Legleye, Karila, Beck, & Reynaud, 2007), the Fagerström Test for Nicotine Dependence (FTND) (Heatherton, Kozlowski, Frecker, & Fagerstrom, 1991), and the Alcohol Use Disorders Identification Test (AUDIT) (Babor, Higgins-Biddle, Saunders, & Monteiro, 2001).

### *4.2.3.2 Experimental assessments*

#### 4.2.3.2.1 Physiological measurements

Body weight, blood pressure and heart rate were measured at baseline. BP and heart rate were monitored throughout drug administration sessions.

#### 4.2.3.2.2 Subjective Ratings

Participants provided ratings from 0 (not at all) to 10 (extremely) for “Stoned”, “High”, “Feel drug effect”, “Like drug effect” “Alert”, “Anxious”, “Paranoid”, “Dry mouth”, “Enhanced colour perception”, “Enhanced sound perception”, “Want to have food”, and “Want to have cannabis”, at a mean time of -6 minutes (apart from “Feel drug effect” and “Like drug effect”), +7 minutes, +34 minutes and +77 minutes (drug administration started at 0 minutes).

#### 4.2.3.2.3 Memory

##### 4.2.3.2.3.1 Prose recall

This episodic memory task was adapted from the Rivermead Behavioural Memory Test battery (Wilson, Cockburn, Baddeley, & Hiorns, 1989). Participants listened to a 30s story and then for 1 minute wrote down what they remembered immediately and again after ~1 hour. Each story contained 21 'idea units' and scoring was standardised.

#### 4.2.3.2.3.2 Spatial N-back

A computerised spatial version of the n-back task (Braver et al., 1997; T. P. Freeman et al., 2012) was used to assess spatial working memory. Stimuli appeared sequentially in one of six possible locations on screen, around a fixation cross. Participants responded 'yes' (signal trial) or 'no' (no-signal trials) as to whether the stimulus was in the same position as the stimulus one before (low load; '1-back') or two before (high load; '2-back'). Performance was indexed by discriminability (*d*'), and reaction time for correct trials. Each load consisted of 50 trials, with seven practice trials preceding each load. For each load, 48% of the trials were signal trials. Each trial started with a smiley face being presented on screen for 600ms, followed by 1500ms inter-stimulus interval where only the fixation cross was visible on the screen; each trial therefore lasted 2100ms. Participant responses were recorded from stimulus-onset until the start of the next trial.

Discriminability (*d*'), which indexed a person's ability to differentiate between signal and no-signal trials, was calculated using the following formula (Stanislaw & Todorov, 1999):

$$Discriminability = Z(H) - Z(F)$$

On signal trials, a correct response (i.e. 'yes') represents a hit, and an incorrect response (i.e. 'no') represents a miss. On no-signal trials a correct response (i.e. 'no') represents a correct negative, and an incorrect response (i.e. 'yes') represents a false alarm. In the above formula 'H' represents the hit rate (i.e. total number of hits divided by the total number of signal trials), and 'F' represents the false alarm rate (i.e. total number of false alarms divided by the total number of no-signal trials). Hit and false alarm rates of 1 and 0 were corrected by substituting hit rates of 1 by (n-0.5)/n, where n represents the total number of signal trials, and by substituting false

alarm rates of 0 by 0.5/n, where n represents the total number of no-signal trials (Macmillan & Kaplan, 1985).

#### 4.2.3.2.4 Response inhibition

##### 4.2.3.2.4.1 Stop-signal

A up/down staircase tracking version of the stop-signal was used to measure response inhibition (Verbruggen, Logan, & Stevens, 2008). Stimuli (white arrows) appeared sequentially in the centre of the screen; participants responded when the white arrow pointed left or right by pressing either the right or left arrow key. On 25% of trials, the arrow became blue following a variable delay (signal trials) and participants were instructed to not press either arrow key (i.e. inhibit the prepotent response). For signal trials, the initial variable delay between stimulus onset and signal was 250ms. The staircase tracking protocol proceeded as follows: if the participant successfully inhibited their response on a signal trial then on the next signal trial the delay was reduced by 50ms; however, if the participant failed to inhibit their response on a signal trial, then on the next signal trial the delay was increased by 50ms. This protocol is intended to control the accuracy of stopping, to locate the delay at which participants successfully stopped with 50% probability and therefore ensure reliable modelling of the stop-signal reaction time (SSRT). Each trial lasted 1500ms from stimulus (white arrow) onset, with an inter-trial interval of 500ms. There were 2 blocks of 96 trials, separated by a 15 second break. The 2 blocks were preceded by 32 practice trials with immediate feedback. Performance was assessed with SSRT, and accuracy and reaction times on no-signal trials.

### 4.2.4 Procedure

Following screening participants attended a 1-hour baseline session during which they provided informed consent, completed baseline measures, drug histories and problematic use questionnaires, task training, and physiological measurements.

Participants then completed two test sessions separated by at least seven days at least one week (>3 times the elimination half-life of THC) to minimise carryover effects (D'Souza et al., 2004; Hindocha, Freeman, et al., 2015). Participants first provided baseline subjective ratings, and BP and heart rate were measured (Time 1; T1). Active or placebo cannabis was then administered and participants again completed subjective ratings, BP and heart rate measures (Time 2; T2). Tasks and state questionnaires were then completed in the following order; prose recall (immediate), subjective ratings (Time 3; T3), spatial N-back, stop-signal, prose recall (delayed), subjective ratings (Time 4; T4), BP and heart rate (T4). Test sessions finished 80 minutes after drug inhalation.

### 4.2.5 Power calculation

To detect a medium effect size (f= 0.25) for the key interaction of interest (group x drug), with 80% power at an alpha of 5%, we required a sample size of 34. To account for drop-out and task adherence issues we tested 40 in total.

### 4.2.6 Statistical Analysis

All analyses were conducted with SPSS 21.0. Syntax and data are available from CM on request. Outliers and normality were assessed via diagnostic plots for all analyses. Extreme outliers (>3 times interquartile range) were winsorized within-group. Greenhouse-Geisser corrections were applied for violations of sphericity. Independent t-test, chi-square, or Mann-Whitney analyses were conducted as appropriate to compare groups (adult, adolescent) on demographic and baseline measures.

Mixed ANOVA was conducted for all test outcomes, with the between-subjects factor of group (adolescent, adult; coded as 1, 2) and within-subjects factor of drug (placebo, cannabis; coded as 1, 2). Additional within-subjects factors were included for relevant analyses: time (T1, T2, T4; coded as 1, 2, 3) for physiological data; time (T1-T4; coded as 1, 2, 3, 4) for subjective ratings (only T2-T4 (coded as 1, 2, 3) were analysed for *stoned* (due to floor effects) and *feel drug*

*effect* & *like drug effect* (as these were not collected at T1)); N-back memory load (low, high; coded as 1, 2); prose recall delay (immediate, delayed; coded as 1, 2). Main effects and interactions with time were explored via Helmert contrasts (comparing Pre-drug (T1) to Post-drug (mean of T2-T4)), to reduce the number of comparisons. Other interactions were explored via pairwise comparisons with local Bonferroni-correction. Drug order was added as an additional between-subjects factor (placebo-first, cannabis-first; coded as 1, 2) and results were compared to reported primary analyses; unless otherwise noted results were unaffected by drug order. All statistical tests were two-tailed.

## 4.3 Results

### 4.3.1 Demographics (Table 4.1.)

Adolescents were younger, and had lower body weight. Groups did not differ on verbal IQ, BAI, BDI-II, SUPPS-P, or SPQ. Adolescents currently used for more days per month than the adults, and the age of first cannabis use was younger for the adolescents compared to the adults, but overall the adults had used for longer. Groups did not differ on CAST score, time since last use, or likelihood of a positive THC urine screen at baseline. Table 4.2. displays baseline instant drug screen results.

***Table 4.1.*** *Demographic and baseline variables for adolescents and adults; values reflect mean (SD) unless otherwise stated; p-values reflect independent t-test comparing mean, Mann-Whitney U-test comparing median, or chi-square comparing frequency (as appropriate), by age group.*

| | **Adolescents (n= 20)** | **Adults (n= 20)** | | |
|---|---|---|---|---|
| **Demographics** | **Mean (SD)** | **Mean (SD)** | **Test statistic** | **p-value** |
| Age (years) | *17.08 (0.44)* | *25.49 (1.07)* | *U= 400.000* | *<.001*[2] |
| Body weight (kg) | *66.40 (10.30)* | *74.96 (10.12)* | *U= 296.000* | *.009*[2] |
| Cannabis weight (mg) | *58.90 (7.65)* | *65.44 (6.56)* | *U= 299.500* | *.006*[2] |
| Verbal IQ (n=39) | *110.20 (11.29)* | *115.11 (8.70)* | *U= 245.000* | *.127* |
| **Baseline questionnaires** | | | | |
| Beck Anxiety Inventory | *4.55 (4.62)* | *6.45 (7.09)* | *U= 234.500* | *.355* |
| Beck Depression Inventory | *6.35 (4.66)* | *4.55 (4.38)* | *U= 152.000* | *.201* |
| SUPPS-P Impulsive Behaviour Scale | *45.55 (8.00)* | *45.40 (5.94)* | $t_{38}$*= 0.067* | *.947* |
| Schizotypal Personality Questionnaire | *20.90 (10.90)* | *15.21 (11.24)* | *U= 145.000* | *.142* |
| **Cannabis use** | | | | |
| Age first tried cannabis (years) | *14.73 (1.25)* | *17.71 (3.00)* | *U= 338.000* | *<.001*[2] |
| Last used cannabis (days) | *3.35 (2.52)* | *4.75 (3.78)* | *U= 259.500* | *.108* |
| Duration of cannabis use (years) | *2.35 (1.24)* | *7.78 (2.85)* | *U= 378.500* | *<.001*[2] |
| Cannabis use frequency (days per month) | *10.58 (4.33)* | *7.94 (5.27)* | *U= 121.000* | *.033*[2] |
| Positive THC urine at baseline (n=37); %(n) | *83.33 (15)* | *63.16 (12)* | $\chi^2_1$*= 1.908* | *.167* |
| Cannabis Abuse Screening Test | *6.45 (2.72)* | *5.60 (3.56)* | $t_{38}$*= 0.848* | *.402* |
| **Cigarette use** | | | | |
| Ever used cigarettes; %(n) | *95.00 (19)* | *75.00 (15)* | $\chi^2_1$*= 3.137* | *.077* |
| Age first tried cigarettes (years)[3] | *15.06 (1.49)* | *17.21 (2.61)* | *U= 279.000* | *.003*[2] |
| Duration of cigarette use (years) | *1.91 (1.41)* | *7.60 (3.44)* | *U= 356.500* | *<.001*[2] |
| Cigarette use frequency (days per month) | *19.28 (12.36)* | *10.37 (11.62)* | *U= 120.500* | *.030*[2] |
| Cigarettes per day | *3.74 (2.83)* | *1.84 (2.06)* | *U= 107.500* | *.011*[2] |
| Fagerström Test for Nicotine Dependence | *1.30 (1.03)* | *0.20 (0.70)* | *U= 81.000* | *<.001*[2] |
| Carbon Monoxide at baseline (ppm; n=38) | *6.00 (4.55)* | *5.68 (3.96)* | *U= 163.000* | *.624* |
| **Alcohol use** | | | | |
| Ever used alcohol; %(n) | *100.00 (20)* | *100.00 (20)* | *n/a* | *n/a* |
| Age first tried alcohol (years) | *14.07 (14.07)* | *14.56 (3.22)* | $t_{28}$*= -0.611*[1] | *.546* |
| Duration of alcohol use (years) | *3.01 (1.63)* | *10.93 (3.71)* | *U= 399.000* | *<.001*[2] |
| Alcohol use frequency (days per month) | *5.80 (4.83)* | *9.78 (6.00)* | *U= 283.500* | *.023*[2] |
| Alcohol units per typical drinking session[4] | *9.81 (6.92)* | *8.43 (2.82)* | *U= 190.000* | *.799* |
| Alcohol Use Disorders Identification Test | *8.95 (5.53)* | *8.95 (4.82)* | *U= 214.000* | *.718* |

[1]Levene's test for homogeneity of variance violated
[2]p<.05
[3]calculated only on those who had ever used cigarettes (n=34)
[4]units= standard UK units of alcohol; equivalent to 8g of pure alcohol or approximately 3/5ths of a NIAAA standardized drink

***Table 4.2.*** *Frequency of positive/negative baseline session urine instant screen results, by age group. Due to an administrative error, data is missing for 2 adolescents and 1 adult.*

| | **Adolescents** | **Adults** |
|---|---|---|
| | n= 18 | n= 19 |
| | pos/neg | pos/neg |
| **Amphetamine** | *0/18* | *0/19* |
| **Barbiturates** | *0/18* | *0/19* |
| **Benzodiazepines** | *0/18* | *1/18* |
| **Cocaine** | *0/18* | *3/16* |
| **MDMA** | *0/18* | *0/19* |
| **Methamphetamine** | *0/18* | *0/19* |
| **Methadone** | *0/18* | *0/19* |
| **Opiates** | *0/18* | *1/18* |
| **Oxycodone** | *0/18* | *0/19* |
| **Phencyclidine** | *0/18* | *1/18* |
| **THC** | *15/3* | *12/7* |

### 4.3.2 Physiological data (Figure 4.1.)

#### *4.3.2.1 Heart rate*

An interaction of drug x time ($F_{1,38}$= 82.879, p< .001, $\eta^2p$= 0.69) was found, with heart rate increasing from Pre-drug to Post-drug after cannabis (p< .001, $\eta^2p$= 0.65) but not placebo (p= .449, $\eta^2p$= 0.01). Main effects of drug ($F_{1,38}$= 89.327, p< .001, $\eta^2p$= 0.70) and time ($F_{1,38}$= 44.141, p< .001, $\eta^2p$= 0.54) also emerged.

#### *4.3.2.2 Systolic BP*

No main effects or interactions were found.

#### *4.3.2.3 Diastolic BP*

Interactions of drug x group x time ($F_{1,38}$= 4.393, p= .043, $\eta^2p$= 0.10), drug x group ($F_{1,38}$= 4.744, p= .036, $\eta^2p$= 0.11), and drug x time ($F_{1,38}$= 4.977, p= .032, $\eta^2p$= 0.12) emerged. For

adolescents there was no drug x time interaction ($p = .919$, $\eta^2p < 0.01$); while for adults a drug x time interaction ($p = .010$, $\eta^2p = 0.30$) revealed an increase in diastolic BP from Pre-drug to Post-drug for cannabis ($p = .016$, $\eta^2p = 0.27$), but no change over time for placebo ($p = .060$, $\eta^2p = 0.17$). Main effects of drug ($F_{1,38} = 7.390$, $p = .010$, $\eta^2p = 0.16$) and group ($F_{1,38} = 7.998$, $p = .007$, $\eta^2p = 0.17$) also emerged.

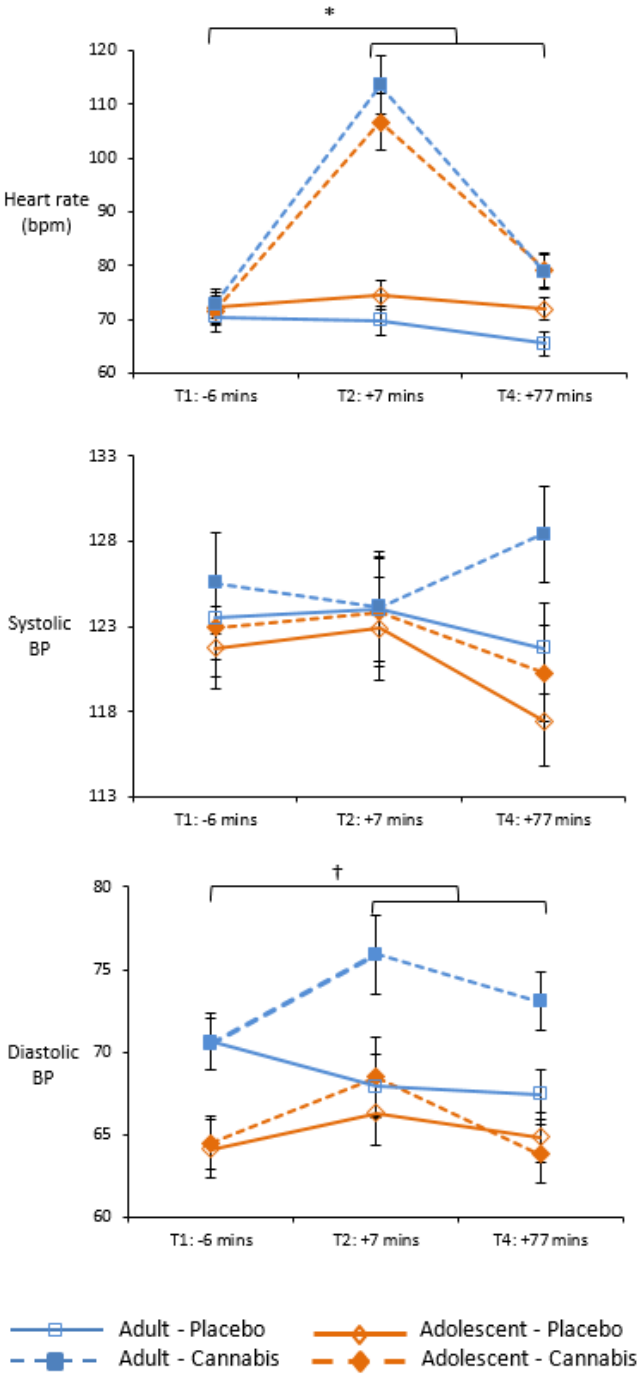


***Figure 4.1***. *Mean (SE) values for heart rate (bpm), systolic and diastolic blood pressure, for adolescents and adults on cannabis and placebo.* = heart rate increased from Pre-drug to Post-drug for cannabis (p< .001) but not placebo (p= .449); † = for adults diastolic BP increased from Pre-drug to Post-drug on cannabis (p= .016) but not placebo (p= .060).*

### 4.3.3 Subjective Ratings (Figure 4.2.)

#### *4.3.3.1 'Stoned'*

There was an interaction of drug x group ($F_{1,38}$= 4.893, p= .033, $\eta^2p$= 0.11). Ratings of both adolescents (p< .001, $\eta^2p$= 0.65) and adults (p< .001, $\eta^2p$= 0.78) were greater after cannabis compared to placebo, however the increase was larger in adults. Main effects of drug ($F_{1,38}$= 200.055, p <.001, $\eta^2p$= 0.84) and time ($F_{2,63}$= 8.271, p= .001, $\eta^2p$= 0.18) also emerged.

#### *4.3.3.2 'Feel drug effect'*

There was an interaction of drug x group ($F_{1,38}$= 8.877, p= .005, $\eta^2p$= 0.19), with adolescents feeling the drug effect less than adults after cannabis (p= .017, $\eta^2p$= 0.14), but not after placebo (p= .565, $\eta^2p$= 0.01). Main effects of drug ($F_{1,38}$= 297.629, p< .001, $\eta^2p$= 0.89) and time ($F_{2,65}$= 9.629, p< .001, $\eta^2p$= 0.20) also emerged.

#### *4.3.3.3 'Alert'*

There was an interaction of drug x group ($F_{1,38}$= 9.123, p= .004, $\eta^2p$= 0.19), with adolescents rating no difference in alertness on cannabis compared to placebo (p= .955, $\eta^2p$< 0.01), whereas adults rated lower alertness on cannabis compared to placebo (p< .001, $\eta^2p$= 0.33). There was also an interaction of drug x time ($F_{1,38}$= 42.844, p< .001, $\eta^2p$= 0.53); with alertness decreasing from Pre-drug to Post-drug in both sessions, though the decrease was larger for cannabis (p< .001, $\eta^2p$= 0.65) than for placebo (p= .005, $\eta^2p$= 0.19). Main effects of drug ($F_{1,38}$= 9.613, p= .004, $\eta^2p$= 0.20) and time ($F_{1,38}$= 60.071, p< .001, $\eta^2p$= 0.61) also emerged.

#### *4.3.3.4 'Anxious'*

There was an interaction of drug x group ($F_{1,38}$= 4.272, p= .046, $\eta^2p$= 0.10), with adolescents reporting no difference in anxiety on cannabis compared to placebo (p= .516, $\eta^2p$= 0.01), but adults reporting more anxiety on cannabis compared to placebo (p= .001, $\eta^2p$= 0.25). There was also an interaction of drug x time ($F_{1,38}$= 9.914, p= .003, $\eta^2p$= 0.21); with no change in anxiety

after taking cannabis (p= .275, $\eta^2p$= 0.03) and a decrease in anxiety after taking placebo (p< .001, $\eta^2p$= 0.39). A main effect of drug ($F_{1,38}$= 8.969, p= .005, $\eta^2p$= 0.19) also emerged.

#### *4.3.3.5 'Dry mouth'*

There were interactions of drug x group x time ($F_{1,38}$= 9.417, p= .004, $\eta^2p$= 0.20), drug x group ($F_{1,38}$= 6.436, p= .015, $\eta^2p$= 0.15), and drug x time ($F_{1,38}$= 72.572, p< .001, $\eta^2p$= 0.66). Both adolescents (p< .001, $\eta^2p$= 0.52) and adults (p< .001, $\eta^2p$= 0.72) reported an increase in dry mouth from Pre-drug to Post-drug on cannabis, though the increase was greater for adults. On placebo there was no change in dry mouth over time for adolescents (p= .495, $\eta^2p$= 0.03) or adults (p= .244, $\eta^2p$= 0.07). Main effects of drug ($F_{1,38}$= 44.682, p< .001, $\eta^2p$= 0.54) and time ($F_{1,38}$= 46.168, p< .001, $\eta^2p$= 0.55) also emerged.

#### *4.3.3.6 'Want to have cannabis'*

There was an interaction of group x time ($F_{1,38}$= 9.661, p= .004, $\eta^2p$= 0.20). From Pre-drug to Post-drug, wanting of cannabis increased in the adolescents (p= .048, $\eta^2p$= 0.19) and decreased in the adults (p= .031, $\eta^2p$= 0.22). There was also an interaction of drug x time ($F_{1,38}$= 5.933, p= .020, $\eta^2p$= 0.14); wanting of cannabis increased after taking placebo (p= .037, $\eta^2p$= 0.11), but did not change after taking cannabis (p= .177, $\eta^2p$= 0.05).

#### *4.3.3.7 Other subjective ratings*

Comparable analyses revealed that compared to placebo, cannabis increased subjective ratings for 'paranoid', 'mentally impaired', 'high', 'like drug effect', 'want to have food', 'enhanced colour perception', and 'enhanced sound perception' (all p's < .05). However, there were no group-related differences or interactions for any of these ratings (all p's > .05).

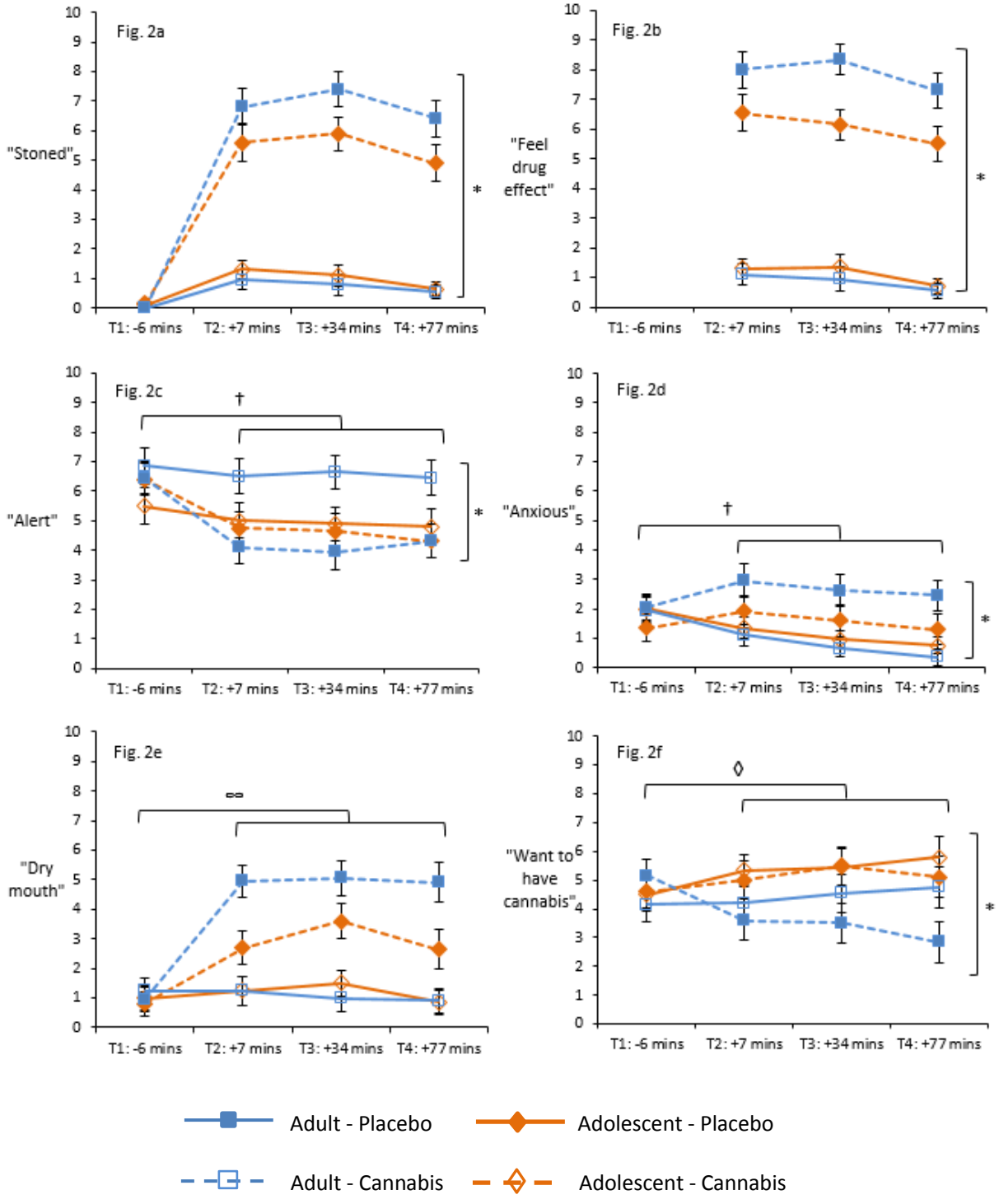


***Figure 4.2.*** Mean (SE) values for subjective ratings (0-10) for 'stoned', 'feel drug effect', 'alert', 'anxious', 'dry mouth', 'want to have cannabis', for adolescents and adults on placebo and cannabis. * = drug x group interaction ($p \leq .046$); † = drug x time interaction ($p \leq .003$); ∞ = drug x group x time interaction ($p = .004$); ◊ = group x time interaction ($p = .004$).

### 4.3.4 Memory

#### *4.3.4.1 Spatial N-back*

Five participants were excluded (3 adults, 2 adolescents) due to <50% accuracy. Table 4.3. contains descriptive data for the task.

##### 4.3.4.1.1 Discriminability

Main effects of drug ($F_{1,33}$= 30.495, p< .001, $\eta^2p$= 0.48) and load ($F_{1,33}$= 26.054, p< .001, $\eta^2p$= 0.44) were found. Discriminability was poorer on cannabis than placebo, and on high load than low load.

##### 4.3.4.1.2 Reaction time (correct trials)

Initial analyses demonstrated main effects of drug ($F_{1,33}$= 12.221, p= .001, $\eta^2p$= 0.27) and load ($F_{1,33}$= 44.430, p< .001, $\eta^2p$= 0.57), with no interactions. Reaction times were longer on cannabis than placebo, and on high load than low load. However, after adding drug order to the model, an interaction of drug x group ($F_{1,31}$= 4.447, p= .043, $\eta^2p$= 0.13) also emerged. For adolescents there was no difference in reaction times between cannabis and placebo (p= .076, $\eta^2p$= 0.10), while for adults reaction times were longer after cannabis than placebo (p< .001, $\eta^2p$= 0.41).

***Table 4.3.*** *Means and standard deviations for spatial N-back, prose recall and stop-signal tasks, by drug and age group.*

| | **Adolescents** | | **Adults** | |
|---|---|---|---|---|
| | **Placebo** | **Cannabis** | **Placebo** | **Cannabis** |
| | **Mean (SD)** | **Mean (SD)** | **Mean (SD)** | **Mean (SD)** |
| **Spatial N-back** | n=18 | | n=17 | |
| *Low load (1-back)* | | | | |
| Dicriminability | *3.28 (0.59)* | *2.75 (0.72)* | *3.28 (0.60)* | *2.94 (0.54)* |
| Reaction time correct trials (ms) | *536.74 (88.43)* | *565.36 (149.39)* | *532.16 (67.09)* | *633.55 (126.34)* |
| *High load (2-back)* | | | | |
| Dicriminability | *2.75 (0.91)* | *1.91 (0.93)* | *3.06 (0.63)* | *2.25 (1.14)* |
| Reaction time correct trials (ms) | *642.76 (135.29)* | *699.89 (170.10)* | *694.08 (174.09)* | *790.35 (222.01)* |
| **Prose recall** | n=20 | | n=20 | |
| Immediate | *6.80 (2.57)* | *4.70 (2.94)* | *6.53 (1.96)* | *4.03 (1.73)* |
| Delayed | *6.08 (2.68)* | *4.55 (2.89)* | *6.68 (1.90)* | *3.45 (1.81)* |
| **Stop-signal** | n=19 | | n=18 | |
| SSRT (ms) | *209.43 (64.95)* | *228.12 (67.35)* | *214.71 (46.51)* | *198.90 (43.61)* |
| Accuracy on no-signal trials | *0.990 (0.012)* | *0.965 (0.041)* | *0.989 (0.010)* | *0.986 (0.013)* |

#### *4.3.4.2 Prose recall (Figure 4.3a)*

Table 4.3. contains descriptive data for the task.

There was an interaction of drug x delay x group ($F_{1,38}$= 5.518, p= .024, $\eta^2p$= 0.13), with adolescents recalling fewer items after cannabis than placebo, both immediately (p= .002, $\eta^2p$= 0.22) and after the delay (p= .038, $\eta^2p$= 0.11). Adults also recalled fewer after cannabis than placebo, both immediately (p< .001, $\eta^2p$= 0.28) and after the delay (p< .001, $\eta^2p$= 0.35); however, the reduction in items recalled after cannabis compared to placebo for delayed recall was twice as large in adults than adolescents. A main effect of drug ($F_{1,38}$= 25.869, p< .001, $\eta^2p$= 0.41) also emerged.

### 4.3.5 Response inhibition

#### *4.3.5.1 Stop-signal*

Two participants (one adult, one adolescent) had missing data due to technical issues; one adult was excluded due to an improbable SSRT (<50ms (Congdon et al., 2012)). Table 4.3. contains descriptive data for the task.

##### 4.3.5.1.1 SSRT

No main effects of drug ($F_{1,35}$= 0.015, p= .903, $\eta^2p$< 0.01) or group ($F_{1,35}$= 0.674, p= .417, $\eta^2p$= 0.19) were found. No interaction of drug x group was found ($F_{1,35}$= 2.160, p= .151, $\eta^2p$= 0.06).

##### 4.3.5.1.2 Accuracy on no-signal trials (Figure 4.3b)

There was an interaction of drug x group ($F_{1,35}$= 4.906, p= .033, $\eta^2p$= 0.12), with adolescents being less accurate on cannabis compared to placebo (p= .001, $\eta^2p$= 0.28), whereas drug did not affect adults' accuracy (p= .644, $\eta^2p$= 0.01). A main effect of drug ($F_{1,35}$= 8.306, p= .007, $\eta^2p$= 0.19) also emerged.

##### 4.3.5.1.3 Reaction times on no-signal trials

No main effects of drug ($F_{1,35}$= 0.903, p= .349, $\eta^2p$= 0.03) or group ($F_{1,35}$= 1.749, p= .195, $\eta^2p$= 0.05) were found. No interaction of drug x group was found ($F_{1,35}$= 0.013, p= .909, $\eta^2p$< 0.01).

**Figure 4a. Prose recall**

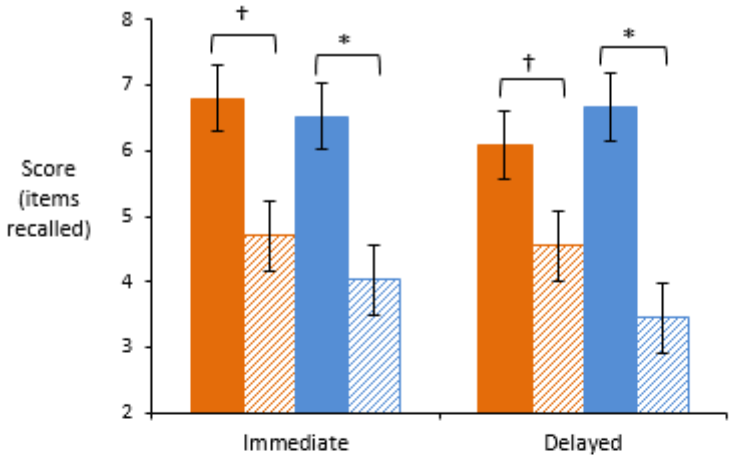


**Figure 4b. Stop-signal**

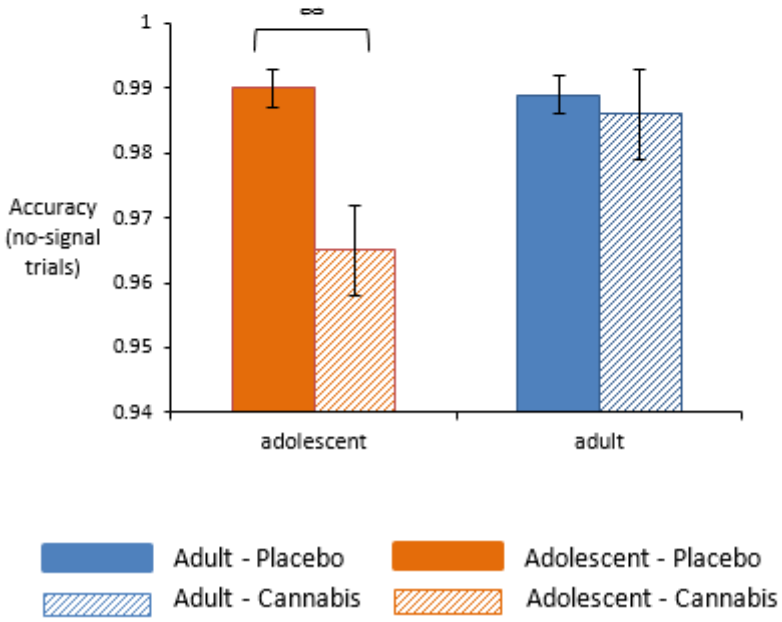


***Figure 4.3.*** *Mean (SE) values for a) prose recall score (number of items recalled, out of a total of 21), and b) stop-signal accuracy (proportion of no-signal trials with a correct (i.e. no button press) response), for adolescents and adults on placebo and cannabis. * = adult scores after taking cannabis were lower than after taking placebo (p< .001); † = adolescent scores after taking cannabis were lower than after taking placebo (p≤ .038); ∞ = adolescents were less accurate after taking cannabis than placebo (p= .001).*

### 4.3.6 Correlations

Within-group correlations were conducted between all cannabis session outcomes in which we found group main effects or interactions, and variables showing baseline group differences (at $p < .10$; Table 4.1.), including administered cannabis weight. Cannabis weight was not found to correlate with any outcome in either group. None were found to correlate (at $p < .10$) with any outcome measure in both the adolescent and adult groups, and so were not entered into models.

## 4.4 Discussion

In the first study to examine the causal effects of acute cannabis administration in human adolescence and adulthood, I found two differing profiles of effects. Compared with adults, adolescents experienced blunted subjective and physiological effects of cannabis, while cannabis impaired inhibitory processes in adolescents but not adults. Specifically, on cannabis adolescents reported feeling less stoned, feeling less effect of the drug and less dry mouth. The adults were also markedly more anxious and less alert during the cannabis session than the placebo session, while no session difference was found for the adolescents (however, since these group differences did not differ over time, these may be session effects rather than effects of cannabis). Indeed, there was no subjective rating on which adolescents reported greater drug effect than adults. Further, adults' but not adolescents' diastolic blood pressure rose after cannabis.

Intriguingly, I found opposing effects between age groups on wanting of cannabis following drug administration. The adolescents did not show a typical satiety effect, wanting more cannabis post-drug regardless of whether they had taken cannabis or placebo. Meanwhile the adults wanted less cannabis post-drug, an effect that appears to be driven by a decrease in wanting following cannabis but not after placebo (although this putative interpretation remains tentative in the absence of a group x drug x time interaction).

In terms of cognitive effects, when intoxicated with cannabis adults showed greater impaired recall of prose following a delay than adolescents. After adjusting for drug order the adults also had longer response times on the spatial working memory task following cannabis, while the adolescents were not affected. While neither group were impaired at inhibiting a pre-potent response following cannabis, the adolescents but not adults were less accurate on the response inhibition task after cannabis.

These results are in line with my first hypothesis that adolescents would be less sensitive to physiological, intoxication and anxiogenic effects compared with adults. These findings accord with the preclinical evidence which shows reduced anxiogenic, aversive and locomotor effects in adolescent rodents (Acheson et al., 2011; Carvalho et al., 2016; Quinn et al., 2008; Schramm-Sapyta et al., 2007). The age group difference in the effect of cannabis on anxiety is intriguing, and may have implications for understanding the aetiology of clinical anxiety- though whether cannabis is a useful pharmacological model of anxiety needs to be determined by future work delineating the specific components of anxiety that are induced by cannabis.

Additionally, partial support for my second hypothesis, that I would see greater cognitive impairment following cannabis in adolescents than adults, was seen in greater impairment of response inhibition accuracy following cannabis in the adolescents compared to adults. However contrary to expectations I did not see greater cannabis-related memory impairment in the adolescents, instead finding evidence of greater impairment in adults. Preclinical evidence for greater adolescent sensitivity to acute memory-impairing effects of cannabis is however inconsistent (Realini, Rubino, & Parolaro, 2009). As described in chapter 1, in adult humans cannabis appears to selectively impair verbal and working memory domains (Broyd et al., 2015), apparently leaving other memory domains intact (Curran et al., 2002), while rodents typically become impaired on a wide range of memory tasks across domains including object recognition and spatial learning, implying that preclinical findings for cannabis and memory may be somewhat limited in translation. However, as described in chapter 1, while much work has addressed verbal and working memory effects of cannabis, there has been considerably less

research into the effects in other memory domains, so whether these preclinical effects translate to human adults or adolescents is not known.

The inconsistency of previous findings for working memory impairments following cannabis that I described in chapter 1 are surprising, given the large drug main effects I found on discriminability (f= 0.96) and reaction time (f= 0.61). It is possible that previous studies were underpowered, but such effect sizes would be detectable in a within-subjects sample of 18 (at 80% power with a 5% alpha), which most studies did achieve. It is possible that the large effect on working memory in this study to some extent reflects a general intoxication effect rather than a memory-specific effect, however this is unlikely given that the average peak rating for feeling stoned was only 7 on a scale of 0-10, which is comparable to previous studies.

Meanwhile, adults but not adolescents were found to have longer reaction times on cannabis than placebo on the working memory task. This secondary outcome group difference may suggest a spatial working memory deficit in adults following cannabis, but an alternative explanation is that this effect instead suggests a psychomotor slowing effect of cannabis in adults but not adolescents. Indeed, this explanation would be broadly in line with preclinical findings of reduced locomotor-suppression effects of cannabis in adolescent relative to adult rats (Schramm-Sapyta et al., 2007). Importantly however, cannabis had no effect on no-signal (that is, trials on which the participant does not need to inhibit their response) reaction times in either group on the stop-signal task, potentially arguing against a general psychomotor slowing interpretation of the working memory findings in adults.

Relatedly, the key indicator of response inhibition on the stop-signal task is stop-signal reaction time, for which I again found no effect of cannabis in either group. As described in chapter 1, previous findings in adults for the effects of cannabis on the stop-signal task are mixed, with many finding effects on accuracy or reaction times, but fewer finding effects on stop-signal reaction time. As such, this null effect was not overly surprising. Meanwhile, on the secondary task outcome, adolescents did have lower accuracy on no-signal trials after cannabis, while the

adult's accuracy was unaffected. Given that accurate responding on non-signal trials simply requires execution of a basic learned response (i.e. left-pointing arrow on screen= press left arrow key, right-pointing arrow on screen= press right arrow key), poorer accuracy potentially suggests a general reduction in task engagement, rather than a domain-specific effect on response inhibition.

### 4.4.1 Limitations

The study is not without limitations, and these will be discussed in detail in the final chapter of this thesis, since many of the issues are relevant across all of my acute studies comparing adolescents and adults. To briefly highlight the key issues, firstly I cannot speak to mechanism of the reported age-related sensitivities. While the findings may represent age-related neural sensitivities to cannabis, there are a number of alternative explanations, as will be discussed in chapter 7. Secondly, while the groups were well-matched for cannabis abuse symptomology and days since last cannabis use, the adolescents did report more days of cannabis usage per month than the adults (11 days versus 8 days). Additionally, while the adults had been using for more years, they had started using from an older age. This raises the possibility that the adolescents may have developed increased tolerance to cannabis relative to the adults. Lastly, since participants were given a weight-adjusted dose (because adolescents typically weigh less than adults (Sutton, 2012)), on average the adolescents received a lower dose. This means that I cannot rule out the possibility that the blunted effects seen in the adolescents are due to the reduced dose, though other groups have commonly administered a weight-adjusted cannabis dose via inhalation without reporting any dose effects (Ramaekers et al., 2008; Ramaekers et al., 2006; Ramaekers et al., 2016). Moreover, critically the weight of cannabis administered did not correlate with any outcome in either group. The potential impact of these limitations and discussion of how to address these issues in future can be found in chapter 7.

### 4.4.2 Conclusions

In this chapter I set out to answer the question:

"Are adolescents more vulnerable to the acute subjective, physiological, memory and inhibition effects of cannabis than adults?"

Compared to adults, adolescent cannabis users experienced blunted subjective, physiological, and memory impairing effects of cannabis. Further, adolescents were not satiated by cannabis and the drug impaired their inhibitory processes while leaving those of adults intact. In agreement with preclinical cannabinoid administration studies, I found evidence to suggest that human adolescents and adults are differentially sensitive to the acute effects of cannabis. Longitudinal research is now needed to determine the degree to which age-related sensitivities are indeed contributing to escalated use and increased risk of cannabis-related harms in adolescent cannabis users.

# 5 Chapter 5: The effects of acute cannabis administration on psychotomimetic symptoms and speech illusion in adolescents and adults

## 5.1 Introduction

In the previous two chapters I focused mainly on the cognitive effects of cannabis use in adolescence, both non-acute (in Chapter 3) and acute (in Chapter 4). Here I move on to the acute psychotomimetic effects of cannabis.

As described in Chapter 2, a wealth of evidence demonstrates acute psychotomimetic effects of cannabis. However, few studies have addressed the psychological mechanisms through which cannabis influences specific psychotic symptoms and typically studies only report general increases in psychotomimetic symptoms as indexed by self- or clinician-rated scales, without looking at the specific symptoms affected by cannabis. No study to date has looked specifically at whether cannabis acutely leads to the experience of auditory-verbal hallucinations (AVH), and whether such cannabis-induced experiences are similar to those experienced by clinically psychotic patients. Further, as discussed in Chapter 2, it is probable that some cannabis use behaviours, such as using at a younger age or using CBD-lacking cannabis, are more likely to lead to psychotic-like effects than others.

As such in this chapter, using self-rated measures alongside a task assessing the experience of AVH previously shown to be sensitive to psychosis vulnerability, I ask firstly whether adolescent cannabis users are more susceptible to the acute psychotomimetic effects, including AVH, of cannabis than adults, and secondly whether using cannabis with higher CBD content can offset the acute psychotomimetic effects, including AVH, of cannabis.

### 5.1.1 Auditory-verbal hallucinations

Auditory-verbal hallucinations (AVH) are a common positive symptom of psychotic disorders, with the majority of patients experiencing them at some point during their illness, with estimates for the prevalence of AVH in schizophrenia patients ranging from 40% to 80% (Larøi et al.,

2012; Vercammen, De Haan, & Aleman, 2008). AVH are also fairly common in the general population, with a recent review estimating a median prevalence of 13%, though estimates vary widely across studies (Beavan, Read, & Cartwright, 2011). AVH have therefore often been described as existing along a continuum, ranging from infrequent and non-problematic hallucinations in healthy individuals, to patients with schizotypal or borderline personality disorder, to, at the most extreme end, patients with severe psychotic disorders such as schizophrenia who experience regular and often distressing hallucinations (Daalman et al., 2011; Van Os, Hanssen, Bijl, & Ravelli, 2000). Whether this is an accurate description, such that AVH experienced by healthy and clinical populations arise through qualitatively similar but quantitatively different mechanisms, remains debated. Indeed, such an explanation does not explain the content of AVH, such that patients typically experience AVH with distressing or emotional content, while non-clinical AVH are typically more neutral in content (Daalman et al., 2011; Daalman, Verkooijen, Derks, Aleman, & Sommer, 2012).

#### *5.1.1.1 Experimental manipulation of AVH*

To investigate the experience of AVH a number of studies have attempted to experimentally manipulate the experience of AVH in both people with psychosis and healthy controls. These tasks typically aim to provoke the experience of hearing voices in white noise (i.e. random noise) in the absence of actual speech, i.e. elicit AVH, often termed a 'speech illusion'.

As discussed above, AVH are not only experienced in the context of clinical psychotic disorder. To investigate this Merchelbach & van de Ven (2001) played white noise to healthy undergraduate students, instructing them to respond when they heard a well-known song ("White Christmas" by Bing Crosby) play. Thirty-two percent indicated they heard song, despite the music never being played (Merckelbach & van de Ven, 2001). Those who reported hearing the song also reported higher baseline levels of fantasy-proneness and hallucinations, indicating that the likelihood of experiencing AVH in a paradigm designed to increase

expectancy of speech is associated with variation of psychosis-related experiences in a healthy population.

Using a related methodology with prodromal patients, Hoffman and colleagues (Hoffman et al., 2007) played multi-speaker babble to prodromal patients with and without recently reported AVH. Participants were asked to write down any words or phrases they heard amongst the babble. No difference in the rate of speech illusion was found between hallucinating and non-hallucinating patients, however the length of the speech illusion (i.e. the number of words within each phrase reported) was found to predict subsequent conversion to psychotic disorder. While interpretation of this post-hoc finding is limited without replication, it suggests that certain qualities of AVH, here the complexity of the AVH, may be able to differentiate between those at risk of psychotic disorder and those not.

Tasks designed to elicit speech illusion have also been utilised to explore the cognitive mechanisms via which AVH arise. For instance, Vercammen and colleagues (2008) found that schizophrenia patients prone to auditory hallucinations, but not non-hallucination prone patients, were more likely than healthy controls to erroneously report that a spoken word they heard matched a previously presented word masked to varying degrees by white noise. They then used signal detection theory to demonstrate that the patients prone to auditory hallucinations had a more liberal criterion when deciding whether a word matched a previous stimuli or not (i.e. they were more willing to indicate that the words matched at higher levels of ambiguity), alongside a greater sensitivity for detecting matched words, relative to non-hallucinating patients. These findings suggest that AVH may be explained by patients having a lower threshold of certainty for determining whether a perception reflects an actual stimulus or not, such that noise in the environment may be misclassified as a true signal (Dolgov & McBeath, 2005; Rimvall et al., 2016; Vercammen et al., 2008).

Vercammen and Aleman later explored whether an overreliance on sematic top-down processing may explain the experience of speech illusion, in healthy undergraduates who scored

either high or low on a scale of hallucination proneness (Vercammen & Aleman, 2010). Participants were presented with sentences in which the final word was either masked by white noise or replaced by white noise. The last word of the sentence was either semantically expected or unexpected in relation to the rest of the sentence. Baseline hallucination proneness positively correlated with the number of top-down errors (that is, the number of times they reported hearing a word that fit the sentence context when it was not actually presented), suggesting that semantic top-down errors may lead to the experience of AVH. Using the same task, Daalman et al (2012) assessed whether overreliance on sematic top-down processing may explain the experience of AVH in psychotic patients, non-psychotic individuals who experience regular AVH, and healthy controls. Contrary to their expectations, the non-psychotic individuals who experienced regular AVH made more top-down errors than the psychotic patients. Together these two studies therefore suggest that while semantic top-down processing may influence the formation of AVH in non-clinical populations, this does not appear to be the case for psychotic patients. This potentially suggests a differing mechanism by which clinically relevant and non-relevant AVH are formed.

Given the findings described here, demonstrating that the likelihood of experiencing AVH in such experimental manipulations is associated with trait schizotypal experiences in healthy controls, future diagnosis of psychotic disorder, and hallucinations in psychotic patients, it seems that such paradigms are a valid method of investigating AVH, and may be able to differentiate between AVH experienced by clinical and non-clinical populations.

### 5.1.2 White Noise task

Recently Galdos and colleagues developed the White Noise (WN) task to further investigate the experience of AVH in patients with psychosis (Galdos et al., 2011). Similar to previous methodologies described above, the task provokes the experience of hearing voices in white noise in the absence of actual speech (i.e. speech illusion).

Adding to previous paradigms, the WN task also requires participants to classify speech illusions according to valence, i.e. positive, negative or neutral. This follows evidence that, psychotic patients experience higher levels of emotional AVH than both healthy controls (Daalman et al., 2011) and non-psychotic individuals who regularly experience AVH (Daalman et al., 2012). Emotional content of AVH is more common in individuals experiencing regular AVH who have sought professional help relative to those who have not (Kråkvik et al., 2015). Further, people diagnosed with schizophrenia who experience delusions are more likely than healthy controls and non-delusional people diagnosed with schizophrenia to attribute emotional valence to presented word stimuli (Holt et al., 2006). Assigning emotional valence to anomalous experiences is therefore more likely in psychotic patients than other groups who experience AVH.

#### *5.1.2.1 Previous findings*

Galdos and colleagues administered the WN task to 30 psychotic disorder patients, 28 of their siblings, and 307 controls (Galdos et al., 2011). They found that patients were more likely to experience any speech illusion (that is, positive, negative and/or neutral illusions), relative to controls, even after controlling for age, gender, years of education and cognitive ability (OR= 3.8, 95% CIs: 1.0, 14.1). Further, the effect was larger for illusions perceived as affective, with positive speech illusion 9.4 times more likely in patients than controls, and negative speech illusion 8.6 times more likely in patients than controls. They also found increasing likelihood of speech illusion with familial vulnerability; 9% of the controls, 14% of the siblings, and 30% of the patients experienced any speech illusion. Finally, in controls and siblings, speech illusion was predicted by clinically-assessed positive but not negative schizotypy.

In a replication attempt of the original paper, Catalan and colleagues (Catalan et al., 2014) administered the WN task to 54 psychosis patients and 150 controls. They again found that patients were more likely to experience any speech illusion (33%), relative to controls (9%), even after controlling for age, gender, and IQ (OR= 3.4, 95% CIs: 1.4, 8.3), and again the effect

was larger for illusions perceived as affective. However, while they found that speech illusion was predicted by clinically-assessed positive symptoms in patients, in this study positive schizotypy did not predict illusions in controls.

The WN task has also since been administered to 1486 children aged 11-12 years from the Copenhagen Child Cohort 2000 population study (Rimvall et al., 2016). It was found that 10% of the children experienced any speech illusion, similar to the previous estimates for control adults. Having experienced hallucinations in the past month and negative affect in both the past month and in their lifetime, all predicted experience of speech illusions, particularly for illusions perceived as affective, and even after controlling for gender and cognitive ability (Rimvall et al., 2016).

These studies therefore demonstrate that the WN task is sensitive to schizophrenia diagnosis, psychosis-vulnerability, baseline psychotic symptoms in patients and children, and may be related to baseline positive schizotypal experiences in non-clinical populations. Both studies with patients demonstrated that affective illusions in particular are more likely in clinical populations than healthy controls, such that psychotic patients were more likely to assign emotional valence to AVH than non-clinical populations. This finding is in line with past work showing that AVH experienced by clinically psychotic patients are more likely to have an affective component than those experienced by healthy individuals (Daalman et al., 2011; Daalman et al., 2012), suggesting that the AVH elicited by the WN task may bare some similarity to of real-life AVH experiences.

### 5.1.3 Auditory-verbal hallucinations and cannabis

While research with schizophrenia patients has utilised tasks such as the WN task and those described in section 5.1.1.1 to investigate AVH, acute cannabis administration research has primarily focused on clinician- and self-rated measures of psychotomimetic effects in general, as discussed in Chapter 2 (Sherif et al., 2016). Indeed, to date, no study has experimentally manipulated AVH following cannabis administration, and the degree to which cannabis

specifically induces AVH (or whether indeed it does) has not been reported from a controlled study. Relatedly, no study has yet explored the affective component of cannabis-induced anomalous experiences. Assessing whether cannabis-induced AVH have an affective component similar to the WN task findings with psychosis patients described above (Catalan et al., 2014; Galdos et al., 2011) can help address the suggestion that psychotic experiences resulting from cannabis intoxication may arise through similar mechanisms as those seen in psychotic disorder (M. Bloomfield et al., 2016).

### 5.1.4 Age

As described in chapter 2, evidence from longitudinal cohort and case-control research suggests that adolescent cannabis use may confer greater risk of psychosis outcomes, relative to adult cannabis use. Further, as also described in chapter 1, there is a mixed preclinical body of work demonstrating differences between adolescent and adult rodent behavioural responses to acute cannabinoid administration. One major gap in our knowledge therefore is whether cannabis acutely produces greater psychotomimetic effects in adolescents than adults. As described above, the WN task is sensitive to AVH in young adolescents, with prevalence estimates similar to the rates seen in adults (Rimvall et al., 2016).

### 5.1.5 CBD

As described in chapter 2, case-control and patient group evidence has suggested variation in the risk of psychosis associated with different cannabis types (Di Forti et al., 2015; Di Forti et al., 2009; Di Forti et al., 2014). This variation has been suggested to result from differences in the CBD-content of cannabis types, following demonstration of CBD's putative antipsychotic effects in schizophrenia patients (Leweke et al., 2012), reduced non-acute psychotic symptoms in cannabis users with CBD present in their hair (Morgan & Curran, 2008; Morgan et al., 2012), and evidence of a protective effect of oral CBD against the acute psychotomimetic effects of IV THC administration (Englund et al., 2013). These findings therefore suggest that using cannabis containing CBD may reduce the psychotic-like effects of cannabis, including AVH. However,

given that Morgan et al did not find a protective effect CBD-rich cannabis against psychotomimetic effects in participants smoking their own cannabis, it is important to investigate these findings in a controlled study (Morgan et al., 2010). To date no study has directly compared the effects of a controlled dose of CBD-rich and CBD-lacking cannabis on psychotic-like symptoms including AVH.

### 5.1.6 Present studies

I therefore conducted two studies to address three research questions;

a) Does cannabis increase the incidence of speech illusions?
b) Are adolescents more vulnerable to the psychotomimetic effects, including AVH, of cannabis than adults (Study 3)?
c) Do higher levels of CBD in cannabis offset the psychotomimetic effects, including AVH, of cannabis in adults (Study 4)?

For both studies we administered cannabis in a double-blind, placebo-controlled, crossover design. Study 3 compared the psychotomimetic effects of Cann-CBD (cannabis containing high-levels of THC and negligible levels of CBD) in adolescents (aged 16-17 years) with adults (aged 24-28 years). Study 4 compared the psychotomimetic effects of Cann-CBD with Cann+CBD (cannabis containing high-levels of THC and high-levels of CBD) in adults-only.

For both studies we administered the WN task alongside self-rated measures of psychotomimetic experiences. These two studies are the first to assess whether cannabis acutely increases experiences of speech illusion. Furthermore, while the primary aim of these studies was to explore the effects of cannabis on AVH, and the influence of age and cannabis type on such experiences, these studies are also the first to explore the causal relationship between a drug that induces psychotic-like experiences and likelihood of experiencing AVH in a speech illusion task. Previous work has necessarily been case-control or population-based, such that comparisons are always between different groups of people (e.g. patients vs. controls, low vs.

high hallucination proneness), and as such differences in rates of speech illusion may be confounded by other group differences (as discussed in chapter 2), and not in fact directly related to psychosis and real-world experiences of AVH. Here I present two placebo-controlled studies in which I assessed experience of AVH in the same individuals on placebo and cannabis. As such, a secondary aim of this study was to assess the sensitivity of the WN task to experimentally drug-induced psychotic-like symptoms in a within-subject design, and to assess whether AVH incidence is related to cannabis-induced increases in self-reported perceptual distortion severity. If AVH incidence on the WN task is increased by an acute cannabis dose, and this is related to hallucination-like symptom severity, this would support the experimental pharmacological cannabis model of AVH and suggest the WN task as a potential simple outcome for experimental medicine study designs.

#### *5.1.6.1 Hypotheses*

##### 5.1.6.1.1 Study 3

1. Following extensive evidence demonstrating that cannabis acutely increases psychotomimetic experiences in adults as indexed by clinician- and self-rated scales (Sherif et al., 2016), alongside evidence that the experience of speech illusions on the WN task is sensitive to psychotic patient status, psychosis vulnerability and symptomology (Catalan et al., 2014; Galdos et al., 2011; Rimvall et al., 2016), I hypothesised that cannabis (Cann-CBD) would increase the likelihood of experiencing speech illusion relative to placebo.
2. Following putative evidence that adolescent cannabis use increases the risk of psychosis relative to adult use (Arseneault et al., 2002), I hypothesised that adolescents would be more vulnerable to the psychotomimetic effects (as indexed by both self-ratings and experiences of speech illusion) of cannabis than adults.

I also explored whether cannabis would increase the likelihood of experiencing affective illusions to a greater extent than neutral illusions, similar to that seen with psychosis patients

and those with psychosis vulnerability or symptomology. Due to a lack of previous research, there were no directional hypotheses for this component.

#### 5.1.6.1.2 Study 4

As described above for study 3 (Hypothesis 1), I again hypothesised that cannabis (Cann-CBD) would increase the likelihood of experiencing speech illusion relative to placebo.

3. Following putative evidence of the anti-psychotic properties of CBD (Leweke et al., 2012), alongside its ability to buffer the acute psychotomimetic effects of THC (Englund et al., 2013), I hypothesised that psychotomimetic effects (as indexed by both self-ratings and experiences of speech illusion) would be lesser following Cann+CBD relative to Cann-CBD.

I also explored whether Cann+CBD relative to Cann-CBD would have a specific effect on the experience of affective illusions or neutral illusions. Due to a lack of previous research, there were no directional hypotheses for this component.

##### 5.1.6.1.2.1 Predictors of speech illusion

In order to assess whether previously reported associations between likelihood of speech illusions and schizotypy were apparent following cannabis administration, I assessed the following hypothesis:

4. Following previous findings (Galdos et al., 2011) suggesting positive trait schizotypy predicts likelihood of speech illusion, I hypothesised that positive but not negative schizotypy (as indexed by the SPQ) would predict likelihood of speech illusions following cannabis.

In order to assess whether the likelihood of speech illusions following cannabis were related to self-rated increases in hallucination-like symptoms, I assessed the following hypothesis:

5. Following previous findings (Catalan et al., 2014; Rimvall et al., 2016) suggesting positive symptoms predict likelihood of speech illusion, I hypothesised that self-rated hallucination-like symptoms (as indexed by the Psychotomimetic States Inventory (PSI) subscale of perceptual distortion) would predict likelihood of speech illusions following cannabis.

## 5.2 Study 3

### 5.2.1 Methods (Study 3)

#### *5.2.1.1 Design and Participants*

As described in chapter 4, section 4.2.1.

#### *5.2.1.2 Drug administration*

As described in chapter 4, section 4.2.2. For consistency with Study 4 described below, here active cannabis will be referred to as Cann-CBD.

#### *5.2.1.3 Measures*

##### 5.2.1.3.1 Baseline Assessments

###### 5.2.1.3.1.1 Questionnaires

As described in chapter 4, section 4.2.3.1.1. Positive and negative schizotypy subscales were calculated and reported for the SPQ (Vollema & Hoijtink, 2000).

###### 5.2.1.3.1.2 Drug use

As described in chapter 4, section 4.2.3.1.2.

##### 5.2.1.3.2 Experimental assessments

###### 5.2.1.3.2.1 Subjective Ratings

As described in chapter 4, section 4.2.3.2.2, participants provided ratings from 0 (not at all) to 10 (extremely) for "Stoned" at a mean time of -6 minutes, +7 minutes, +34 minutes and +77 minutes (drug administration started at 0 minutes).

###### 5.2.1.3.2.2 Psychotic-like symptoms

Participants completed the PSI, a self-report questionnaire previously shown to be sensitive to the psychotomimetic effects of cannabis and ketamine (Mason et al., 2008). The PSI comprises 48 items covering six domains measuring positive-like symptoms (thought distortion, perceptual distortion, mania and paranoia) and negative-like symptoms (cognitive disorganisation, anhedonia). Items are rated on a 4-point scale, ranging from not at all to strongly. Higher scores indicate greater levels of psychotomimetic symptoms.

5.2.1.3.2.3 White Noise task

Participants were presented (via headphones) with one of three auditory stimuli types, sequentially in a randomised order. Stimuli were fragments of either:

- white noise only (consisting of only white noise);
- white noise plus clearly audible speech (consisting of white noise simultaneously overlaid with clear speech);
- white noise plus barely audible speech (consisting of white noise simultaneously overlaid with barely audible speech).

There were 25 trials for each of the stimuli, resulting in a total of 75 trials. Following each fragment participants indicated their opinion about what they just heard, selecting one of the following responses (numbers refer to required keyboard response); 1= "I heard something positive", 2= "I heard something negative", 3= "I heard something neutral", 4= "I heard nothing", 5= "Don't know". Prior to starting the task participants were informed that their responses should be in reference to any spoken language they heard during each sound fragment (rather than in reference to the white noise). Reminders of the response options and associated statements appeared on screen following each clip, until the participant responded (no time limit to make a selection was imposed). The incidence of reporting speech heard (i.e. keyboard response 1, 2 or 3) on the white noise only trials was the key variable of interest. Responses on trials containing white noise plus clearly/ barely audible speech were presented only to create an expectancy of hearing speech, aiming to increase the likelihood of hearing speech on white

noise only trials, and responses were not recorded. The task was delivered via E-prime 1.1. (Psychology Software Tools, Pittsburgh, Pennsylvania), and was provided by Galdos and colleagues (Galdos et al., 2011). The original fragments were shortened to a duration of 1 second, with the aim of increasing uncertainty in the task and thus increasing the base-rate of experiencing speech illusion.

#### *5.2.1.4 Procedure*

As described in chapter 4, section 4.2.4.

#### *5.2.1.5 Power calculation*

As described in chapter 4, section 4.2.5.

#### *5.2.1.6 Statistical Analysis*

##### 5.2.1.6.1 Data preparation

All analyses were conducted with SPSS 21.0. Outliers and normality were assessed via diagnostic plots for all analyses. Extreme outliers (>3 times interquartile range) were winsorized within-group and Greenhouse-Geisser corrections were applied for violations of sphericity.

##### 5.2.1.6.2 Analysis

Independent t-test, chi-square, or Mann-Whitney analyses were conducted as appropriate to compare groups (adult, adolescent) on demographic and baseline measures. Mixed ANOVA was conducted for all test outcomes, with the between-subjects factor of group (adult, adolescent) and within-subjects factor of drug (Cann-CBD, placebo). Additional within-subjects factors were included for relevant analyses: time (T2-T4) for subjective ratings of stoned (T1 (i.e. Pre-drug) was not included in analyses due to floor effects); PSI subscale (thought distortion, perceptual distortion, cognitive disorganisation, anhedonia, manic experience, paranoia). Interactions were explored via pairwise comparisons with local Bonferroni-correction. Drug order was added as an additional between-subjects factor (placebo-first, Cann-

CBD-first) and results were compared to reported primary analyses; unless otherwise noted results were unaffected by drug order.

Generalised estimating equation (GEE) models were used to assess the likelihood of experiencing speech illusion after placebo and Cann-CBD. GEE analyses were necessary due to the repeated measures design and binary outcome. GEE models also allow inclusion of participant data with missing occasions. The outcome was the experience of speech illusion, which following previous work was defined as having reported speech illusion on at least two white noise-only trials (Catalan et al., 2014; Rimvall et al., 2016). This binary outcome has been implemented in previous work and here due to the skewed distribution of the AVH incidence data. The initial model (Model 1a) included main effects of drug (Cann-CBD, placebo) and group (adolescent, adult), with the reference categories of placebo and adult. The second model (Model 1b) included both main effects and the interaction of drug x group. Within-group correlations were run between all outcomes reported and: cannabis use variables, administered cannabis weight, and any other variables showing group differences (at $p < .10$) at baseline (Chapter 4, Table 4.1.).

### 5.2.2 Results (Study 3)

Demographic and baseline data are displayed in Chapter 4 Tables 4.1. and 4.2.

To briefly summarise, adolescents were younger, and had lower body weight. Groups did not differ on verbal IQ, BAI, BDI-II, SUPPS-P, or SPQ. Adolescents currently used cannabis for more days per month than the adults, and the age of first cannabis use was younger for the adolescents compared to the adults, but overall the adults had used for longer. Groups did not differ on CAST score, time since last use, or likelihood of a positive THC urine screen at baseline.

#### *5.2.2.1 Subjective ratings*

##### 5.2.2.1.1 'Stoned'

As described in chapter 4, section 4.3.3.1.

### *5.2.2.2 Psychotomimetic symptoms*

#### 5.2.2.2.1 PSI (Figure 5.1.)

There were interactions of drug x subscale x group ($F_{5,190}$= 6.114, p< .001, $\eta^2p$= 0.14), subscale x group ($F_{5,190}$= 4.768, p< .001, $\eta^2p$= 0.11) and drug x subscale ($F_{3,132}$= 31.762, p <.001, $\eta^2p$= 0.46). Neither group had greater thought distortion or paranoia following cannabis compared to placebo (all p's≥ .065, all $\eta^2p\leq$ 0.09). Both groups had greater perceptual distortion, manic experience and cognitive disorganisation on cannabis compared to placebo (all p's≤ .001, all $\eta^2p\geq$ 0.27). On cannabis adults reported greater cognitive disorganisation than adolescents (p= .009, $\eta^2p$= 0.17). Lastly, cannabis increased anhedonia in adults (p= .001, $\eta^2p$= 0.25) but not adolescents (p= .925, $\eta^2p$< 0.01). Main effects of drug ($F_{1,38}$= 57.871, p< .001, $\eta^2p$= 0.60) and subscale ($F_{3,114}$= 55.961, < .001, $\eta^2p$= 0.60) also emerged.

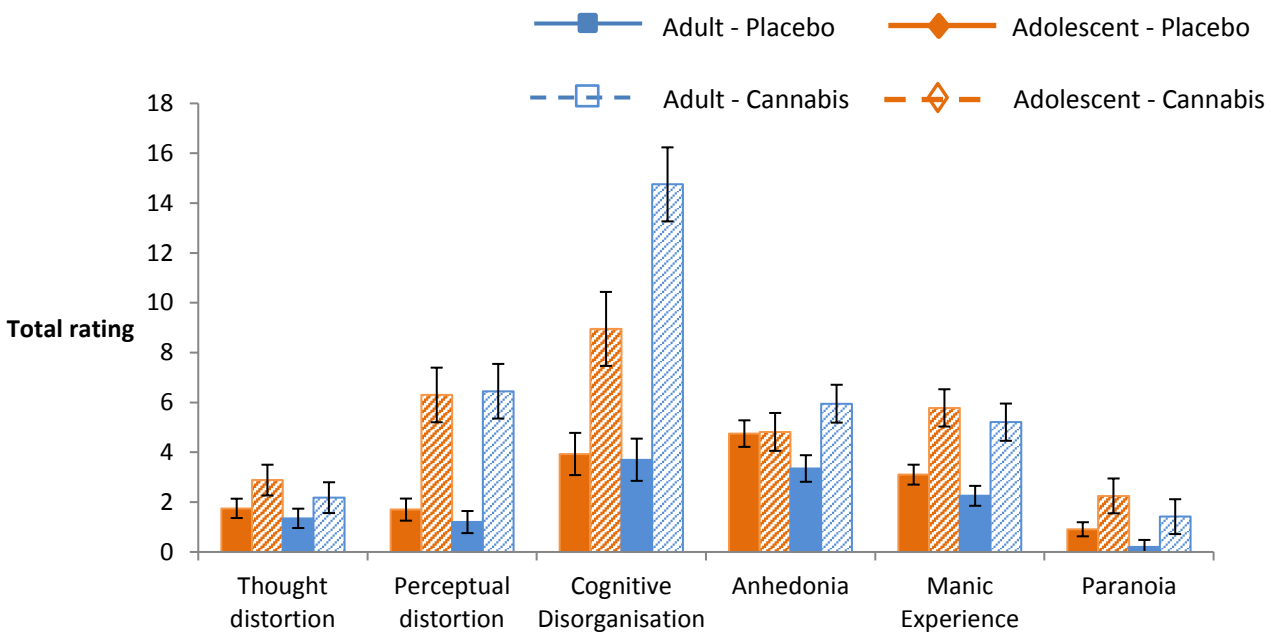


***Figure 5.1.*** *Mean (SE) values for total ratings of each subscale of the Psychotomimetic States Inventory (PSI), for adolescents and adults on placebo and cannabis.*

*5.2.2.3 White Noise (Table 5.1.)*

5.2.2.3.1 Any speech illusion (Table 5.2. Model 1)

Drug predicted the experience of speech illusion in Model 1a (p= .009). Relative to placebo, active cannabis led to a greater likelihood of experiencing speech illusion (b=1.128, SE=0.433, OR=3.09, 95% CIs: 1.32, 7.22). Drug also predicted experience of speech illusion in Model 1b (p= .029). Group did not predict the experience of speech illusion in either model (ps≥ .154). There was no interaction of drug x group in Model 1b (p= .428).

***Table 5.1.*** *Study 3 incidence % (n) of speech illusion on placebo and Cann-CBD, for adolescents and adults.*

| | **Adolescent** | | **Adult** | |
|---|---|---|---|---|
| | **Placebo** | **Cann-CBD** | **Placebo** | **Cann-CBD** |
| **Positive speech illusion** | *0.0 (0)* | *10.0 (2)* | *0.0 (0)* | *20.0 (4)* |
| **Negative speech illusion** | *0.0 (0)* | *15.0 (3)* | *0.0 (0)* | *20.0 (4)* |
| **Neutral speech illusion** | *35.0 (7)* | *40.0 (8)* | *15.0 (3)* | *40.0 (8)* |
| **Any speech illusion** | *35.0 (7)* | *55.0 (11)* | *15.0 (3)* | *45.0 (9)* |

***Table 5.2.*** *Generalised estimating equation models, study 3*

**Model 1a.** Study 3 generalised estimating equations model predicting any speech illusion from drug (Cann-CBD, **placebo**) and group (adolescent, **adult**). Reference categories in bold.

| | | | | | **95% CIs** | |
|---|---|---|---|---|---|---|
| | **Beta** | **SE** | **p-value** | **OR** | **Lower** | **Upper** |
| Cann-CBD vs. **placebo** | *1.128* | *0.433* | *.009* | *3.088* | *1.321* | *7.219* |
| Adolescent vs. **adult** | *0.680* | *0.531* | *.200* | *1.975* | *0.698* | *5.586* |

**Model 1b.** Study 3 generalised estimating equations model predicting any speech illusion from drug (Cann-CBD, **placebo**), group (adolescent, **adult**), and the interaction of drug x group. Reference categories in bold.

| | | | | | **95% CIs** | |
|---|---|---|---|---|---|---|
| | **Beta** | **SE** | **p-value** | **OR** | **Lower** | **Upper** |
| Cann-CBD vs. **placebo** | *1.534* | *0.701* | *.029* | *4.636* | *1.174* | *18.312* |
| Adolescent vs. **adult** | *-1.116* | *0.782* | *.154* | *3.051* | *0.659* | *14.137* |
| Drug x Group | *-0.714* | *0.901* | *.428* | *0.490* | *0.084* | *2.860* |

5.2.2.3.2 Positive & negative speech illusion

GEE analysis was not possible for positive or negative speech illusion independently, since 0% reported affective speech illusion on placebo.

#### *5.2.2.4 Group differences*

No baseline variables were found to correlate (at $p < .10$) with any outcome measure in both the adolescent and adult groups, and so were not entered into models.

## 5.3 Study 4

### 5.3.1 Methods (Study 4)

#### *5.3.1.1 Design and Participants*

A within-subjects, double-blind, cross-over design was used to compare the acute effects of cannabis with high-levels of THC and negligible levels of CBD (Cann-CBD), cannabis with high-levels of THC and high-levels of CBD (Cann+CBD), and placebo cannabis on adult cannabis users. Treatment order was randomised within gender and counterbalanced for task version. Randomisation was based on a Latin Square design. The target sample size was 18 (9 female), however due to drop-outs we reached 17 participants; so the Latin square was not completed (one treatment order was repeated twice while all other orders were repeated three times).

We recruited adult cannabis users through word-of-mouth. The following inclusion criteria were assessed at telephone screening: aged between 18 and 70 years; current cannabis use 3 days/ week or less; have smoked cannabis 4 or more times in the past year; alcohol use on fewer than 5 days per week; no other illicit drug use more than 2 times per month, no current or history of psychosis; no MRI contraindications, right handed (for additional fMRI assessments). Participants were asked to remain abstinent from all drugs including alcohol but not cigarettes for 24 hours before each testing session.

The study was approved by UCL Research Ethics Committee. All participants provided written informed consent. Participants were reimbursed for their time (£7.50 per hour).

#### *5.3.1.2 Drug administration*

Medicinal-grade cannabis (Bedrobinol®, THC 12.0% CBD <0.1% (as described in chapter 4; Cann+CBD; Bediol®; THC 6% CBD 7.5%) and placebo (THC <0.3% CBD <1%) cannabis were imported under UK Home Office licence from Bedrocan® in The Netherlands. Following previous protocols, on each session participants received one of the following: (1) Cann-CBD: 66.7mg of Bedrobinol® plus 66.7mg of placebo (equivalent to approximately 8.0mg THC and 0.0mg CBD; placebo was added to ensure the same weight of plant material was loaded into the vaporiser for each treatment while matching THC doses between active treatments); (2) 133.4mg of Cann+CBD (approximately 8.0mg THC and 10mg CBD); (3) 134.4mg placebo, followed by a 50% top-up dose approximately 120 minutes later.

Drug was administered via vaporiser, as described in chapter 4, section 4.2.2.

#### *5.3.1.3 Measures*

##### 5.3.1.3.1 Baseline assessments

Depression and anxiety were assessed on the BDI-II (Beck et al., 1996) and BAI (Beck et al., 1988), as described in chapter 4, section 4.2.3.1.1. The SPQ (Raine, 1991) indexed schizotypy, as described chapter 4, section 4.2.3.1.1. Positive and negative schizotypy subscales were calculated and reported for the SPQ (Vollema & Hoijtink, 2000).

##### 5.3.1.3.2 Drug use

A structured interview recorded: lifetime use (yes/no); time since last use (days); duration of use (years); frequency (days/month); and amount per session (alcohol units; cigarettes/day; other illicit drugs grams/ pills/ tabs). Instant urine drug screens assessed recent use of illicit drugs. Problematic drug use was assessed using the cannabis-adapted SDS. The SDS is a 5 item self-

report scale to measure psychological aspects of drug dependence. Response options vary by item, each item being rated between 0 and 3, with higher scores reflecting more problematic use. Adult scores ≥ 3 are indicative of DSM-III-TR diagnosis of cannabis dependence (Swift et al, 1998).

5.3.1.3.3 Subjective Ratings

Participants provided ratings from 0 (not at all) to 10 (extremely) for "Stoned" at approximately -10 minutes, +20 minutes, +100 minutes, +130 minutes and +190 minutes (drug administration started at 0 minutes).

5.3.1.3.4 Psychotic-like symptoms

Participants completed the PSI as described in chapter 5, section 5.2.1.3.2.2.

*5.3.1.4 Procedure*

Following screening participants attended a baseline session during which they provided informed consent, completed baseline measures, drug histories and problematic use questionnaires and task training.

Participants then completed three test sessions separated by at least seven days. Participants first provided a urine sample for instant drug screen and pregnancy test and provided baseline subjective ratings (Time 1; T1). Cann-CBD, Cann+CBD or placebo cannabis was then administered and participants again completed subjective ratings (Time 2; T2). Participants next completed an MRI scanning session for 1 hour (as part of a larger study; findings to be reported elsewhere), followed by more subjective ratings (Time 3; T3) and then top-up drug administration. Participants then completed further subjective ratings (Time 4; T4), and completed the task and state questionnaires in the following order; White Noise task, PSI and subjective ratings (Time 5; T5). Test sessions finished approximately 190 minutes after initial drug inhalation. Blood pressure and heart rate were monitored throughout.

#### *5.3.1.5 Power calculation*

The WN task was included in this study protocol as part of a wider study on which I collaborated. The sample size was therefore decided according to other outcome measures. I therefore calculated the sensitivity of the design with this sample size. The target sample size of 18 allowed for detection of a medium effect size (f= 0.32) for the main effect of drug, with 80% power at an alpha of 5%.

#### *5.3.1.6 Statistical Analysis*

All analyses were conducted with SPSS 21.0. Outliers and normality were assessed via diagnostic plots for all analyses. Extreme outliers (>3 times interquartile range) were winsorized within-group. Greenhouse-Geisser corrections were applied for violations of sphericity.

Repeated measures ANOVA was conducted for all test outcomes, with the within-subjects factor of drug (Cann-CBD, Cann+CBD, placebo). Additional within-subjects factors were included for relevant analyses: time (T2-T5) for ratings of stoned (T1 (i.e. Predrug) was not included in analyses due to floor effects); PSI subscale (thought distortion, perceptual distortion, anhedonia, cognitive disorganisation, manic experience, paranoia). Interactions were explored via pairwise comparisons with local Bonferroni-correction.

GEE models were used, as described in study 3, to assess the likelihood of experiencing speech illusion after placebo, Cann-CBD and Cann+CBD. The model (Model 2) included the main effect of drug (Cann-CBD, Cann+CBD, placebo), with planned comparisons made between Cann-CBD and placebo (reference category= placebo), and Cann-CBD and Cann+CBD (reference category= Cann+CBD).

Drug order was added as an additional between-subjects factor (placebo-first, Cann-CBD-first, Cann+CBD-first) to all analyses and results were compared to reported primary analyses; unless otherwise noted results were unaffected by drug order.

### 5.3.2 Results (Study 4)

Demographic and baseline data are displayed in Table 5.3. The 17 participants (9 female) had a mean age of 26.1 years. The mean age of first cannabis use was 17.3 years, and the mean duration of use was 8.9 years. Participants reported cannabis use on 8.1 days per month and a mean time since last use of 19.3 days, with 52.9% testing positive for THC at occasion 1.

***Table 5.3.*** *Demographic and baseline variables for Study 4 participants; values reflect mean (SD) unless otherwise stated.*

| | | Range | |
|---|---|---|---|
| **Demographics** | **Mean (SD)** | **Lower** | **Upper** |
| Female; % (n) | *52.94 (9)* | *n/a* | *n/a* |
| Age (years) | *26.18 (7.13)* | *19.00* | *50.00* |
| **Baseline questionnaires** | | | |
| BAI (n= 15) | *3.73 (2.76)* | *0.00* | *11.00* |
| BDI (n= 15) | *3.62 (3.07)* | *0.00* | *9.00* |
| SPQ (n=16) | *15.26 (6.41)* | *4.00* | *25.00* |
| **Cannabis use** | | | |
| Age first tried cannabis (years; n= 16) | *17.31 (1.78)* | *14.00* | *20.00* |
| Last used cannabis (days; n= 16) | *19.25 (45.28)* | *1.00* | *180.00* |
| Duration of cannabis use (years; n= 16) | *8.94 (7.02)* | *2.00* | *32.00* |
| Cannabis use frequency (days per month; n= 16) | *8.06 (5.48)* | *1.00* | *20.00* |
| Positive THC urine at occasion 1; % (n) | *52.94 (9)* | *n/a* | *n/a* |
| Cannabis Severity of Dependence Scale (n= 15) | *1.00 (1.20)* | *0.00* | *3.00* |
| **Cigarette use** | | | |
| Ever used cigarettes (n= 16); % (n) | *0.94 (15)* | *n/a* | *n/a* |
| Age first tried cigarettes (years)[1] | *17.60 (2.38)* | *15.00* | *23.00* |
| Duration of cigarette use (years) | *8.13 (7.15)* | *3.00* | *32.00* |
| Cigarette use frequency (days per month) | *11.30 (10.27)* | *1.00* | *31.00* |
| Cigarette use amount (cigs per session; n= 14) | *3.89 (3.61)* | *1.00* | *10.00* |
| **Alcohol use** | | | |
| Ever used alcohol (n=16); %(n) | *100.00 (16)* | *n/a* | *n/a* |
| Age first tried alcohol (years; n=16) | *15.44 (1.86)* | *12.00* | *18.00* |
| Duration of alcohol use (years; n=16) | *10.81 (7.71)* | *4.00* | *35.00* |
| Alcohol use frequency (days per month; n=16) | 10.81 (4.86) | 4.00 | 25.00 |

*5.3.2.1 Subjective ratings*

5.3.2.1.1 'Stoned' (Figure 5.2.)

There were main effects of drug ($F_{2,32}$= 56.346, p< .001, $\eta^2p$= 0.78) and time ($F_{3,48}$= 18.490, p< .001, $\eta^2p$= 0.54). Ratings were higher on Cann-CBD (p< .001) and Cann+CBD (p< .001) compared to placebo, however ratings did not differ between Cann-CBD and Cann+CBD (p> .999). Ratings were greater at T2 than at T3 (p= .004) and T5 (p< .001), however there was no difference between ratings at T2 and T4 (p= .382) demonstrating that the top-up drug administrations achieved similar levels of intoxication as the initial dose. No interaction of drug x time emerged ($F_{6,96}$= 1.374, p= .233, $\eta^2p$= 0.08).

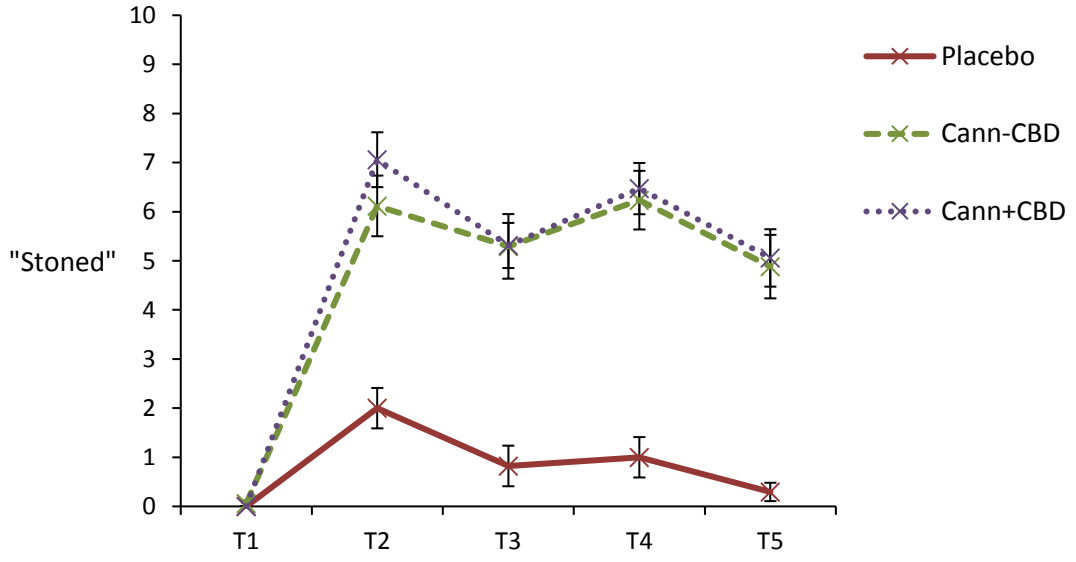


***Figure 5.2.*** *Study 4 mean (SE) values for subjective ratings (0-10) for 'stoned', on placebo, Cann-CBD, and Cann+CBD.*

*5.3.2.2 Psychotomimetic symptoms*

5.3.2.2.1 PSI (Figure 5.3.)

There was an interaction of drug x subscale ($F_{4,69}$= 6.195, p< .001, $\eta^2p$= 0.28). Compared to placebo ratings were higher on both Cann-CBD and Cann+CBD for the subscales of thought distortion (p< .001 and p= .002), perceptual distortion (p= .001 and p= .003), cognitive disorganisation (p= .001 and p< .001), and manic experiences (p= .002 and p= .032). There were no differences between placebo and Cann-CBD or Cann+CBD for anhedonia or paranoia (all ps≥ .107). There were no differences between Cann-CBD and Cann+CBD on any of the subscales (all ps≥ .824). There were also main effects of drug ($F_{2,32}$= 15.804, p< .001, $\eta^2p$= 0.50) and subscale ($F_{3,47}$= 38.757, p< .001, $\eta^2p$= 0.71).

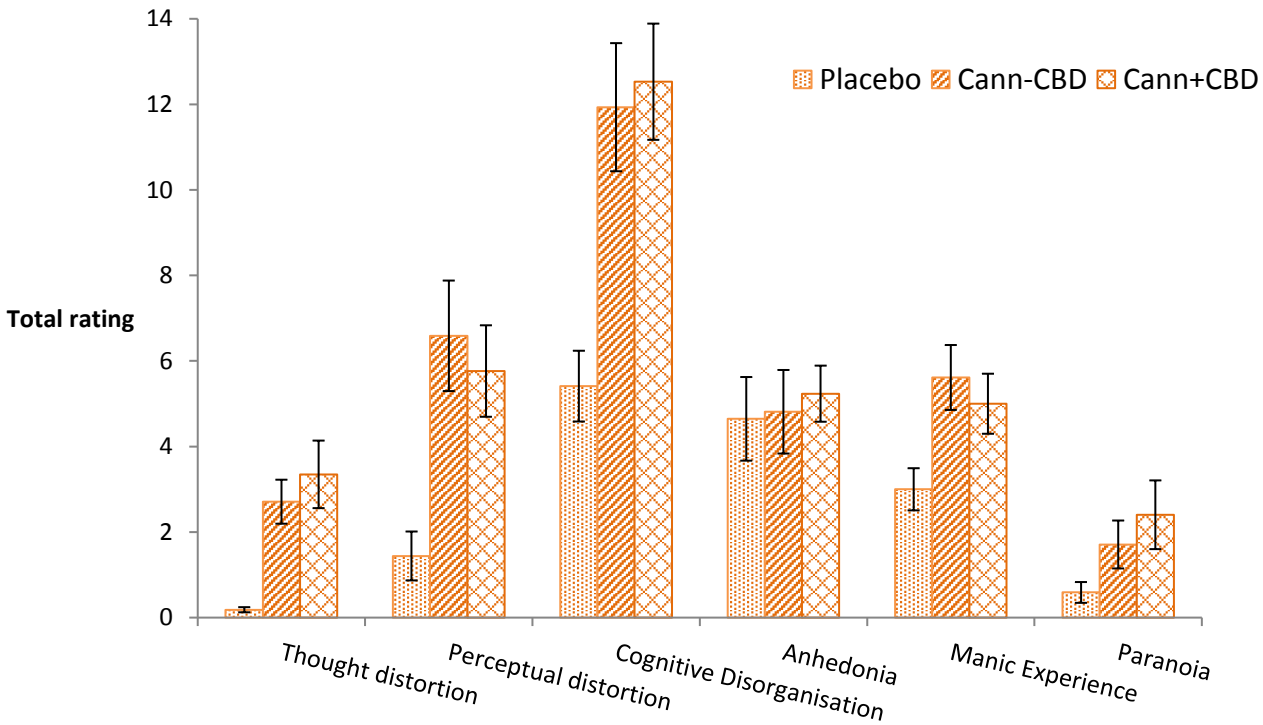


***Figure 5.3.*** *Study 4 mean (SE) values for total ratings of each subscale of the Psychotomimetic States Inventory (PSI), on placebo, Cann-CBD, and Cann+CBD.*

*5.3.2.3 White Noise (Table 5.4.)*

5.3.2.3.1 Any speech illusion (Table 5.5. Model 2)

Relative to placebo, Cann-CBD did not increase the likelihood of experience of speech illusion (b= 0.945, SE= 0.680, OR= 2.57, 95% CIs: 0.68, 9.76, p= .164). Relative to Cann-CBD, Cann+CBD did not lead to a lower likelihood of experiencing speech illusion (b= 0.474, SE= 0.667, OR= 0.62, 95% CIs: 0.17, 2.30, p= .477).

5.3.2.3.2 Positive & negative speech illusion

GEE analysis was not possible for positive or negative speech illusion independently, since 0% reported affective speech illusion on placebo.

***Table 5.4.*** *Study 4 incidence % (n) of speech illusion on placebo, Cann-CBD and Cann+CBD. Due to technical error, one participant's data was missing for placebo.*

| | **Placebo (n=16)** | **Cann-CBD (n=17)** | **Cann+CBD (n=17)** |
|---|---|---|---|
| **Positive speech illusion** | *0.0 (0)* | *5.9 (1)* | *5.9 (1)* |
| **Negative speech illusion** | *0.0 (0)* | *11.8 (2)* | *17.6 (3)* |
| **Neutral speech illusion** | *37.5 (6)* | *52.9 (9)* | *41.2 (7)* |
| **Any speech illusion** | *37.5 (6)* | *58.8 (10)* | *47.1 (8)* |

***Table 5.5.*** *Generalised estimating equation models, study 4*

Model 2. Study 4 generalised estimating equations model predicting any speech illusion from drug (Cann-CBD, Cann+CBD, placebo). Reference categories (in bold) were pre-defined to address specific hypotheses: Cann-CBD vs. **placebo** and Cann-CBD vs. **Cann+CBD**.

| | | | | | **95% CIs** | |
|---|---|---|---|---|---|---|
| | **Beta** | **SE** | **p-value** | **OR** | **Lower** | **Upper** |
| Cann-CBD vs. **Placebo** | *0.945* | *0.680* | *.164* | *2.574* | *0.679* | *9.757* |
| Cann-CBD vs. **Cann+CBD** | *0.474* | *0.667* | *.477* | *1.607* | *0.435* | *5.943* |

## 5.4 Combined Results

### 5.4.1 Mega-analysis

Given the similarity of the experimental protocols I then combined the two datasets, aiming to increase power and assess whether the effect of cannabis differed between the two studies. For consistency I only included data from Cann-CBD and placebo, excluding study 4 data for Cann+CBD. The initial model (Model 3a) included main effects of drug (Cann-CBD, placebo) and study (study 3, study 4). The second model (Model 3b) included both main effects and the interaction of drug x study.

#### *5.4.1.1 Drug effect comparison check*

5.4.1.1.1 'Stoned'

There was a main effect of drug ($F_{1,55}$= 199.415, p< .001, $\eta^2p$= 0.78), with ratings higher on cannabis (M= 6.44, SE= 0.38) than placebo (M= 0.98, SE= 0.24). There was no main effect of study ($F_{1,55}$= 0.135, p= .715, $\eta^2p$< 0.01) and no interaction of drug x study ($F_{1,55}$= 0.360, p= .551, $\eta^2p$= 0.01).

5.4.1.1.2 PSI (Figure 5.4.)

There was an interaction of drug x subscale ($F_{3,177}$= 29.248, p< .001, $\eta^2p$= 0.35). Compared to placebo ratings were higher on Cann-CBD for the subscales of thought distortion (p< .001), perceptual distortion (p< .001), cognitive disorganisation (p< .001), manic experiences (p< .002) and paranoia (p= .005). There was no difference between placebo and Cann-CBD for anhedonia (p= .194). There were also main effects of drug ($F_{1,55}$= 60.714, p< .001, $\eta^2p$= 0.53) and subscale ($F_{3,170}$= 69.849, p< .001, $\eta^2p$= 0.56). There was no main effect or interactions with study (all ps≥ .251).

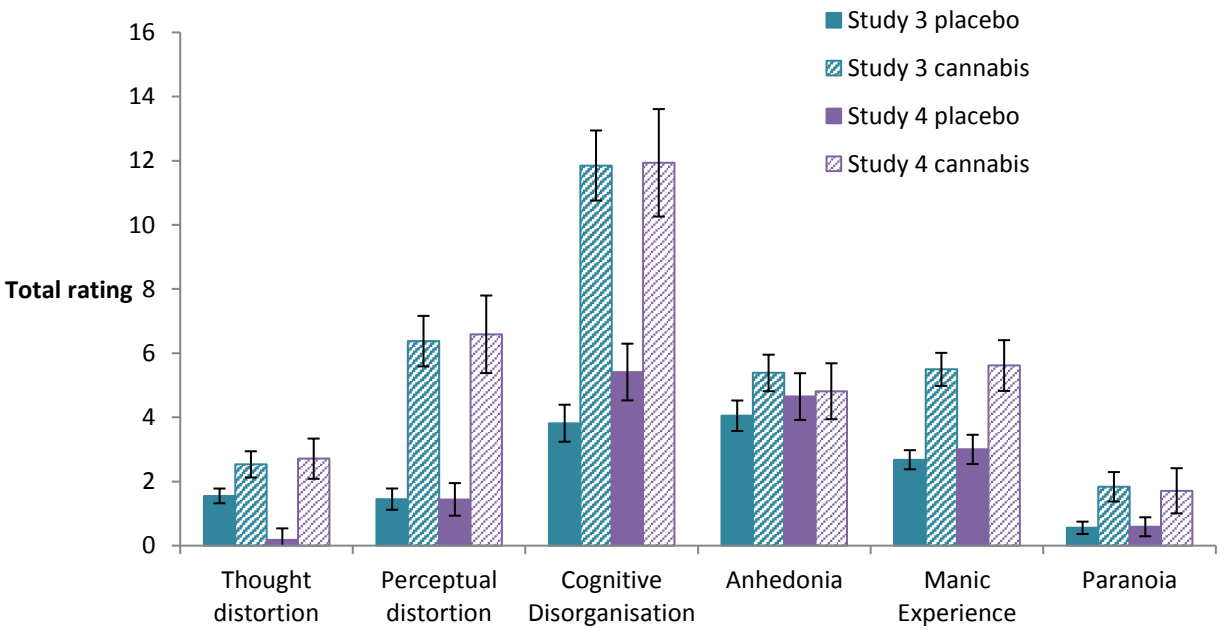


***Figure 5.4.*** *Mean (SE) values for total ratings of each subscale of the Psychotomimetic States Inventory (PSI), on placebo and Cann-CBD, by study.*

*5.4.1.2 White Noise (Table 5.6.)*

5.4.1.2.1 Any speech illusion (Table 5.7. Model 3)

Drug predicted the experience of speech illusion in both Model 3a (p= .004) and 3b (p= .010). Relative to placebo, active cannabis led to a greater likelihood of experiencing speech illusion (b= 1.036, SE= 0.362, OR= 2.82, 95% CIs: 1.39, 5.73). Study did not predict the experience of speech illusion in either model (both ps> .330) and there was no interaction of drug x study in Model 3b (p= .804).

***Table 5.6.*** *Incidence % (n) of speech illusion on placebo and Cann-CBD, for Study's 3 and 4 combined. Due to technical error in Study 4, one participant's data was missing for placebo.*

| | **Placebo (n=56)** | **Cann-CBD (n=57)** |
|---|---|---|
| **Positive speech illusion** | *0.0 (0)* | *12.3 (7)* |
| **Negative speech illusion** | *0.0 (0)* | *15.8 (9)* |
| **Neutral speech illusion** | *28.6 (16)* | *43.9 (25)* |
| **Any speech illusion** | *28.6 (16)* | *52.6 (30)* |

***Table 5.7.*** *Generalised estimating equation models, combined study 3 and 4*

Model 3a. Combined generalised estimating equations model predicting any speech illusion from drug (Cann-CBD, **placebo**) and study (**Study 3**, Study 4). Reference categories in bold.

| | | | | | **95% CIs** | |
|---|---|---|---|---|---|---|
| | **Beta** | **SE** | **p-value** | **OR** | **Lower** | **Upper** |
| Cann-CBD vs. **placebo** | *1.036* | *0.362* | *.004* | *2.818* | *1.385* | *5.733* |
| Study 4 vs. **Study 3** | *0.448* | *0.460* | *.330* | *1.565* | *0.636* | *3.854* |

Model 3b. Combined generalised estimating equations model predicting any speech illusion from drug (Cann-CBD, placebo), study (Study 3, Study 4) and the interaction of drug x study. Reference categories in bold.

| | | | | | **95% CIs** | |
|---|---|---|---|---|---|---|
| | **Beta** | **SE** | **p-value** | **OR** | **Lower** | **Upper** |
| Cann-CBD vs. **placebo** | *1.099* | *0.424* | *.010* | *3.000* | *1.306* | *6.891* |
| Study 4 vs. **Study 3** | *0.558* | *0.636* | *.380* | *1.748* | *0.502* | *6.079* |
| **Drug x Study** | *-0.202* | *0.811* | *.804* | *0.817* | *0.167* | *4.009* |

#### *5.4.1.3 Predictors of speech illusion*

To investigate whether self-rated measures of schizotypy (as indexed by the SPQ subscales) and hallucination-like psychotic symptoms (as indexed by the PSI subscale of perceptual distortion) were associated with the experience of speech illusion following cannabis, I then conducted a series of logistic regressions.

##### 5.4.1.3.1 Schizotypy

Baseline scores on the positive subscale of the SPQ did not predict likelihood of experiencing AVH following cannabis (b= 0.007, Wald $\chi^2_1$= 0.023, OR= 1.01 (95% CIs: 0.92, 1.10), p= .880). Baseline scores on the negative subscale of the SPQ did not predict likelihood of experiencing AVH following cannabis (b= 0.015, Wald $\chi^2_1$= 0.147, OR= 1.02 (95% CIs: 0.94, 1.09), p= .702).

##### 5.4.1.3.2 Hallucination-like symptoms

The cannabis session perceptual distortion subscale of the PSI predicted increased likelihood of experiencing AVH following cannabis (b= 0.123, Wald $\chi^2_1$= 3.872, OR= 1.13 (95% CIs: 1.00, 1.28), p= .049).

## 5.5 Discussion

### 5.5.1 Summary of findings

Together the two studies described in this chapter demonstrate that cannabis acutely (Cann-CBD) increases the likelihood of experiencing speech illusion during the white noise task, and that likelihood of experiencing speech illusion following cannabis is predicted by self-rated increases in hallucination-like symptoms, but not by self-rated positive or negative schizotypy scores at baseline. Moreover, Study 3 demonstrated that adolescents did not differ from adults in the likelihood of experiencing speech illusion, though the increase in self-rated psychotomimetic symptoms following cannabis was greater in adults. Specifically, cognitive disorganisation was especially elevated in adults relative to adolescents after cannabis, and cannabis increased anhedonia symptoms in adults but not adolescents. Furthermore, Study 4 demonstrated no difference in the likelihood of speech illusion or in the extent of self-rated psychotomimetic symptoms following CBD-lacking relative to CBD-rich cannabis.

### 5.5.2 Study 3

In study 3, as predicted with my first hypothesis, I found that Cann-CBD increased the likelihood of experiencing speech illusion, relative to placebo. Also in line with my first hypothesis, Cann-CBD led to an increase in self-rated psychotomimetic symptoms of perceptual distortion, manic experience, and cognitive disorganisation in both groups, though cannabis did not increase self-rated thought distortion or paranoia in either group.

Contrary to my second hypothesis, I found no difference in rate of speech illusion between the adolescents and adults. Furthermore, while my second hypothesis predicted a greater degree of psychotomimetic effects following cannabis in the adolescents compared to the adults, I instead found the opposite: cognitive disorganisation was especially elevated in adults compared to adolescents after cannabis. This unexpected finding is however in agreement with the findings I described in chapter 4, of lesser intoxication effects in general in adolescents relative to adults,

perhaps suggesting a common mechanism by which adolescents are resilient to the acute negative effects of cannabis. It may also reflect an awareness in adults of the greater cognitive impairments they were experiencing (as described in chapter 4), rather than amplified psychotic-like effects of cannabis per se. I also found that cannabis increased anhedonia symptoms in adults but not adolescents, suggesting heightened dysphoric effects in adults, which may reflect the heightened negative drug effects experienced by the adults in general. Of note however, on placebo the adolescents had (non-significantly) higher levels of anhedonia than the adults, potentially highlighting a baseline dependency effect.

Of interest, unlike other subscales of the PSI, cognitive disorganisation and anhedonia are not 'psychotic-like' as I defined in chapter 2. These findings therefore demonstrate that there were no differences between age groups for positive-like symptoms (that is, AVH on the WN task, alongside self-rated thought distortion, perceptual distortion, manic experiences and paranoia), but adults experienced greater cannabis-induced negative-like symptoms (that is, anhedonia and cognitive disorganisation) than adolescents. Importantly therefore, while the cannabis pharmacological model of psychosis (that is, specifically positive-like symptoms) appears to be a potentially useful model in 16-17-year-old boys as well as adults, cannabis may not induce the similar negative-like symptoms in adolescent and adult males. I will re-visit this in the next chapter, where I specifically assess the acute effects of cannabis on anhedonia, a common negative symptom of schizophrenia and of depression, in adolescents and adults, and evaluate cannabis intoxication as a pharmacological model of anhedonia.

Furthermore, these findings suggest that adolescents are less vulnerable to some of the unwanted side-effects of cannabis, which in the short-term is a promising finding, however as discussed in the previous chapter, such negative drug effects may be use-limiting. A reduction in acute negative effects may facilitate heavier cannabis use, including consumption of higher THC doses per session, which as described in chapter 2 has been linked to increased risk of psychosis. Interestingly, Di Forti et al found that younger age of cannabis use onset predicted psychosis before but not after adjusting for type of cannabis used and frequency of use, with

those with younger age of onset also reporting more frequent use and greater preference for higher potency cannabis (Di Forti et al., 2014). This potentially alludes to a role of younger age in consuming higher doses more regularly, and subsequently increasing the risk of psychosis. However, without longitudinal studies aiming to assess the role of reduced negative effects of cannabis in adolescence on cannabis use patterns and long-term outcomes, such potential consequences of these findings are purely speculative.

### 5.5.3 Study 4

In study 4, contrary to my first hypothesis, Cann-CBD did not increase the likelihood of speech illusion, relative to placebo. However, in line with my first hypothesis and in line with the findings of study 3, Cann-CBD led to an increase in self-rated psychotomimetic symptoms of thought distortion, perceptual distortion, manic experience, and cognitive disorganisation, but not anhedonia or paranoia.

Meanwhile, contrary to my third hypothesis, I did not find a difference in the rate of speech illusion after Cann+CBD relative to Cann-CBD. Furthermore, there was no difference in self-rated psychotomimetic symptoms between Cann-CBD and Cann+CBD, suggesting that CBD did not blunt the psychotomimetic effects of cannabis. The findings of no difference between Cann-CBD and Cann+CBD were consistent across all psychosis related measures (AVH and all subscales of the PSI), this strengthens my confidence in the null findings, however lacking statistical power may be an issue.

These findings are in line with those of Morgan et al (2010), who found no reduction in the self-rated psychotomimetic effects of cannabis in those smoking their own CBD-rich or CBD-lacking cannabis (Morgan et al., 2010). Englund et al however found that an oral dose of CBD prior to IV THC reduced the likelihood of a clinically relevant increase in positive psychotic symptoms following THC (Englund et al., 2013). Together these findings tentatively suggest that while a pre-dose of CBD may be protective against psychotomimetic effects of subsequent THC, consuming CBD-rich cannabis may not be sufficient to offset such effects. However,

Englund et al did not find consistent effects across all measures- they did not find a significant reduction in self-rated Positive and Negative Syndrome Scale (PANSS) positive scores in those who received the pre-dose of CBD. It is also difficult to compare THC:CBD ratios between Morgan et al and Englund et al to identify whether this may explain their potentially contradictory results, since the routes of administration differ. A dose-response study with differing doses and ratios of THC is needed. Alternatively, the putative anti-psychotic effects of CBD may result from extended exposure to CBD, which would reconcile my findings with findings discussed in chapter 2 of lower rates of psychotic disorder amongst cannabis users who preferentially use lower potency cannabis types that likely contain more CBD than higher potency types (Di Forti et al., 2014).

### 5.5.4 Combined data

After combining the data from both studies, and in line with my first hypothesis, I found evidence of an overall effect of Cann-CBD on speech illusions, with participants 2.8 times more likely to experience speech illusion on Cann-CBD than placebo. Given the small sample size of study 4, it is possible that a lack of statistical power explains the potentially contradictory findings from the two studies. This is supported by the similarity of the effect size and overlapping confidence intervals when comparing the rate of experiencing any speech illusion on Cann-CBD to placebo for study 3 (OR= 3.09, 95% CIs: 1.32, 7.22) and 4 (OR= 2.57, 95% CIs: 0.68, 9.76). Incidence of AVH and ratings of stoned and psychotomimetic symptoms did not differ between studies (as indicated by the lack of any 'study' main effects or interactions in any analyses), reflecting the similarity of the study designs and dosing. Of note, after combining data from both studies Cann-CBD was shown to increase psychotomimetic effects on all subscales of the PSI apart from anhedonia, whereas from the individual studies it was unclear whether cannabis increased thought distortion and paranoia (study 3) and paranoia (study 4) since differences were at trend-level (i.e. $p < .10$).

Of interest, the combined summary effect size of 2.8 when comparing Cann-CBD to placebo is not dissimilar to that reported in previous papers when comparing rate of any speech illusion in patients with psychosis to controls (OR= 3.8 (Galdos et al., 2011) and OR= 3.4 (Catalan et al., 2014)), with confidence intervals clearly overlapping. These findings therefore demonstrate that acutely cannabis can increase AVH in otherwise healthy cannabis users who were screened to exclude those at high risk of psychosis (according to personal and family history). Of further interest, higher self-rated perceptual distortion following cannabis predicted increased likelihood of AVH, demonstrating that the WN task is sensitive to experimentally drug-induced psychotic-like symptoms- supporting previous assertions that the task is related to psychosis-related symptom severity (Catalan et al., 2014; Galdos et al., 2011). Neither positive nor negative schizotypy at baseline predicted AVH following cannabis, contradicting previous findings that positive schizotypy predicts AVH incidence (Galdos et al., 2011). One possible explanation for this null association is that the cannabis-induced psychotic symptoms 'uncoupled' baseline schizotypal symptoms from on-drug symptoms, though previous research has found that baseline schizotypy predicts psychotomimetic effects of cannabis (Barkus & Lewis, 2008; Barkus et al., 2006; Mason et al., 2009). Of note however, others have also found no association between schizotypy and AVH on the WN task (Catalan et al., 2014).

That cannabis acutely increases the likelihood of experiencing AVH in otherwise healthy volunteers, suggests that cannabis may be useful pharmacological model of AVH. The lengthy and expensive process of testing novel medications in clinical populations has increased calls for experimental medicine models to allow testing of such treatments in healthy populations. Experimental medicine can use pharmacological models of symptoms, such as AVH, to identify promising (and discount less promising) drugs that are worth taking forward to clinical trial. Given that the WN task is sensitive to psychotic symptom severity in patients with psychotic disorder, and as the results of this chapter have now shown, cannabis-induced psychotic symptoms, this task may be a useful outcome in such studies. A first step would therefore be to

assess whether existing medications for the treatment of psychosis reduce the incidence of speech illusions on the WN task.

#### *5.5.4.1 Affective speech illusions*

While we were unable to statistically analyse the data separately for affective speech illusions, we found no one in either study who reported an affective speech illusion on placebo, while 7 (12%) participants reported positive illusions and 9 (16%) reported negative illusions while on Cann-CBD. While this is a dramatic finding, the increase was of similar magnitude, in terms of actual numbers of participants, to the increase in participants who reported neutral speech illusions following cannabis compared to placebo (rising from 16 (29%) to 25 (44%) participants). This finding therefore likely reflects a baseline difference in the experience of affective and non-affective speech illusions, and demonstrates a non-specific effect of cannabis on all speech illusions, rather than a cannabis-related increase in *affective* speech illusions. Nevertheless, it is of interest to note that on placebo none of the cannabis users reported affective speech illusions, a finding which concords with previous uses of the WN task which have found healthy controls had an affective speech illusion rate of 1-2% (Catalan et al., 2014; Galdos et al., 2011), despite our attempts to increase the illusion rate by increasing ambiguity in the task.

That cannabis did not appear to preferentially increase affective speech illusions is interesting because it suggests that AVH in psychosis and AVH from cannabis may be qualitatively different, potentially suggesting a differing mechanism by which they occur. Of note however, it has been suggested that the affective component of AVH experienced clinically may reflect a general state of emotional turmoil in patients, rather than providing evidence for a different mechanism by which clinical and non-clinical AVH arise (Catalan et al., 2014). Clearly a major gap in the literature is identifying the mechanisms by which cannabis induces its psychotomimetic effects.

### 5.5.5 Strengths of the studies

Previously the WN task has been used only in cross-sectional between-subjects designs, and as such the increased rate of speech illusions demonstrated in patients with psychosis relative to controls may be confounded by other differences between the groups. Importantly our design was repeated measures, whereby we directly manipulated a participant's level of psychotic-like symptoms with cannabis administration. Comparing rates of illusion on cannabis to placebo therefore provides non-confounded evidence to validate the WN task is sensitive to psychotic symptomology. Our finding that self-rated hallucination-like symptoms following cannabis administration predicted the likelihood of speech illusions further supports the task as an index of psychotic symptoms.

Secondly, the similarity of the effect size derived from both experiments (Studies 3 and 4), despite wide confidence intervals, again increases confidence in a true effect of cannabis on the experience of speech illusions. The combination of the data from the two studies is a further strength, given the increase in sample size and thus increase in power, and the ability to compare the studies in a more meaningful way than the qualitative significant/ non-significant nomenclature. Of course, the smaller sample size of study 2 (n= 17) is a limitation, and a larger sample size would have been preferable to improve confidence in the independent findings of this study.

Thirdly, administering the cannabis via a more ecologically valid method (inhalation of vaporised plant material) is a strength of both studies, likely providing a more realistic estimation of the magnitude and type of psychotomimetic symptoms recreational users typically experience. Many studies assessing the psychotomimetic effects of cannabinoids have used either intravenous administration (29% of studies assessing clinician- or self-rated psychotomimetic symptoms cited in a recent review (Sherif et al., 2016)), an invasive procedure which has been suggested to artificially increase the psychotic-like effects of cannabis, or oral administration (33% from the same review (Sherif et al., 2016)), which may result in under-estimation of the psychotomimetic effects of cannabis given that peak subjective intoxication

appears to be lower following oral than inhaled administration (Chait & Zacny, 1992; Hart et al., 2002; Ohlsson et al., 1981).

Finally, a real strength of these studies is the attempt to better describe the specific psychotic-like symptoms induced by cannabis, rather than only describing a general increase in self- or clinician-rated measures of psychotic-like symptoms. Pharmacological models are unlikely to be able to model all aspects of a disorder such as schizophrenia, but, as I discussed in chapter 2, taking a symptom-based approach to identify the basic aspects of disorders that can be modelled pharmacologically can lead to improved understanding of the aetiology of individual symptoms and lead to more refined methods of testing potential new treatments.

### 5.5.6 Limitations of the studies

As described in chapter 2, the psychotomimetic effects of cannabis are thought to be heightened in patients with psychotic disorder and those with higher baseline schizotypy. However, I specifically excluded those with a current or past diagnosis, or family history of psychosis, to reduce the risk of participation. As such, the studies described in this chapter may have underestimated the full magnitude of acute psychotomimetic effects of cannabis in vulnerable individuals. Relatedly, given that the interquartile range of age of first psychotic disorder treatment is estimated to fall between ages 19 and 27 years (Kessler et al., 2007), it is possible that some included adolescents in study 3 will go onto develop psychosis, while it is more likely that the adults would have already experienced their first episode and therefore would have been excluded. However, this is unlikely, given both the rarity of psychosis in the general population (as described in chapter 2) and the exclusion of those at risk of psychosis as indicated by family history of psychosis. Importantly, however, if it is the case that I inadvertently included adolescents in study 3 who go on to develop psychosis in future, I would expect this to inflate the psychotomimetic effects of cannabis in the adolescent group; yet I found that adults had increased cannabis-induced psychotomimetic symptoms relative to adolescents.

Importantly, findings in this chapter cannot be used to infer causal relationships between cannabis use and psychotic disorder; as discussed in chapter 2, the acute effects of a drug are often different to the long-term effects of repeated consumption. Indeed, as described in chapter 2, while experiencing acute psychotomimetic effects from cannabis is very common, the number of cannabis users who will develop psychotic disorder is very low. Despite the similarity between the acute psychotomimetic effects of cannabis and clinical psychotic symptoms, we cannot yet know whether there is a common mechanism by which cannabis acutely and chronically leads to psychotic symptoms.

A further limitation is that I cannot address the mechanism by which cannabis induced AVH, though this was not my aim. It is however possible that cannabis altered participant's sound perception, potentially leading to increased reporting of AVH due to increased task difficulty. Future studies could test hearing sensitivity and potentially adjust the volume of stimuli on cannabis and placebo if necessary. While the association with self-rated perceptual distortion importantly demonstrates a relationship between self-reported hallucination-related symptom severity and incidence of AVH, the subscale of perceptual distortion includes items referring to both changes in perception (for instance, "your hearing has become very sensitive") and actual experiences of hallucinations (for instance, "you have seen a person's face in front of you when no one was in fact there"), and so this association does not aid the separation of potential lower-level perceptual changes from higher-level mechanisms. Importantly, if the mechanisms by which clinical and cannabis-induced AVH arise are dissimilar then this would question the clinical utility of an acute cannabis pharmacological model of AVH. Of note, while clinical AVH often have an affective component, cannabis did not appear to preferentially induce affective AVH in my studies; this potentially suggests a different mechanism via which clinical and cannabis-induced AVH occur.

Following previous work, I analysed the data in terms of incidence of AVH, rather than mean number of AVH experienced on each drug. This is necessary with this task since the data are highly skewed; some participants experience many AVH while others experience very few or

none. Cannabis clearly does not increase the likelihood of AVH in all participants (for instance, on Cann-CBD, 53% of participants across both studies experienced AVH). However, the dichotomisation of the outcome also loses the richness of the data within those who did experience speech illusion, since some participants experienced one or two illusions, while others experienced many. Development of an improved task which creates a more spread distribution of AVH would be preferable for future studies. Future research with larger samples should also aim to identify the predictors of cannabis-induced AVH and psychotomimetic symptoms in general, as I did here with age, as this can identify important at risk groups. While small sample sizes in study 4 precluded it here, future work should assess the effect of CBD content of cannabis specifically in those who did experience an increase in AVH incidence following cannabis. This would more directly address the question of whether CBD can prevent the cannabis-inducing increase in AVH.

Additionally, as described in chapter 4, it is important to note the baseline differences between the adolescent and adult groups, for example the higher cannabis use frequency in the adolescent groups, which may have influenced the blunted effects of cannabis on cognitive disorganisation and anhedonia in the adolescents. This issue will be discussed in more detail in the final chapter.

Finally, as with most acute cannabis studies, here I have focused on positive-like symptoms induced by cannabis; whether cannabis also induces negative-like symptoms and other mood-related symptoms, such as anhedonia, remains much less explored. While after combining both study's data there was no evidence of an overall increase in anhedonia following cannabis, for study 3 I found an effect of age group. Cannabis increased self-rated anhedonia in the adults but not adolescents, though adolescents had (non-significantly) higher anhedonia on placebo. As such, my next chapter will investigate whether acutely cannabis induces anhedonia on a number of different measures, and explore further whether such effects differ in adolescents and adults.

### 5.5.7 Conclusions

In this chapter I have replicated previous findings that cannabis acutely induces psychotomimetic effects, and demonstrated for the first time evidence that cannabis acutely induces AVH. Moreover, incidence of AVH following cannabis was predicted by hallucination-related symptom severity, but not with baseline positive or negative schizotypy. Additionally, I found no evidence that cannabis with high CBD content results in blunted psychotomimetic effects relative to CBD-lacking cannabis.

In relation to the over-arching question of whether adolescents are more sensitive to the acute psychotomimetic effects of cannabis, I found no evidence of this. No age group differences were apparent for incidence of AVH or any positive-like effects of cannabis, but contrary to my expectations adults reported heightened negative-like effects: cognitive disorganisation was especially elevated in adults relative to adolescents after cannabis, and cannabis increased anhedonia symptoms in adults but not adolescents.

Together these findings suggest that cannabis may be a useful pharmacological model of AVH, though further work is required to assess the mechanisms by which cannabis induces AVH. The lack of differences in positive-like effects of cannabis suggest that adolescents are not at heightened vulnerability to the acute psychotic-like effects of cannabis, though whether such findings would translate to longer-term effects of repeated cannabis use on psychosis cannot be determined.

In the next chapter I will return to the acute effects of cannabis on anhedonia in more detail, implementing a reward sensitivity task conceptualised to index anhedonia alongside self-rated measures.

# 6 Chapter 6: The acute effects of cannabis on anhedonia in adolescent and adult cannabis users.

## 6.1 Introduction

In the previous chapter I focused on the acute effects of cannabis on psychotic-like experiences, and on AVH in particular. In this chapter I now investigate the acute effects of cannabis on anhedonia. As described in chapter 2, while considerable research to date has focused on the links between cannabis use and psychosis, the question of whether acutely cannabis also induces negative-like and mood related symptoms, such as anhedonia, has received much less attention (Morrison & Stone, 2011).

Anhedonia is a common symptom experienced by patients with schizophrenia, depression and substance use disorders, and has been linked with repeated cannabis use. Whether cannabis acutely leads to anhedonia is not known. In a similar vein to the previous chapter, here I take a symptom-based approach to better define the specific acute effects of cannabis. If indeed cannabis acutely leads to anhedonia, then it could be a useful pharmacological model for the investigation of clinical anhedonia. Here I will use a number of measures to index anhedonia following cannabis, including a probabilistic reinforcement learning task. Importantly, the RDoC has identified probabilistic reinforcement learning as a key construct in understanding human behaviour. As such if cannabis does impact upon probabilistic reinforcement learning, then it could also be a useful pharmacological model for the investigation of this construct.

### 6.1.1 Anhedonia

Definitions of anhedonia vary, though DSM-IV describes anhedonia as “deficits in the capacity to feel pleasure and take interest in things” (APA, 2013). Others more specifically highlight the role of motivation and sensitivity to reward, for instance “decreased motivation for and sensitivity to rewarding experiences” (Treadway, Buckholtz, Schwartzman, Lambert, & Zald, 2009), and “loss of pleasure or lack of reactivity to pleasurable stimuli” (Pizzagalli, Jahn, &

O'Shea, 2005). As such, in my opinion, anhedonia is a multi-faceted construct, relating to hedonic capacity (that is, the ability to experience pleasure from rewarding stimuli), but also to motivation for reward, and to reward learning (i.e. the ability to modulate behaviour in response to reward, also termed reward sensitivity) (Gold, Waltz, Prentice, Morris, & Heerey, 2008).

### 6.1.2 Measuring anhedonia

Both clinically and in much psychological research, anhedonia is often indexed via self- or clinician-rated measures. For instance, the clinician-rated Scale for the Assessment of Negative Symptoms (Andreasen, 1989) and the widely used PANSS (Kay, Flszbein, & Opfer, 1987); and the self-rated Revised Physical and Social Anhedonia Scales (Chapman, Chapman, & Raulin, 1976) and the anhedonia items of the BDI-II (Beck et al., 1996). However, such scales are typically broad in their content, for instance with items covering ability to enjoy social events alongside items assessing whether patients are actually attending such events. Furthermore, different scales do not necessarily measure similar concepts (Foussias & Remington, 2010). Such measures are not necessarily able to distinguish between the different aspects of anhedonia as described above. While a clinician or patient might easily recognise a lack of reward-seeking behaviour (for example, not meeting up with friends), they cannot identify the multiple underlying cognitive mechanisms that may be contributing to such behaviour.

#### *6.1.2.1 Reward learning*

Pizzagalli et al developed the Probabilistic Reward Task (PRT; Figure 6.1.), to more objectively index anhedonia, characterising anhedonia as reduced sensitivity to reinforced stimuli (Pizzagalli et al., 2005). The PRT is a signal-detection task which uses an asymmetrical reinforcement schedule to intermittently reward correct responses (Pizzagalli et al., 2005). Participants are simply required to indicate which one of two possible stimuli has been visually presented to them. Critically, correct identification of one stimulus is monetarily rewarded more than correct identification of the other stimulus, thus typically producing a systematic preference for the response paired with the more frequent reward (in other words, the

reinforcement schedule creates a response bias whereby participants become more likely to identify either stimulus as the stimulus associated with more frequent reward). This response bias therefore reflects an individual's propensity to modulate behaviour as a function of reward, with a lower bias reflecting lower reward learning. Reduced response bias has been found to correlate with higher self-reported anhedonic symptoms in undergraduates, patients with depression, and patients with bipolar disorder (Bogdan & Pizzagalli, 2006; Pizzagalli, Goetz, Ostacher, Iosifescu, & Perlis, 2008; Pizzagalli, Iosifescu, Hallett, Ratner, & Fava, 2008; Pizzagalli et al., 2005), and predicts occurrence of these symptoms one month later (Pizzagalli et al., 2005).

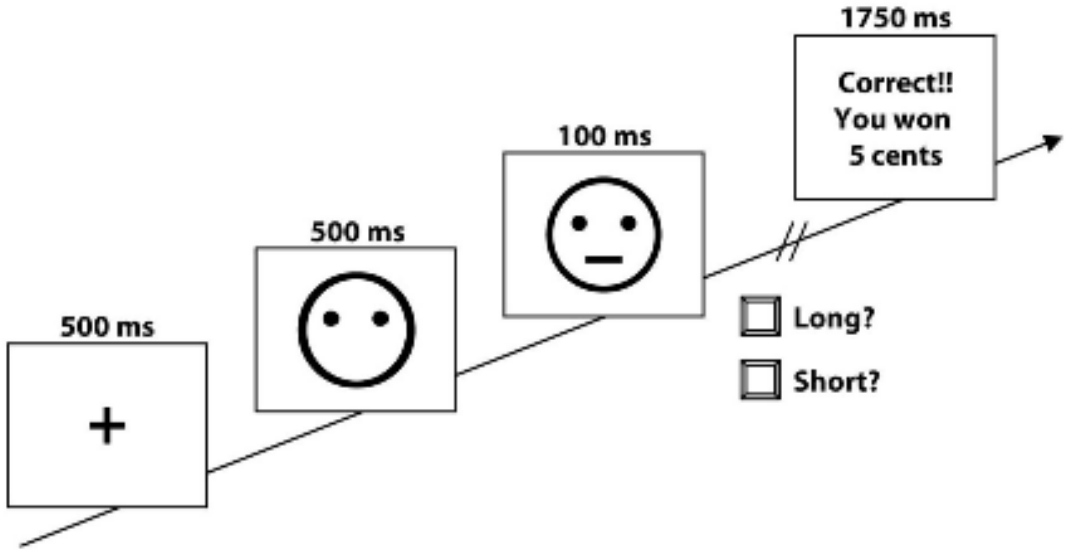


***Figure 6.1.*** *A diagrammatic representation of the Probabilistic Reward Task (taken from Pizzagalli et al., 2005). At the start of each trial, a fixation cross is displayed on screen for 500ms. A mouthless face is the displayed for 500ms, then a long (13.0mm) or short (11.5mm) mouth is displayed for 100ms. The participant responds as to whether they saw the long or the short mouth, then feedback is displayed for 1750ms. One of the mouths is reinforced three times more frequently than the other mouth, and so a response bias tends to develops towards responding that they saw the more reinforced mouth.*

### 6.1.3 Cannabis and anhedonia

#### *6.1.3.1 Associations between cannabis use and anhedonia*

Few studies have directly addressed links between cannabis and anhedonia. Non-acutely some studies (Bovasso, 2001; Dawes et al., 2011; Dorard et al., 2008) but not all (Johnson et al., 2009) have reported associations between cannabis use and anhedonia as indexed by self- and clinician-rated scales.

While anhedonia can form a key component of a diagnosis of schizophrenia and related disorders, whether cannabis is also associated with negative symptoms such as anhedonia has received less attention than the links between cannabis and psychosis. As described in chapter 2, psychosis refers only to the positive symptoms that may lead to a diagnosis of schizophrenia or related disorders. Importantly, the two key meta-analyses described in chapter 2 demonstrating robust associations between cannabis and psychosis did not assessing associations with negative symptomology such as anhedonia (Gage et al., 2016; T. H. Moore et al., 2007). Indeed, of the studies included in the meta-analyses, some had an outcome of psychotic disorder (including schizophrenia), but most had an outcome of psychotic experiences. Though, as described in chapter 2, Moore et al (2007) also reported an association between cannabis use and depression, of which anhedonia is a key symptom, and many studies have reported links between cannabis use or dependence and depression.

While again not specific to anhedonia, some reports have linked repeated cannabis use to *reductions* in negative symptoms in patients with schizophrenia (Compton, Furman, & Kaslow, 2004; Maremmani et al., 2004; Peralta & Cuesta, 1992). However a recent meta-analysis demonstrated no robust difference in negative symptoms between patients with psychotic disorder who continued to use cannabis relative to both ex-users and non-users (Schoeler, Monk, et al., 2016). However, the meta-analysis did detect a small reduction in negative symptoms in ex-users relative to non-users, though this finding was based on only 3 studies with a total of 220 patients.

#### *6.1.3.2 Acute effects of cannabis on anhedonia*

A few studies have reported acute effects of cannabis of schizophrenia-like negative symptoms. D'Souza and colleagues (D'Souza et al., 2004; D'Souza et al., 2005) reported that IV THC increased negative symptoms as indexed by the clinician-rated PANSS in both healthy controls and patients with schizophrenia. More recently Morrison and Stone reported that IV THC in healthy controls increased negative symptoms, as indexed on the self-rated Community Assessment of Psychic Experiences (CAPE) scale, independently of increases feelings of sedation (Morrison & Stone, 2011). The authors reported the most commonly endorsed items on the CAPE following THC, with two of the four items clearly relating to anhedonia ("Do you feel that you are lacking in energy/ motivation/ spontaneity?" and "Do you feel that you experience few or no emotions at this time?"). However, the authors also reported no increase in negative symptoms as indexed by the clinician-rated PANSS, contrary to the previous findings from D'Souza and colleagues (2004, 2005). Morrison et al (2011) suggests that the clinician-rated measures may not be sensitive enough to reliably pick up drug-induced state changes in negative symptomology, given that such changes can present more subtly than positive symptoms.

In study 3 I reported that adults but not adolescents showed increases on the anhedonia subscale of the Psychotomimetic States Inventory (PSI) following acute cannabis administration. However, in study 4 I found no increase on the same anhedonia subscale following both Cann-CBD and Cann+CBD. Two further studies have reported increases on the PSI anhedonia subscale in cannabis users smoking their own cannabis (Mason et al., 2008) and following oral THC (Stokes et al., 2009).

As described above however, anhedonia is a broad concept, and the findings described so far linking cannabis and anhedonia are all based on broad clinician- or self-rated scales. To my knowledge, only one study to date has specifically assessed hedonic capacity in cannabis users. Lawn et al administered both a trait and state measure of hedonic capacity in a sample of

infrequent cannabis users at baseline (non-acute) and following acute administration of cannabis and placebo in a within-subjects placebo-controlled study (Lawn, Freeman, Pope, Joye, Harvey, Hindocha, Mokrysz, Moss, Wall, & Bloomfield, 2016). At baseline cannabis users scored at typical levels on the trait scale, with similar mean scores for both anticipatory and consummatory pleasure to previous healthy control samples (Gard, Gard, Kring, & John, 2006), though there was no direct comparison to a non-user group. Acutely, cannabis did not alter hedonic capacity. In the same study, acutely cannabis was found to have no effect on liking of clips of classical music (T. Freeman, Pope, & Curran, in prep.).

#### 6.1.3.2.1 Cannabis and reward

As described above, anhedonia relates not only to the ability to experience pleasure, but also to motivation to gain rewards, and sensitivity to rewards.

Surprisingly, given the critical role of the eCB system in reward processing (beyond the scope of this thesis, but for review see (Curran et al., 2016)), few studies have addressed whether cannabis influences reward learning. While some have shown no difference between heavy and light cannabis users on motivation for monetary and cannabis reward (Mello & Mendelson, 1985; Mendelson, Kuehnle, Greenberg, & Mello, 1976), daily adolescent cannabis users were found to have lower motivation for monetary reward than non-users (Lane, Cherek, Pietras, & Steinberg, 2005). In the same study assessing trait and state hedonic capacity described above, Lawn et al (2016) also assessed the acute effects of cannabis on the willingness to work for rewards, finding that cannabis reduced likelihood of an individual choosing a hard task with a greater reward value other an easier task with a lower reward value.

More directly assessing reward sensitivity (rather than motivation for rewards), two studies compared neural responses to anticipation of monetary reward in cannabis users relative to healthy controls but found opposing results (Nestor, Hester, & Garavan, 2010; van Hell et al., 2010). In a small study (n= 8) Lane et al assessed the acute effects of cannabis on sensitivity to reward frequency using a task in which monetary reinforcement to one of two responses was

declining (Lane & Cherek, 2002). After smoking cannabis cigarettes (THC 1.77% and 3.58%), participants allocated a higher proportion of their responses to the stimuli with a decreasing reward frequency, relative to placebo and a lower THC dose (THC 0.89%). This pattern suggests that following cannabis the participants were less able to modulate their behaviour in response to reward. Though, after cannabis participants also responded fewer times overall, potentially suggesting decreased motivation for reinforcement alongside decreased reward sensitivity.

Notably, Lawn et al recently administered the PRT to dependent cannabis users and healthy controls, finding no difference in the magnitude of response bias between cannabis users and controls, indicating similar sensitivity to reward (Lawn, Freeman, Pope, Joye, Harvey, Hindocha, Mokrysz, Moss, Wall, & Bloomfield, 2016). However, to my knowledge, no study to date has directly investigated the acute effects of cannabis on anhedonia as indexed by reward sensitivity.

### 6.1.4 Research questions and hypotheses

Given the identified gaps in the literature on how acute cannabis influences anhedonia, I designed a study to address two key research questions:

1. Does cannabis acutely induce anhedonia?

Given previous evidence that cannabis acutely increases self-ratings of anhedonia, I hypothesised that participants would be less sensitive to reward following cannabis than following placebo, as indexed by a lower response bias on the PRT. I further hypothesised that self-rated hedonic capacity would be lower after cannabis than after placebo, and that cannabis would increase self-rating of anhedonia as indexed by the PSI subscale.

Note that the findings reported in this chapter are based on the same study protocol and sample as described for study 3; the final aspect of this hypothesis has therefore already been addressed

in the previous chapter: cannabis increased self-rated anhedonia on the PSI in adults but not adolescents.

2. Does cannabis differentially induce anhedonia in adolescents than adults?

Increasing evidence suggests adolescence may be characterised by reward hyper-sensitivity, relative to childhood and adulthood, for instance a recent study administered a probabilistic reinforcement learning task similar to the PRT to adolescents aged 13-17 years and adults aged 20-30 years, finding that adolescents were more sensitive to reinforcement (Davidow, Foerde, Galván, & Shohamy, 2016). As such, I hypothesised that after both cannabis and placebo adolescents would be more sensitive to reward than adults, as indexed by a higher response bias on the PRT.

Adolescent-onset of cannabis use has also been found to be associated with increased risk of cannabis and other drug use disorders (this will be discussed in further detail in chapter 7), though to date no study has investigated whether the pharmacological effects of cannabis on reward related processes in adolescence differs to that in adulthood. As such, I also explored whether cannabis would have a differential effect on anhedonia in adolescents and adults.

## 6.2 Methods

### 6.2.1 Design and Participants

Study design and participant recruitment and inclusion criteria were as described in chapter 4 section 4.2.1. However, due to a technical error, data were lost for 14 participants on the Probabilistic Reward Task (PRT), leaving a sample size of 26 (13 adolescents) for the present study.

### 6.2.2 Drug administration

As described in chapter 4 section 4.2.2.

### 6.2.3 Measures

#### *6.2.3.1 Baseline assessments*

##### 6.2.3.1.1 Questionnaires

As described in chapter 4, section 4.2.3.1.1. Additionally, participants completed the Temporal Experience of Pleasure Scale (TEPS) and Apathy Evaluation Scale (AES). The TEPS indexes ability to experience pleasure (Gard et al., 2006). The scale consists of items with two subscales to measure anticipatory and consummatory pleasure. Items are rated between 1 (very false for me) and 6 (very true for me), with higher scores reflecting greater ability to experience pleasure. The AES indexes apathy, as defined as a lack of motivation (Marin, Biedrzycki, & Firinciogullari, 1991). The scale consists of 18 items rated between 1 (not at all characteristic) to 4 (very characteristic), with higher scores reflecting greater apathy.

##### 6.2.3.1.2 Drug use

As described in chapter 4, section 4.2.3.1.2.

#### *6.2.3.2 Experimental assessments*

##### 6.2.3.2.1 Subjective Ratings

As described in chapter 4, section 4.2.3.2.2.

##### 6.2.3.2.2 Psychotomimetic States Inventory (PSI)

As described in chapter 5, section 5.2.1.3.2.2. The anhedonia subscale only, consisting of 7 items, is reported here. Items included were: “You are enjoying mixing with people” (reverse-coded), “You feel close to people” (reverse-coded), “You feel rather uninvolved with other people”, “You would feel uncomfortable if your friends were to touch you”, “It is fun to do things with other people at the moment” (reverse-coded), “You find usual activities less enjoyable than usual”, “You feel rather indifferent about things”.

6.2.3.2.3 Snaith-Hamilton Pleasure Scale (SHAPS)

Participants completed the SHAPS, which indexes the ability to experience pleasure (Snaith et al., 1995). The scale consists of 14 items rated between 0 (definitely agree) and 3 (definitely disagree), with lower scores reflecting greater ability to experience pleasure. The SHAPS typically refers to "in the last few days", but following previous work (Lawn, Freeman, Pope, Joye, Harvey, Hindocha, Mokrysz, Moss, Wall, & Bloomfield, 2016) the scale was adapted to refer to the participant's experiences "right now" in order to capture acute effects of the drug.

6.2.3.2.4 Probabilistic Reward Task

For each trial participants were presented with one of two stimuli, and were required to identify, as quickly as possible by button press, which of the two stimuli had been presented. Stimuli were two different lengths of mouth ('short' and 'long'), as seen in Figure 6.2. The 'short mouth' measured 8mm and the 'long mouth' 9mm (note that in the original task as detailed in Figure 6.1. the short mouth measured 11.5mm and the long mouth 13.0mm)

Short mouth Long mouth

***Figure 6.2.*** *Diagram shows 'short mouth' and 'long mouth' stimuli as used in Probabilistic Reward Task. When displayed on screen the 'short mouth' measured 8mm and the 'long mouth' 9mm.*

Correct responses were sometimes rewarded with money, using an asymmetrical reinforcement schedule, with one stimulus rewarded more frequently (the 'rich' stimulus) than the other (the

'lean' stimulus), thus producing a response bias towards the more reinforced stimulus. The task comprised of one block of 100 trials, including 50 rich trials (of which 30 had the opportunity for reinforcement) and 50 lean trials (of which 10 had the opportunity for reinforcement). Participants were only rewarded for correct responses (that is, correct identification of the short or long mouth). To try to ensure that all participants had similar numbers of rich and lean reinforced stimuli, if a stimulus scheduled for reinforcement was not correctly identified, the next stimulus of that type (rich or lean) that was not scheduled to be reinforced was then scheduled for reinforcement. Two task versions were created, with order of completion counterbalanced by drug order; in one version the short mouth was the rich stimulus, in the other version the long mouth was the rich stimulus.

An adapted version (adjusted timings; reward in GBP rather than USD; stimuli mouth length changes as described above) of the task pictured in Figure 6.1. was administered. At the start of each trial, a fixation-cross was presented for a jittered time (750ms, 800ms, 850ms, or 900ms). A mouthless face was then presented for 500ms followed by the appearance of the mouth in the face for 97ms. The mouthless face then remained on the screen for up to 1500ms, or until the participant responded with either the 'c' or 'm' key. For all sessions participants pressed the 'c' key if they thought the mouth was short and the 'm' key if they thought the mouth was long. Feedback was then provided for 1500ms, e.g. "Correct!!! You won 5p" or "You did not win anything". There was then an inter-trial interval of 2000ms, in which a blank screen appeared. Trials were pseudo-randomised, with a maximum of 3 rich or lean stimuli appearing consecutively.

Before the task began it was described to participants by the experimenters, with the aid of on-screen instructions to demonstrate the stimuli and button responses. Participants were told that they would only win money on some of their correct responses, but they were not told that one stimuli would be rewarded more than the other. Participants first completed 5 practice trials, before receiving reminder instructions and being given the opportunity to ask questions.

The main task outcome is response bias, which indexed a person's bias towards the more frequently reinforced stimulus. This was calculated using the following formula (Pizzagalli et al., 2005):

$$Response\ Bias = \frac{1}{2} * log \frac{Rich\_correct * Lean\_incorrect}{Lean\_correct * Rich\_incorrect}$$

Secondary task outcomes include discriminability, accuracy and reaction time. Discriminability, which indexed a person's ability to differentiate the stimuli, was calculated using the following formula (Pizzagalli et al., 2005):

$$Discriminability = \frac{1}{2} * log \frac{Rich\_correct * Lean\_correct}{Rich\_incorrect * Lean\_incorrect}$$

Rich_correct and Rich_incorrect refer to the number of rich stimuli that were correctly and incorrectly identified. Lean_correct and Lean_incorrect refer to the number of lean stimuli that were correctly and incorrectly identified.

### 6.2.4 Procedure

As described in chapter 4, section 4.2.4. Additionally, following drug administration participants completed the SHAPS and PRT.

### 6.2.5 Power calculation

As described in chapter 4, section 4.2.5. However, following data loss for 14 participants I ran a sensitivity power calculation at the smaller sample size. With a sample size of 26, at 80% power at an alpha of 5%, I was able to the detect a medium to large effect size (f= 0.29) for the key interaction of interest (group x drug).

### 6.2.6 Statistical Analysis

#### *6.2.6.1 Data preparation*

All analyses were conducted with SPSS 21.0. Outliers and normality were assessed via diagnostic plots for all analyses. Extreme outliers (>3 times interquartile range) were winsorized within-group.

#### *6.2.6.2 Analysis*

Mann-Whitney or chi-square analyses were conducted as appropriate to compare groups (adult, adolescent) on demographic and baseline measures. Mixed ANOVA was conducted for all test outcomes, with the between-subjects factor of group (adult, adolescent) and within-subjects factor of drug (cannabis, placebo). Additional within-subjects factors were included for relevant analyses: time (T2-T4) for stoned (as in chapter 4, T1 was not analysed due to floor effects); stimuli type (rich, lean) for the PRT. Interactions with time were explored via simple contrasts (comparing T2 to T3, and T3 to T4). Other interactions were explored via pairwise comparisons with local Bonferroni-correction. For PRT, after exclusion of all trials with a reaction time quicker than 100ms, outcomes were calculated according to formulae described in above. Drug order was added as an additional between-subjects factor (placebo-first, cannabis-first) and results were compared to reported primary analyses; unless otherwise noted results were unaffected by drug order.

## 6.3 Results

Demographic and baseline data are displayed in Table 6.1. (note that these differ to those displayed in Chapter 4 Table 4.1, as a result of the reduced sample size).

Adolescents were younger, but groups did not differ on body weight, cannabis weight administered, verbal IQ, BAI, BDI-II, SUPPS-P or AES. Adolescents reported lower pleasure from anticipation but not consumption of reward, compared to adults. Age of first cannabis use was younger for the adolescents compared to the adults, but overall the adults had used for longer. Groups did not differ on current days of use per month, CAST score, days since last use, or likelihood of a positive THC urine result at baseline.

***Table 6.1.*** *Demographic and baseline variables for adolescents and adults; values reflect mean (SD) unless otherwise stated; p-values reflect Mann-Whitney U test comparing median or Fischer's exact test comparing frequency (as appropriate), by age group.*

| | **Adolescents (n=13)** | **Adults (n=13)** | | |
|---|---|---|---|---|
| **Demographics** | **Mean (SD)** | **Mean (SD)** | **Test statistic (df)** | **p-value** |
| Age (years) | *17.05 (0.45)* | *25.46 (0.99)* | *U= 169.000* | *<.001*[1] |
| Body weight (kg) | *67.95 (11.36)* | *76.75 (11.35)* | *U= 117.500* | *.091* |
| Cannabis weight (mg) | *59.87 (8.28)* | *66.23 (6.95)* | *U= 120.500* | *.064* |
| Verbal IQ | *113.54 (8.01)* | *115.69 (9.42)* | *U= 105.999* | *.311* |
| **Baseline questionnaires** | | | | |
| Beck Anxiety Inventory | *5.31 (4.44)* | *5.85 (5.94)* | *U= 84.000* | *>.999* |
| Beck Depression Inventory | *5.46 (3.48)* | *4.77 (4.97)* | *U= 67.000* | *.390* |
| SUPPS-P Impulsive Behaviour Scale | *46.46 (7.81)* | *45.23 (6.35)* | *U= 76.500* | *.687* |
| Apathy Evaluation Scale | *57.85 (8.49)* | *60.23 (6.07)* | *U= 95.500* | *.579* |
| Temporal Experience of Pleasure: anticipatory | *38.69 (6.65)* | *45.15 (7.76)* | *U= 131.000* | *.016*[1] |
| Temporal Experience of Pleasure: consummatory | *35.15 (5.64)* | *39.23 (5.40)* | *U= 114.500* | *.125* |
| **Cannabis use** | | | | |
| Age first tried cannabis (years) | *14.78 (0.93)* | *17.42 (2.16)* | *U= 144.000* | *.002*[1] |
| Last used cannabis (days) | *3.69 (3.07)* | *4.38 (3.18)* | *U= 106.500* | *.264* |
| Duration of cannabis use (years) | *2.27 (1.17)* | *8.04 (2.71)* | *U= 167.500* | *<.001*[1] |
| Cannabis use frequency (days per month) | *11.50 (4.45)* | *8.81 (5.62)* | *U= 47.500* | *.057* |
| Positive THC baseline urine (n=24); %(n) | *81.82 (9)* | *61.54 (8)* | $\chi^2_1$*= 2.355* | *.220* |
| Cannabis Abuse Screening Test | *6.54 (2.73)* | *6.69 (3.84)* | *U= 85.000* | *>.999* |
| **Cigarette use** | | | | |
| Ever used cigarettes; %(n) | *92.31 (12)* | *100.00 (13)* | $\chi^2_1$*= 1.040* | *>.999* |
| Age first tried cigarettes (years)[2] | *15.51 (1.04)* | *18.61 (2.99)* | *U= 144.500* | *.001*[1] |
| Duration of cigarette use (years) | *1.54 (1.03)* | *6.85 (3.51)* | *U= 158.500* | *<.001*[1] |
| Cigarette use frequency (days per month) | *19.18 (12.92)* | *11.72 (12.09)* | *U= 59.000* | *.204* |
| Cigarettes per day | *4.48 (3.20)* | *2.12 (2.21)* | *U= 43.500* | *.034*[1] |
| Fagerström Test for Nicotine Dependence | *1.23 (1.01)* | *0.23 (0.83)* | *U= 36.500* | *.012*[1] |
| Carbon Monoxide at baseline (ppm; n=25) | *6.62 (5.27)* | *5.83 (4.15)* | *U= 67.000* | *.574* |
| **Alcohol use** | | | | |
| Ever used alcohol; %(n) | *100.00 (12)* | *100.00 (13)* | *n/a* | *n/a* |
| Age first tried alcohol (years) | *14.20 (1.60)* | *15.57 (1.92)* | *U= 119.000* | *.081* |
| Duration of alcohol use (years) | *2.85 (1.68)* | *9.88 (2.62)* | *U= 168.500* | *<.001*[1] |
| Alcohol use frequency (days per month) | *6.07 (5.55)* | *10.62 (6.50)* | *U= 123.500* | *.044*[1] |
| Alcohol units per typical drinking session[4] | *9.45 (8.08)* | *8.13 (2.37)* | *U= 88.000* | *.880* |
| Alcohol Use Disorders Identification Test | *8.31 (5.45)* | *8.77 (5.15)* | *U= 95.000* | *.614* |

[1]p<.05

[2]calculated only on those who had ever used cigarettes (n=21)

### 6.3.1 Subjective ratings

#### *6.3.1.1 'Stoned' (Figure 6.3.)*

Interactions emerged for drug x group ($F_{1,24}$= 4.242, p= .050, $\eta^2p$= 0.15), and drug x time ($F_{2,48}$= 5.160, p= .009, $\eta^2p$= 0.18). Ratings of both adolescents (p< .001, $\eta^2p$= 0.71) and adults (p< .001, $\eta^2p$= 0.82) were higher after cannabis compared to placebo, however the increase was larger in adults. Main effects of drug ($F_{1,24}$= 166.466, p <.001, $\eta^2p$= 0.87) and time ($F_{2,48}$= 9.053, p< .001, $\eta^2p$= 0.27) also emerged.

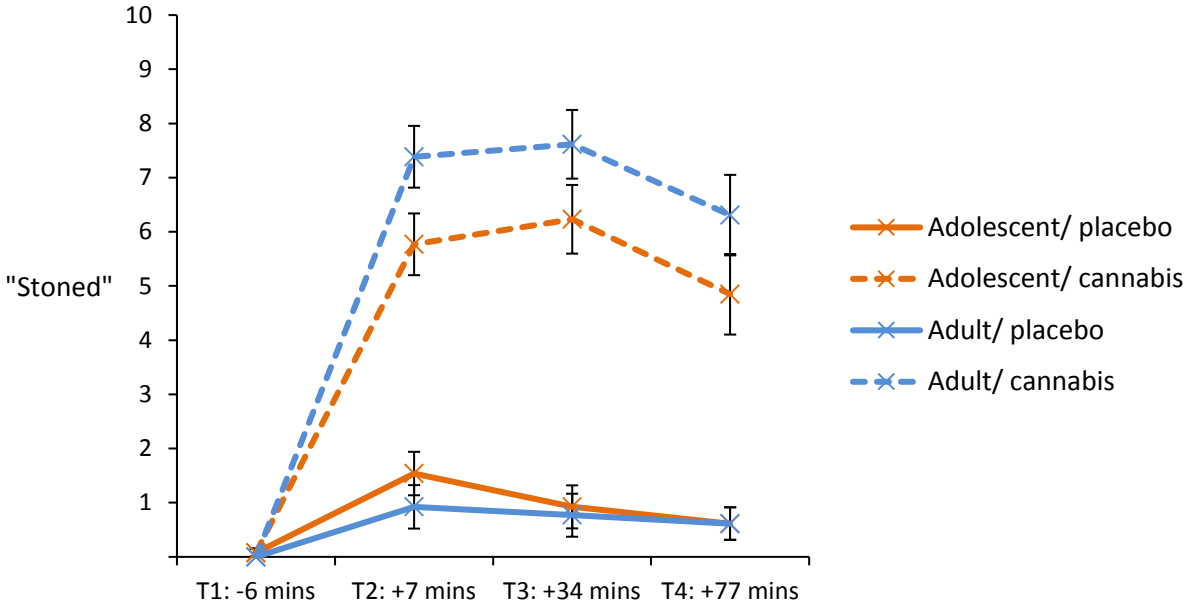


***Figure 6.3.*** *Mean (SE) values for subjective ratings (0-10) for 'stoned', for adolescents and adults on placebo and cannabis.*

### 6.3.2 Anhedonia

#### *6.3.2.1 PSI_A*

An interaction of drug x group ($F_{1,24}$= 5.890, p= .023, $\eta^2p$= 0.20) emerged. Anhedonia increased on cannabis relative to placebo for adults (p< .001, $\eta^2p$= 0.42), but not for adolescents (p= .477, $\eta^2p$= 0.02). A main effect of drug also emerged ($F_{1,24}$= 11.894, p= .002, $\eta^2p$= 0.33).

#### *6.3.2.2 SHAPS*

No main effects or interactions emerged.

***Table 6.2.*** *Means and standard deviations for the anhedonia subscale of the PSI and the SHAPS, by drug and age group.*

| | **Adolescents** | | **Adults** | |
|---|---|---|---|---|
| | **Placebo** | **Cannabis** | **Placebo** | **Cannabis** |
| | **Mean (SD)** | **Mean (SD)** | **Mean (SD)** | **Mean (SD)** |
| | n=13 | | n=13 | |
| **PSI_A** | *4.61 (2.96)* | *5.23 (2.49)* | *2.92 (1.93)* | *6.46 (3.53)* |
| **SHAPS** | *24.46 (4.35)* | *25.00 (5.40)* | *22.85 (6.00)* | *23.46 (4.75)* |

### 6.3.3 Probabilistic Reward Task

#### *6.3.3.1 Response bias (Figure 6.4.)*

No main effects or interactions emerged.

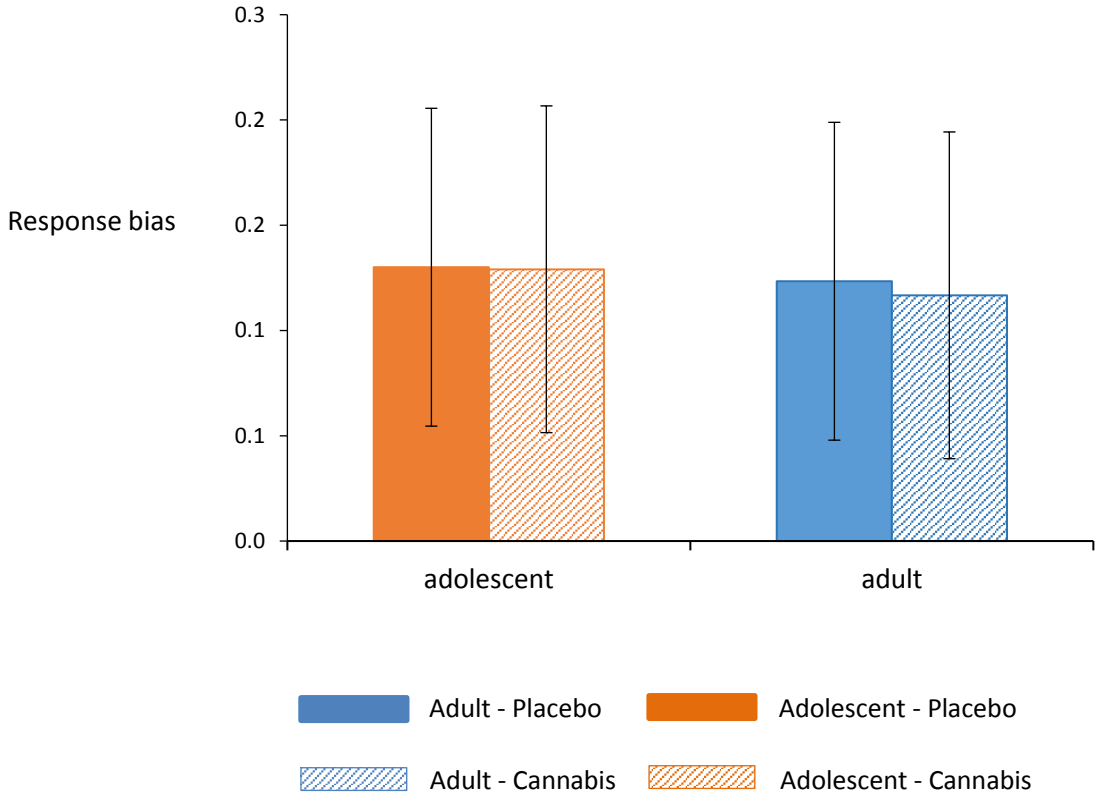


***Figure 6.4.*** *Response bias on the probabilistic reward task (PRT), for adolescents and adults on placebo and cannabis.*

*6.3.3.2 Discriminability (Figure 6.5.)*

No main effects or interactions emerged.

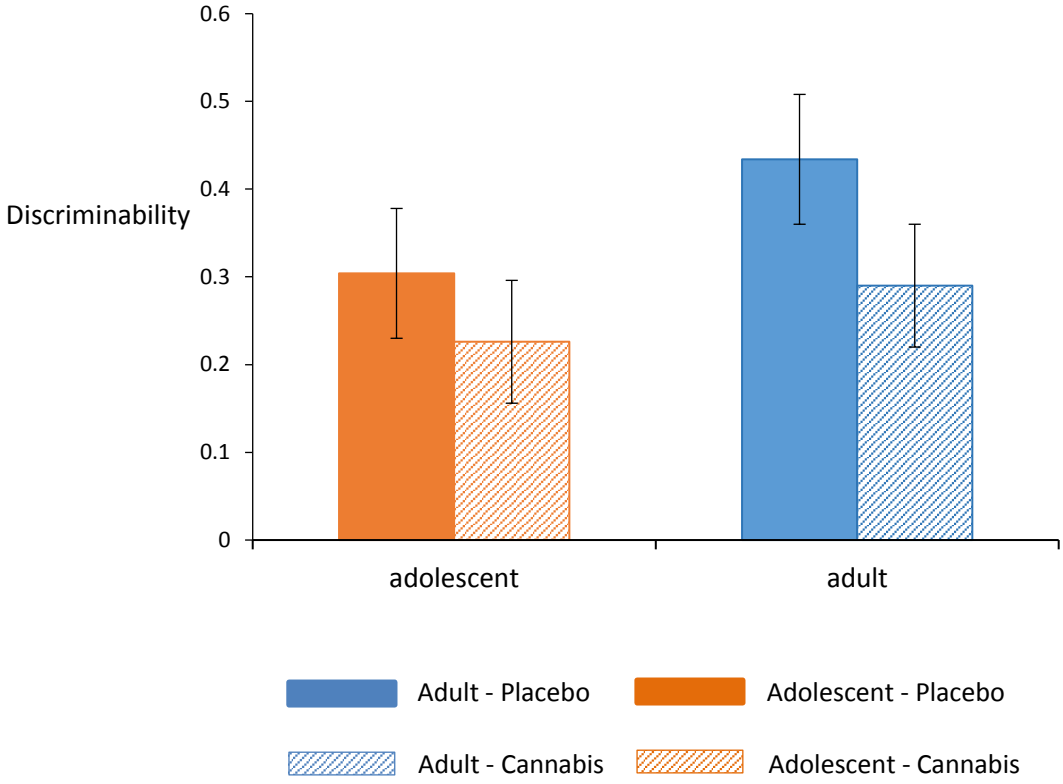


***Figure 6.5.*** *Discriminability on the probabilistic reward task (PRT), for adolescents and adults on placebo and cannabis.*

*6.3.3.3 Accuracy (Figure 6.6.)*

There were main effects of drug ($F_{1,24}$= 4.265, p= .050, $\eta^2p$= 0.15) and stimuli ($F_{1,24}$= 10.911, p= .003, $\eta^2p$= 0.31). Accuracy was lower after cannabis relative to placebo, and for lean relative to rich stimuli.

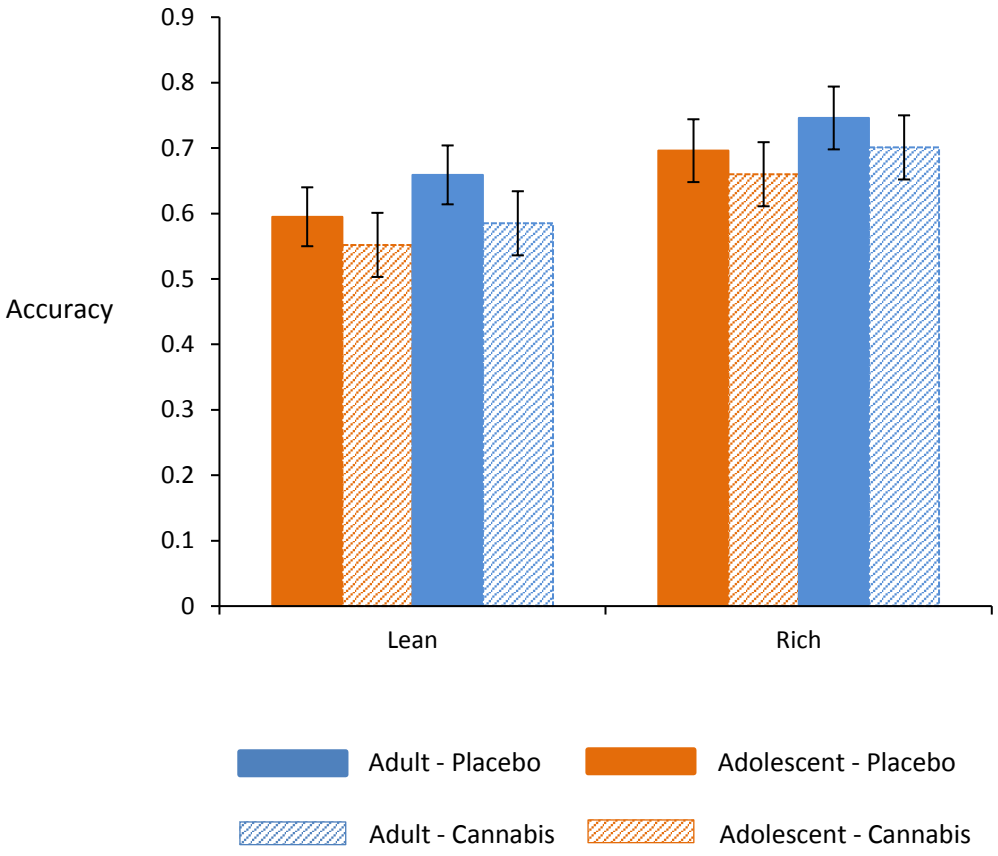


***Figure 6.6.*** *Accuracy on the probabilistic reward task (PRT), for adolescents and adults on placebo and cannabis.*

*6.3.3.4 Reaction times (Figure 6.7.)*

There was a main effect of stimuli ($F_{1,24}$= 6.404, p= .018, $\eta^2p$= 0.21), with longer reaction times for lean relative to rich stimuli.

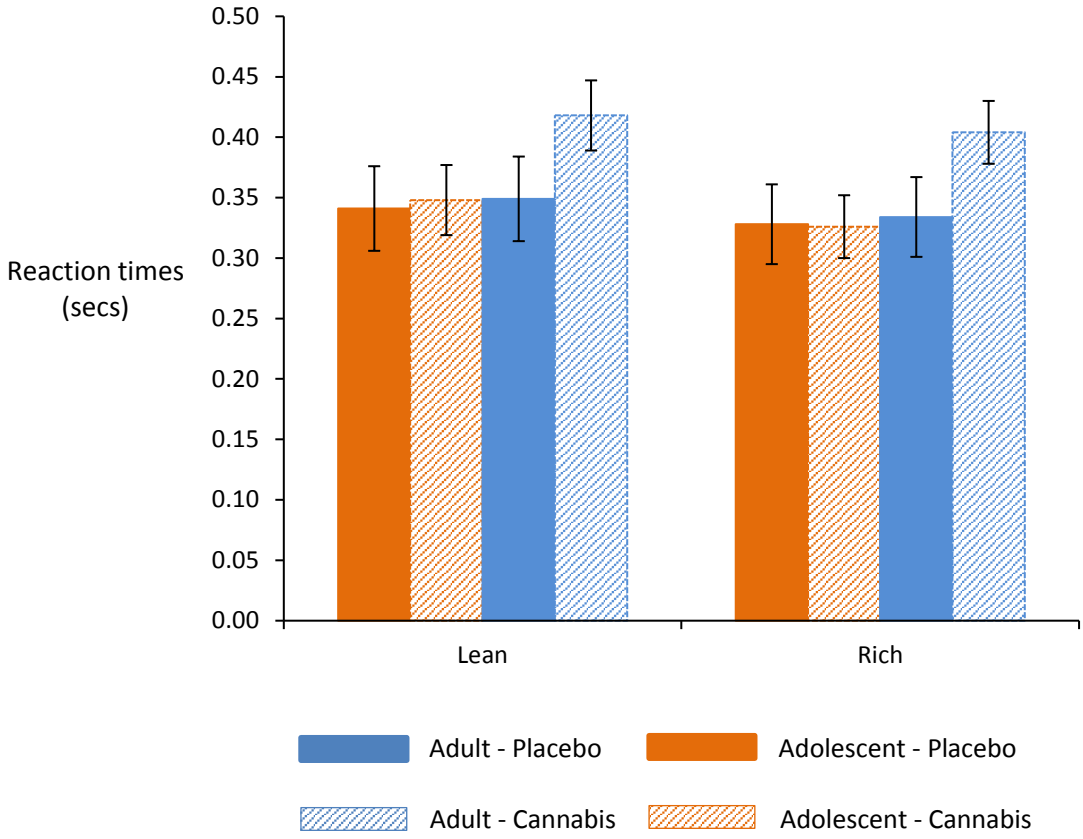


***Figure 6.7.*** *Reaction times (seconds) on the probabilistic reward task (PRT), for adolescents and adults on placebo and cannabis.*

#### *6.3.3.5 Sensitivity analyses*

PRT analyses were the rerun following conservative exclusion criteria (Lawn, Freeman, Pope, Joye, Harvey, Hindocha, Mokrysz, Moss, Wall, & Bloomfield, 2016). Participants were excluded if, on either block, they fulfilled any of the following criteria: more than 20% excluded trials; received reinforcement on fewer than 25 rich stimuli; received reinforcement on fewer than 6 lean stimuli; less than 55% accuracy for the rich stimulus; less than 55% accuracy overall. These sensitivity analyses have been recommended so as to exclude those participants who received little or no reward throughout the task. Such participants would likely demonstrate little or no learning due to a lack of reinforcement, which may obscure any occasion differences due the experimental manipulation (i.e. placebo cannabis versus active cannabis; adolescent versus adult).

Following exclusions, the sample size was reduced to n=14 (5 adolescents). The pattern of results did not change for any of the dependent variables, though these analyses should be treated with caution given the small sample size, particularly of the adolescent group.

### 6.3.4 Within-group correlations

Within-group correlations were conducted between cannabis session PRT response bias and variables showing baseline group differences (at $p < .10$; Table 6.1.), including administered cannabis weight.

TEPS-anticipatory was found to correlate with response bias in both adolescents and adults, though interestingly the relationship was positive ($r = .682$, $p = .010$) for adolescents and negative ($r = -.624$, $p = .023$) for adults (Figure 6.8.). Fisher's r-to-z transformation demonstrated a significant difference between the correlation coefficients ($z = 3.50$, $p < .001$). To explore this further, similar correlations were calculated for placebo response bias scores. No correlations were found in either group between TEPS-anticipatory and response bias on placebo, and the placebo correlation coefficients did not differ by age group (adolescents: $r = -.443$, $p = .130$,

adults= r= -.201, p= .510; z= -0.61, p= .543). TEPS-anticipatory was subsequently entered as a covariate into the response bias model; however, this had no effect on the results and was no longer a significant predictor of response bias in the full model ($F_{1,23}$= 2.830, p= .106, $\eta^2p$= 0.11).

No other variables were found to correlate (at p< .10) with any outcome measure in either the adolescent or adult groups, and so were not entered into models.

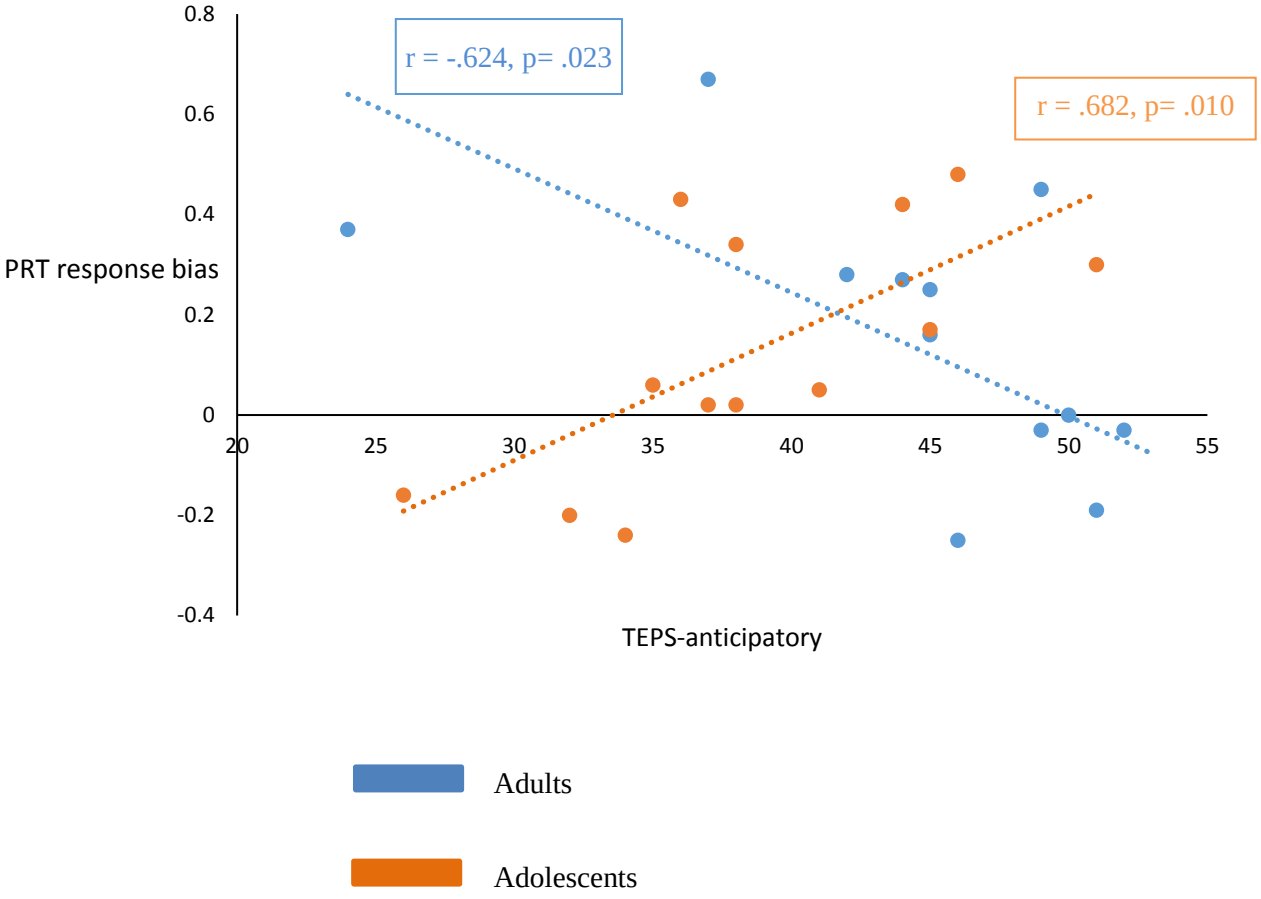


***Figure 6.8.*** *Correlations between TEPS-anticipatory at baseline and PRT response bias on cannabis, for adolescents and adults.*

## 6.4 Discussion

### 6.4.1 Summary of findings

In this study I found that acute administration of cannabis did not result in a lower response bias on the Probabilistic Reward Task (PRT), although cannabis did lead to lower PRT accuracy. Moreover, cannabis did not affect self-ratings of hedonic capacity in either adolescents or adults. Though, as reported in the previous chapter, cannabis increased self-rated anhedonia on the PSI in adults but not adolescents. Additionally, at baseline, adolescents rated themselves as experiencing reduced anticipatory pleasure relative to the adults, but there was no age difference in experience of consummatory pleasure. Moreover, intriguingly, response bias following cannabis was positively correlated with baseline anticipatory pleasure in the adolescents but negatively correlated in the adults. No correlations were found between baseline anticipatory pleasure and response bias following placebo in either group.

### 6.4.2 Does cannabis acutely induce anhedonia?

Contrary to my first hypothesis, participants were not less sensitive to reward following cannabis, as indexed by a lack of difference between response bias on the PRT on cannabis relative to placebo. Furthermore, participants were no less able to experience pleasure after cannabis, as indexed by a lack of difference in self-rated hedonic capacity on cannabis relative to placebo- replicating a previous finding from my research group (Lawn et al., 2016). Though, as described in study 3, cannabis increased self-rated anhedonia on the PSI in adults but not adolescents; however, this was not replicated in the adults in study 4. Together these findings suggest that cannabis has minimal, if any, effects on anhedonia. As such, cannabis does not appear to be a good pharmacological model of anhedonia. Importantly however, given that my target sample size was not reached, these findings should be considered as preliminary, since my design may have lacked statistical power to detect a difference.

Both following acute administration of cannabis in this study, and previously non-acutely in dependent cannabis users compared to healthy controls (Lawn et al, 2006), cannabis was not associated with reduced response bias on PRT. However, I did find lower overall accuracy on the PRT following cannabis. In the absence of an effect of cannabis on discriminability (that is, cannabis did not significantly affect the ability to differentiate between the two stimuli), this reduced accuracy may suggest a general lack of engagement with the task. However, while no significant difference between discriminability on cannabis and placebo was found, descriptively the mean values were somewhat lower on cannabis for both groups. Given the reduced sample size for this study, this lack of significant difference may therefore reflect reduced statistical power rather than a "true null". Given this lower accuracy (and potentially lower discriminability) following cannabis, it is perhaps surprising that the same response bias was achieved.

That cannabis increased anhedonia on the PSI in adults, but on neither of the other indexes of anhedonia, is worthy of further investigation. This finding suggests that the PSI subscale is measuring something different to reward sensitivity or hedonic capacity. Of note, the PSI was specifically designed to be sensitive to drug-related state changes in symptoms, and the questionnaire was indeed first tested in two acute drug studies, one with cannabis and the other with ketamine (Mason et al., 2008). As such, the items in this subscale may have been specifically chosen to tap into expected cannabis effects. Of interest, Freeman and colleagues are in the process of conducting a meta-analysis of the PSI following cannabis, ketamine and sensory deprivation (T. Freeman & Curran, in prep.). Preliminary analyses suggest that across studies cannabis has a small but robust effect on the PSI subscale of anhedonia (d= 0.24, 95% CIs: 0.07-0.41, p=0.006). Further analyses in this large sample of studies may be able to identify whether there are specific PSI items that contribute to this effect, and in turn this may aid interpretation of the discrepant findings between different anhedonia measures in the present study. Importantly, while I measured reward sensitivity and hedonic capacity in this study, anhedonia can also relate to a person's motivation to gain rewards- as described above. Indeed,

Lawn et al (2016) recently showed that cannabis acutely decreases the willingness to work for monetary reward. If the anhedonia subscale of the PSI is capturing reduced motivation, this may explain the discrepant findings between measures in the present study.

Of note, as described in chapter 2, there is previous evidence to suggest that cannabis might be expected to increase reward sensitivity, in opposition to my first hypothesis. Indeed, rimonabant (a $CB_1R$ antagonist) has been found to have depressant effects in rats and prevented conditioned place preference (CPP) to nicotine in rats- suggesting reduced reward sensitivity. Moreover, in humans rimonabant was removed from clinical use as an anti-obesity drug after it was found to be associated with increased risk of depression and suicide (Christensen et al., 2007). Since THC is a partial $CB_1R$ agonist, we would perhaps expect it to have opposite effects to a drug such as rimonabant. In the present study however, I found no evidence of any effect – neither positive or negative – of THC on reward sensitivity or hedonic capacity.

### 6.4.3 Does cannabis differentially induce anhedonia in adolescents than adults?

#### *6.4.3.1 Main effect of age group*

Contrary to my second hypothesis, the adolescents were not more sensitive to reward across sessions than the adults, as indexed by a lack of difference between response bias on the PRT between the age groups, and by a lack of differences in state hedonic capacity, on either placebo or active cannabis. I instead found that adolescents had lower anticipatory hedonic capacity at baseline (TEPS-anticipatory), and they reported somewhat higher (though, non-significantly) anhedonia than the adults on the PSI on placebo.

This is in contrast to past research suggesting that adolescents have a hypersensitivity to reward relative to adults and children, including as was recently found on a task similar to the PRT (Davidow et al., 2016). Importantly however, to my knowledge, no study has yet assessed probabilistic reward learning in a sample of cannabis using adolescents. As such, it is possible

that the past use of cannabis in my adolescent sample may have altered their reward processing, resulting in the lack of an age group difference for reward sensitivity in the present study.

The evidence of higher anhedonia in the adolescents as measured by the TEPS-anticipatory at baseline and (non-significantly) by the PSI anhedonia subscale on placebo, are intriguing, and seem to be specific to anhedonia, since no baseline group differences were apparent on the broader depression (BDI-II) or apathy (AES) scales. Moreover, on the state measure of hedonic capacity (SHAPS) completed during the drug sessions, the adolescents did not score differently to the adults on placebo or cannabis. This contrasts with the trait measure of hedonic capacity (TEPS-anticipatory). However, the SHAPS scale does not separate hedonic capacity into anticipatory and consummatory pleasure, when indeed there is good evidence that these refer to independent constructs (Gard et al., 2006). These discrepant findings therefore suggest that the adolescents specifically experienced lower anticipatory pleasure at baseline, while consummatory pleasure was unaffected. Future longitudinal work is needed to track reward sensitivity and other aspects of anhedonia throughout adolescence, to assess whether cannabis or other substance use does indeed alter typical trajectories.

#### *6.4.3.2 Interaction between drug and age group*

The exploration of whether cannabis would have a differential acute effect on anhedonia in adolescents and adults also led to unclear results, though overall cannabis did not decrease reward sensitivity or hedonic capacity in either group.

In terms of reward sensitivity and hedonic capacity, no effect of cannabis administration was seen in either group, though on the general self-rated anhedonia measure (PSI), cannabis acutely increased anhedonia in adults but adolescents. Furthermore, correlations between response bias and hedonic capacity following cannabis revealed opposite relationships for adolescents and adults; while response bias was positively correlated with baseline anticipatory pleasure in the adolescents, in the adults it was negatively correlated. However, no correlations were found between baseline anticipatory pleasure and response bias following placebo in either group.

These correlations are difficult to interpret, and were not predicted relationships, suggesting they may be anomalous. Though, this finding is in line with the findings reported in chapters 4 and 5 that overall adolescents experienced fewer negative effects of cannabis than adults. Indeed, cannabis may have 'uncoupled' anticipatory pleasure sensitivity from reward learning in the adults, while enhancing this relationship in adolescents.

Finally, I also found a non-significant reaction time impairment in adults but not adolescents. Though this observation should of course be treated with caution, I did find a similar effect in study 2, with longer reaction times on the N-back in adults but not adolescents. As will be discussed in more detail in the next chapter, such effects may represent a psychomotor slowing in the adults following cannabis.

### 6.4.4 Limitations

One limitation of my study was the use of a shorter PRT, with only 1 block of 100 trials as opposed to 2-3 blocks of 100 trials as in previous research. This may have resulted in increased measurement error, and had more trials been included differences in response bias across drug or groups may have emerged. Despite the shorter task however, the magnitude of the response bias in both groups was similar to that reported in previous studies using a task version with 2 blocks (Lawn et al., 2016), suggesting that the task was successful at inducing a response bias despite the smaller number of trials over which to adapt to the reinforcement schedule.

Secondly, as mentioned above, reduced statistical power resulting from a technical error is a clear limitation of this study. Descriptively it is apparent that discriminability was lower following cannabis relative to placebo, and reaction times following cannabis were longer relative to placebo in adults but adolescents. However, these differences were non-significant. Given the small sample sizes (n=13 per group), it is not possible to determine whether these non-significant effects reflect "true nulls", or whether lack of statistical power may be masking true effects. Importantly however, on the key index of reward sensitivity (that is, response bias), performance appears to similar across drugs and groups.

Thirdly, the various findings on different measures of anhedonia are difficult to interpret. Future work should assess the specific factors that different anhedonia scales measure. As discussed above, many anhedonia scales, such as the PSI anhedonia subscale, are broad in their content, so differences between what domain each scale or task specifically indexes may explain the discrepant findings between measures in this study.

Finally, as has been described in chapters 4 and 5, age group differences, such as the lower ratings of anticipatory pleasure in the adolescents at baseline, may have influenced the results in this study. Indeed, the opposite relationship between anticipatory pleasure and reward responsivity in the adults and adolescents is confusing, and may have influenced performance on the PRT. The potential impact of baseline group differences will be discussed in further detail in the final chapter.

### 6.4.5 Conclusions and implications

Anhedonia is a common symptom of schizophrenia and depression, but the broad definition of anhedonia prevents both good description and investigation of the underlying mechanisms of this. Emerging evidence now suggests that patients diagnosed with schizophrenia do not appear to have impaired hedonic capacity or reward sensitivity (Foussias & Remington, 2010; Heerey & Gold, 2007), but that they do have impaired motivation for rewards and reduced valuation of rewards (Gold et al., 2008). This is of interest in the context of cannabis, as the limited studies to date would suggest a similar pattern resulting from acute cannabis administration- impaired motivation for rewards (Lawn et al., 2016), intact hedonic capacity (present study; Lawn et al., 2016) and intact reward sensitivity (present study). In contrast, patients with depression appear to have decreased hedonic capacity (Nakonezny, Carmody, Morris, Kurian, & Trivedi, 2010) and reduced reward sensitivity (Pizzagalli et al., 2005). Referring to anhedonia in both patient groups under broad definitions, is therefore unhelpful. Indeed, even within diagnostic categories there is considerable heterogeneity in patient phenotypes. As described previously, the RDoC initiative aims to reduce this ambiguity by identifying basic symptoms that give rise to certain

symptoms. Indeed, probabilistic reward learning is a key construct within the RDoC, so in the coming years we are likely to see studies with large sample sizes, assessing this construct in many different healthy and clinical populations. I and others (Treadway & Zald, 2011) would also argue that the wider concept of anhedonia needs to be redefined, according to its different components. Of particular importance in relation to cannabis research and following my findings here, is to validate to PSI anhedonia subscale against other tasks and measures so as to better identify what this measure is capturing.

#### ***6.4.5.1*** *Conclusion*

In summary, this study suggests that the effects of cannabis on anhedonia are weak at best, and do not appear to be related to hedonic capacity or to reward sensitivity. As such, cannabis is probably not a good pharmacological model of anhedonia- though as discussed above, better definition of the different aspects of anhedonia is needed to better understand why cannabis does appear to increase self-rated anhedonia on the PSI. Importantly, however, the results described in this chapter should be considered preliminary, given the reduced statistical power. Replication studies with larger samples, and which ideally motivational aspects of anhedonia alongside reward sensitivity and hedonic capacity are required before strong conclusions can be drawn.

# 7 Chapter 7. General Discussion

This thesis set out to address the following question:

Does adolescent cannabis use have greater potential for harm than adult use?

Using a mixed methods approach, including epidemiology and psychopharmacology, I designed a series of studies to address this question. My empirical chapters specifically investigated the following questions:

1. Are IQ and educational outcomes in teenagers related to their cannabis use?
2. Are adolescents more vulnerable to the acute subjective, physiological, memory and inhibition effects of cannabis than adults?
3. Does cannabis increase the incidence of auditory-verbal hallucinations (AVH)?
    a. Are adolescents more vulnerable to the psychotomimetic effects, including AVH, of cannabis than adults?
    b. Do higher levels of CBD in cannabis offset the psychotomimetic effects, including AVH, of cannabis in adults?
4. Does cannabis increase anhedonia, and are adolescents more vulnerable than adults to these effects?

In this final chapter I will bring together the results across each study, and discuss how my findings help to answer these questions and the over-arching question of whether adolescent cannabis use has greater potential for harm than adult use. I will discuss the implications and broader context of my findings, as well as discussing methodological limitations of the studies described in this thesis and suggesting future directions for research.

## 7.1 Summary of findings

In chapter 3 I examined the associations between adolescent cannabis use and IQ and educational attainment in a UK birth cohort. I considered several potential confounders that are commonly associated with teenage cannabis use and that may account for previously reported associations with IQ and educational performance. I hypothesised that cannabis use would be associated with both lower IQ and educational performance (Meier et al., 2012; Silins et al., 2014), but also that these associations may be attenuated by adjusting for potential confounders (Lynskey & Hall, 2000; Rogeberg, 2013; Verweij et al., 2013). As predicted, higher cumulative cannabis use was associated with lower IQ at age 15 and educational attainment at age 16. However, after adjustment for potential confounding variables these associations were attenuated, suggesting that associations between cannabis use and IQ and educational attainment are not causal, but the result of overlapping risk factors increasing the likelihood of both cannabis use and these outcomes. The most dramatic reduction in effect sizes for both IQ and educational attainment occurred following adjustment for cumulative cigarette smoking. Furthermore, after exclusion of all cannabis users, ever having smoked a cigarette remained a predictor of lower IQ and educational attainment. These findings therefore do not suggest that moderate adolescent cannabis use is causally related to IQ or educational attainment, but that co-morbidity between cannabis and cigarette use may be confounding these relationships.

In chapter 4 I described a placebo-controlled study, which for the first time compared the acute effects of active and placebo cannabis on both adolescents and adults. Firstly, I hypothesised that adolescents would be less sensitive to the intoxicating (Carvalho, Reyes, Ramalhosa, Sousa, & Van Bockstaele, 2016; Quinn et al., 2008; Schramm-Sapyta et al., 2007) and anxiogenic (Acheson et al., 2011; Schramm-Sapyta et al., 2007) effects of cannabis compared to adults. Secondly, I hypothesised greater cognitive impairment following acute cannabis in adolescents than adults (Cha et al., 2007; Cha et al., 2006; Schneider et al., 2008), as indexed by spatial working memory, episodic memory and response inhibition. As expected, compared to adults, adolescent cannabis users experienced blunted subjective and physiological effects of cannabis,

but contrary to my second hypothesis they also experienced blunted memory impairing effects. Further, adolescents were not satiated by cannabis and, as predicted, the drug impaired their accuracy on the response inhibition task while leaving those of adults intact. This first demonstration of age-related differences in humans in the acute response to cannabis may have implications for cannabis use patterns by adolescents, as will be discussed in more detail below.

In chapter 5 I first described a placebo-controlled study comparing the acute psychotomimetic effects of active and placebo cannabis on adolescents and adults (study 3). I hypothesised that cannabis would increase the likelihood of experiencing AVH relative to placebo, and that adolescents would be more vulnerable to the psychotomimetic effects, including AVH, of cannabis than adults. Next I described a placebo-controlled study comparing the acute psychotomimetic effects of CBD-lacking, CBD-rich, and placebo cannabis on adult cannabis users (study 4). I hypothesised that both Cann-CBD and Cann+CBD would lead to psychotomimetic effects, including AVH, relative to placebo, but to a greater extent in Cann-CBD. Together the two studies demonstrated that acutely cannabis increases the likelihood of experiencing speech illusion. Contrary to my hypotheses, adolescents did not differ from adults in the likelihood of experiencing speech illusion, and in fact the increase in self-rated psychotomimetic symptoms following cannabis was greater in adults. Moreover, no difference in the likelihood of experiencing speech illusion was found between Cann-CBD and Cann+CBD

In chapter 6 I described a placebo-controlled study comparing the acute effects of active and placebo cannabis on anhedonia in adolescents and adults. I hypothesised that cannabis would increase anhedonia relative to placebo as indexed by reduced reward sensitivity, reduced self-rated hedonic capacity, and increased self-rated anhedonia. Secondly I hypothesised that after both cannabis and placebo, adolescents would be more sensitive to reward than adults, and I explored whether the effect of cannabis on anhedonia would differ between age groups. I found that the acute administration of cannabis did not affect anhedonia as operationalised as reward sensitivity or hedonic capacity. However, as described in chapter 5, cannabis increased self-

rated anhedonia in adults but not adolescents. An intriguing relationship between baseline experience of anticipatory pleasure and reward sensitivity following cannabis was found, with a positive correlation in the adolescents and negative correlation in the adults.

The findings presented in my final two chapters suggest that acute cannabis administration to otherwise healthy adolescent and adult cannabis users induces psychotic-like symptoms, including AVH, but does not induce anhedonia in adolescents. In study 3 (chapter 5; adolescents and adults) cannabis increased anhedonia on only one general self-rated measure in adults, but this was not replicated in study 4 (chapter 5; adults-only and CBD) and in chapter 6 I found no effect of cannabis on two others measures of anhedonia (reward sensitivity or hedonic capacity) in either group. Arguably the effects of cannabis on anhedonia are weak at most. Together these findings further encourage the potential for cannabis to be used as a pharmacological model to investigate novel treatments for psychotic-like symptoms including AVH. They also suggest that cannabis is unlikely to be a useful model of anhedonia.

## 7.2 Does adolescent cannabis use have greater potential for harm than adult use?

The aim of this thesis was to determine whether adolescent cannabis use has greater potential for harm than adult use. Firstly, in chapter 3 I found no robust evidence of differences in IQ and educational attainment between adolescent cannabis users and non-users. Then, as described in chapters 4-6 I found a number of key differences, but also apparent similarities, between adolescent and adult acute responses to cannabis.

### 7.2.1 Are IQ and educational outcomes in teenagers related to their cannabis use?

In study 1, even prior to any adjustment for potential confounders, the most experienced cannabis users had an IQ score only 3 points below those who had never used, and gained the equivalent of six grades lower on one subject of a total of eight at Key Stage 4 relative to never-users. Moreover, after adjustment these effects were attenuated, with confidence intervals crossing the null.

At the start of my PhD I would have found this surprising; however, as is apparent from the literature described in chapters 1 and 2, the evidence that adolescent cannabis use is associated with increased negative outcomes is inconsistent and lacking. Indeed, few studies have found impaired performance on memory or response inhibition tasks in adolescent cannabis users following two weeks or more of abstinence, and evidence linking earlier age of cannabis use onset to poorer cognitive and psychosis outcomes is limited. As I concluded in chapter 1, studies assessing cognitive performance in non-intoxicated cannabis users relative to non-users tend to result in similar findings in adults and adolescents; though to my knowledge none to date have directly compared adolescent and adult cannabis users.

Nevertheless, a number of cross-sectional studies have found evidence of impaired memory and other cognitive performance in adolescent cannabis users relative to non-users, and in a prospective cohort study Meier et al (2012) demonstrated associations between cannabis use and considerable IQ decline in adolescent-onset (≤17 years old) but not adult-onset (≥17 years old) users. However, there are many possible explanations for such findings. While Meier et al (2012) ran a series of sensitivity analyses in an attempt to weed out certain alternative explanations, including excluding those with a diagnosis of schizophrenia or persistent other substance use disorders, the analysis comparing adolescent-onset and adult-onset users was not adjusted for any potentially confounding variables.

Degenhardt and colleagues (2016) discuss the many vulnerability and protective factors that have been identified as likely contributors to an adolescent initiating substance use, including contextual factors (such as drug availability), fixed markers of risk (including individual risk factors such as being male, familial factors such as low parental education, and structural factors such as being an member of a racial minority), and individual and interpersonal risk factors (such as personality factors and stressful life events). Many studies have demonstrated that adolescent cannabis users differ from their peers, from an early age (that is, prior to cannabis exposure), on many factors including antisocial behaviour, rebelliousness and poor educational attainment (Hall & Degenhardt, 2007). Further, adolescent cannabis users are typically also

heavier users of alcohol, tobacco and other drugs, and are more likely to have drug-using peers (Agrawal et al., 2007; Fergusson & Horwood, 2000; Lynskey, Vink, & Boomsma, 2006).

Moreover, risk factors often co-occur, and adolescents with more risk factors are more likely to initiate cannabis use at a young age and to become regular users (van den Bree & Pickworth, 2005). These findings clearly demonstrate that there is selective recruitment to adolescent-onset of cannabis use (that is, adolescent-onset of cannabis use does not occur at random across the population). Crucially, many of the risk factors for cannabis use initiation are thought to overlap with risk factors for a range of negative outcomes, for instance, parental drug use is linked to both earlier initiation of drug use and poorer educational attainment in offspring (Barnard & McKeganey, 2004; Hawkins, Catalano, & Miller, 1992). Without careful consideration of such issues in statistical analyses, associations between cannabis use and poorer cognitive, IQ or educational performance are likely to be influenced by confounding variables, as I demonstrated in chapter 3.

However, statistical adjustment of relationships between the exposure of interest and outcome is limited by imperfect measurement of confounders and the unending possibility that unmeasured confounders (that is, confounders that are unknown or cannot be measured) may be influencing your findings. To my mind, one of the strongest methods for assessing the evidence of links between cannabis use and outcomes, is by use of twin cohorts. As described in chapter 3, twin studies avoid the problems detailed above, since they theoretically isolate the role of substance use in predicting outcomes, by controlling for familial factors (both genetic & environmental) shared by twins discordant for substance use.

Soon after I published the findings of chapter 3, a twin study assessing associations between cannabis use and IQ was also published based on a number of cohorts from the USA. As described in chapter 3, Jackson et al (2016) found no difference in IQ in the late teens (aged 17-20 years) between twins discordant for cannabis use. Furthermore, two studies have found no difference in rates of leaving education early or years of education between twins discordant for

cannabis use (J. D. Grant et al., 2012; Verweij et al., 2013). Such findings therefore support my conclusions in chapter 3, that associations between adolescent cannabis use and poorer IQ and educational outcomes may result from overlapping risk factors (probably environmental more than genetic for both IQ and education, as evidenced by a lack of increased effect size in the cannabis discordant DZ relative to MZ twin pairs (Jackson et al., 2016; Verweij et al., 2013)) rather than from a causal relationship. Though, twin studies are not without their own limitations, including the questions of whether twins can indeed be considered representative of the general population. As such it is important to consider the weight of evidence across different studies, cohorts and methodologies. Together, these three twin studies, alongside my ALSPAC findings reported in chapter 3, and the many previous studies described in chapters 1 and 3 assessing links between cannabis use and cognitive and educational outcomes, suggest that non-causal explanations are not only plausible but indeed likely.

Nevertheless, causality is inherently difficult to address in non-intervention studies. My next chapters described controlled studies, in which causal relationships between acute cannabis exposure and outcome can be directly assessed.

### 7.2.2 Are adolescents more vulnerable to the acute effects of cannabis than adults?

Moving to my acute findings described in chapters 4-6, clear age group differences emerged for a number of outcomes, most notably for the subjective effects of cannabis. Group differences in cognitive effects were also found for verbal memory (delayed verbal recall was affected by cannabis more in adults than adolescents), and on a number of secondary task outcomes (reaction times were longer on the N-back in adults and accuracy was lower on the stop-signal in adolescents). Meanwhile, I found no differences between adolescent and adult cannabis users in the effects of cannabis on any positive psychotic-like symptom (including incidence of speech illusion on the white noise task and self-rated scales), a number of subjective effects (including liking of drug effect, ratings of mental impairment and wanting of food), and many primary outcomes of task performance (including immediate prose recall, discriminability on

the spatial N-back, SSRT on the stop-signal, and response bias on the Probabilistic Reward Task). Intriguingly, when group differences were apparent, across all studies the majority of these were in the direction of heightened effects in adults.

Whether these findings are commensurate with the animal literature, upon which many of my hypotheses were necessarily based, is complicated by the mixed and limited preclinical findings to date. In terms of the generally blunted subjective effects of cannabis in adolescents, this was predicted, and is broadly in agreement with previous animal findings. Indeed, adolescent rats have been found to be less sensitive to the anxiogenic, locomotor-suppressing, and aversion-inducing effects of cannabinoids (Acheson et al., 2011; Carvalho et al., 2016; Quinn et al., 2008; Schramm-Sapyta et al., 2007).

Furthermore, the adolescents became less accurate on the response inhibition task following cannabis, while adults were unaffected, in line with my hypothesis of heightened cognitive effects in adolescents. However, in general my predictions of heightened cognitive and psychotomimetic effects in adolescents were not supported, with instead some evidence of heightened effects on memory and self-rated psychotomimetic effects in adults. While some studies with animals have demonstrated heightened acute effects of cannabinoids on learning and memory in adolescent relative to adult rats (Cha et al., 2007; Cha et al., 2006; Schneider et al., 2008), others have found the opposite (Acheson et al., 2011; Fox et al., 2009), in agreement with my findings. Nevertheless, previous comparisons between adolescent and adult rats for both subjective and cognitive effects should be treated with caution. Many of the studies referenced in this thesis had small samples, and have not been replicated despite key findings having been published approximately 10 years ago and widely reported (e.g. (Cha et al., 2007; Cha et al., 2006; Schramm-Sapyta et al., 2007)). Moreover, as I will discuss in more detail below, there remains controversy about what constitutes the adolescent period in rodents.

#### *7.2.2.1 Potential implications on adolescent cannabis use patterns*

The pattern of effects seen throughout the acute studies – blunted subjective, memory and psychotomimetic effects and a lack of satiety following cannabis in adolescents, alongside potentially impaired response inhibition processes in adolescents – may conceivably influence real-world cannabis use patterns in the different age groups. Importantly, following drug administration the adolescents did not show satiety; instead they wanted more cannabis regardless of whether they had taken active cannabis or placebo, while the opposite was seen for adults. If adolescents do not feel satiated after an acute dose of cannabis whilst also experiencing fewer negative effects, it follows that they may well use more cannabis in a smoking session than adults, potentially contributing to the increased risk of long-term harms that some have found to be associated with younger age of use (Curran et al., 2016). Indeed, though I concluded that the evidence for cannabis use leading to long-term impairments of cognition, IQ and educational performance is weak, there are many other potential consequences of early-onset substance use, including increased risk of addiction (Chen & Anthony, 2003; Chen et al., 2005; Hines et al., 2015; von Sydow et al., 2002). In turn, adults' experience of more negative effects of cannabis may limit their use and reduce their risk of harms, which would concur with the declining prevalence of cannabis use seen from early adulthood (Degenhardt et al., 2008). At the moment however, such suggestions are largely speculative. As will be discussed in further detail below, longitudinal studies are required to follow-up on whether age-related cannabis sensitivities do indeed predict future cannabis use patterns and other outcomes.

Of note, similar hypotheses have been suggested from the preclinical alcohol literature, where the literature base is much more substantial. There is similar evidence of contrasting profiles of heightened positive and blunted negative sensitivities in adolescent and adult rats exposed to alcohol acutely, alongside human epidemiological evidence to support the assertion that binge

drinking is considerably more prevalent among adolescent and young adult drinkers (Kuntsche, Rehm, & Gmel, 2004; Naimi et al., 2003; Research & Evaluation, 2005), and that those started drinking from an earlier age have an increased risk of alcohol addiction (DeWit, Adlaf, Offord, & Ogborne, 2000; B. F. Grant & Dawson, 1997). Again speculatively, it seems plausible that the effects could be promoting heavier and potentially problematic drinking in younger people. In fact, I am currently running a similar study design to that described chapter 4, with alcohol, in human adolescents and adults.

In summary, I found some potentially important differences between the age groups in their reactions to cannabis, which when considered as a whole may have implications for cannabis use patterns and vulnerability to long-term harms in adolescents.

#### *7.2.2.2 General intoxication or domain-specific effects?*

One alternative interpretation of the overall pattern of my results, is that the often heightened effects of cannabis in adults reflect amplified general intoxication (as demonstrated by heightened subjective effects), rather than domain-specific cognitive impairments or psychotomimetic effects. The primary outcome for the spatial working memory task (that is, discriminability) was lowered by cannabis, but did not differ between groups, while the secondary outcome of reaction time was longer in adults but not adolescents following cannabis. Reaction times also appeared to be marginally slowed in the probabilistic reward task in adults but not adolescents (though this was a non-significant effect). Together these findings may suggest a psychomotor slowing effect in the adults, which would be in agreement with previous findings that adolescent rats experienced lesser locomotor-suppression effects of acute THC than adults. Additionally, the blunted effect of cannabis on psychotomimetic effects in adolescents was apparent specifically for the PSI subscales of cognitive disorganisation and anhedonia, with no age group differences on positive psychotic-like symptoms on any measure. Increased self-ratings of cognitive disorganisation may again reflect generally heightened intoxication effects of cannabis in adults, rather than a domain-specific effect. The age group

difference in PSI anhedonia is intriguing, though as I found no evidence of anhedonia increases following cannabis on the same measure in adults in study 4 (chapter 5), and on other anhedonia measures in study 5 (chapter 6), this may be an anomalous finding.

In summary, the overall pattern of results suggests a contrasting profile of resilience and vulnerability to the acute effects of cannabis in adolescents and adults, though whether the differences reflect domain-specific effects is difficult to determine. Given that adolescents experienced reduced accuracy on the stop-signal, the pattern of results cannot be fully explained by heightened intoxication in the adults. Furthermore, as will be discussed in the next section, that the adolescents and adults had opposing patterns of response regarding desire for more cannabis throughout the sessions is intriguing and again suggests a more complex explanation for my findings.

#### *7.2.2.3 Strengths and limitations*

Study-specific strengths and limitations have been described previously in the relevant chapters, however given the similarity of the design of the acute studies, there are a number that apply across studies 2, 3 and 5.

##### 7.2.2.3.1 Strengths

The acute studies described in this thesis have several critical strengths. My groups were well-matched on baseline measures including premorbid IQ and levels of anxiety, depression, impulsivity and schizotypy. This increases confidence that participants in the two age groups were drawn from similar populations, and maximises comparability between groups.

The use of cannabis plant material, rather than extracted or synthetic cannabinoids, via an ecologically valid administration procedure (that is, inhalation) enhances the relevance of my findings to the real world use of cannabis. Meaningful comparisons between studies that have utilised a variety of administration routes (including oral, IV, smoked, vaporised), types of drug (including cannabis plant material, isolated THC or CBD), and doses is problematic. In my

opinion, inhalation of cannabis plant material via a vaporiser is the ideal method of administration in many situations, since it best replicates recreational use but without the health risk associated with smoking or the practicality issues given that UK smoking laws prevent indoor smoking. Relatedly, administration of a known THC dosage in my studies, which closely corresponds to that contained in about a third of a typical joint (van der Pol et al., 2014), increases generalisability of my findings to real-world cannabis use.

Weight-adjustment of the cannabis dose in my adolescent/adult studies is a key strength. While IV THC doses are, to my knowledge, always weight-adjusted, this is not often implemented for inhaled cannabis doses - indeed, weight-adjustment was not implemented in study 4 (chapter 5; adults-only and CBD). Given evidence, as described in chapters 1 and 2, of dose-response relationships between THC and drug effects, failure to weight-adjust doses likely introduces unwanted variability in the data.

Lastly, to my knowledge, this is the first time that cannabis has been administered in a controlled setting to humans under 18 years of age. Indeed, I am aware of no controlled studies in which under 18's have been administered an illicit drug, thus leaving a large gap in our knowledge of the effects of substance use at the very stage that recreational use typically begins. The acute studies described in this thesis therefore represent a significant step forward in the translation of preclinical developmental psychopharmacology. Moreover, in relation to this thesis as a whole, my use of mixed methods – epidemiology and psychopharmacology – with hypotheses often driven by animal work, is a key strength of the research I have described here. Mixed methods, drawing on the strengths of different research methods and translating findings and questions across methodologies, is key to us developing a more comprehensive understanding of the full implications of adolescent cannabis use.

#### 7.2.2.3.2 Limitations

Firstly, I cannot speak to mechanisms of the reported age-related sensitivities. My findings suggest there may be age-related neural sensitivities to cannabis, potentially resulting from the

ongoing maturation of the eCB system throughout adolescence and its putative role in developmental processes in the brain. Given the limited and inconsistent knowledge we have about the development of the eCB system throughout adolescence however, it would be inappropriate to speculate how this may relate to my specific findings. There are also a number of alternative explanations for the findings, other than age-related neural sensitivities. Adolescents have a higher basal metabolism than adults (Black, Coward, Cole, & Prentice, 1996; Manini, 2010), alongside lower percentage body fat (Forbes & Reina, 1970; Guo, Chumlea, Roche, & Siervogel, 1998), potentially affecting the speed of THC metabolism differentially in the two groups. Should THC and its by-products be metabolised more quickly in adolescents than adults, this could potentially result in the reduced subjective and memory effects seen in adolescents; however, if drug metabolism in the adolescents was faster, a quicker decline of drug effects would be expected, which does not appear to be the case. Further this would not explain the adolescent's impaired response inhibition accuracy when the adults were unaffected. As will be discussed below, future work should focus on identifying the mechanisms by which the differential effects I found occurred.

As discussed above, a key strength was that participants were given a weight-adjusted dose. However, since adolescents typically weigh less than adults (Sutton, 2012), on average this resulted in them receiving a lower dose than the adults. This was expected, and was in fact the key initial motivation for implementing weight-adjusted doses. Unfortunately, this also means that I cannot rule out the possibility that the blunted effects seen in the adolescents are due to the reduced dose- however again this would not explain the overall pattern of results including the adolescents' (but not adults') impaired response inhibition accuracy, or the lack of differences between the groups on a number of other measures. Moreover, critically the weight of cannabis administered did not correlate with any outcomes reported across chapters 4-6, in either of the age groups. To address this, groups could potentially be matched for body weight in future research. However, this would result in a biased adult sample that does not reflect the population as a whole, reducing generalisability of results. Nevertheless, comparison of results

from such a study to my findings could be informative. For instance, recruiting lighter adults to match to a normative weight sample of adolescents would help to ensure that adolescent development was at a similar stage to that in the studies described here, but would remove the potential confounds of differential body weight and dose.

The within-subjects placebo-controlled design is a clear strength of the acute studies described in this thesis, allowing causal statements to be made about drug effects within each age group. Nevertheless, the issues of confounding, described above in relation to my ALSPAC study, are relevant for the between-subjects comparisons (adolescent versus adult) in my acute studies. It is important to bear in mind that my adolescent and adult groups differed not only in age, but also on a number of other measured variables, and likely will have differed on a number of unmeasured variables too, and such differences may have influenced the acute effects of cannabis. While many have suggested that there is individual variation in responses to cannabis, few studies have explored the baseline characteristics that may influence the acute effects of cannabis. Importantly however, the groups were matched for baseline schizotypy, which has been linked to both the psychotic-like and pleasurable effects of cannabis (Barkus & Lewis, 2008; Barkus et al., 2006; Mason et al., 2009).

Relatedly, all participants were necessarily regular cannabis users, raising the possibility that my findings may be affected by group differences in past cannabis use. While the groups were statistically matched for cannabis abuse symptomology and days since last cannabis use (adolescents= 3 days, adults= 5 days), the adolescents did report slightly more days of cannabis use per month than the adults (11 days versus 8 days); further, while the adults had been using for more years, they had started using from an older age. Acute subjective and cognitive effects of cannabis may be blunted in more frequent cannabis users, and tolerance to some cannabis effects following frequent use has been reported (including for spatial working memory and episodic memory (Broyd et al., 2015)), however findings are limited and inconsistent (Ramaekers et al., 2016). As such, it is possible that differing cannabis use histories and patterns between the groups may have contributed to differences in outcomes. Importantly however,

cannabis use frequency did not correlate with any outcome in either the adolescent or adult groups, somewhat reducing these concerns.

The adolescents were also more frequent and heavier cigarette smokers, with higher nicotine dependence scores, and they had started tobacco smoking from a younger age than the adults. The groups were well-matched for age of first alcohol use, but the adolescents were less frequent alcohol drinkers. It is possible that cross-tolerance to cannabis from previous alcohol or tobacco use may occur, though I am not aware of evidence demonstrating such an effect. A recent ecological momentary assessment study suggested that acutely tobacco use may offset acute impairment of working memory from cannabis (Randi Melissa Schuster, Mermelstein, & Hedeker, 2016), though this has yet to be replicated in a controlled study. If more adolescents smoked a cigarette immediately prior to the testing sessions, it is possible that the recently consumed nicotine may have helped to protect against the memory impairing effects of cannabis in this group. Given that cigarette use was found to be a key confounder of associations between cannabis use and IQ and educational performance in chapter 3- and that cigarette use was associated with lower IQ and educational performance- it is clearly important to consider cigarette use when working with cannabis using populations.

While pharmacological studies of cannabis typically have a double-blind design, cannabis intoxication effects are likely to result in un-blinding of both participant and experimenter, potentially influencing responses. This is unfortunately an issue with all pharmacological studies in which the drug under investigation has clear subjective effects. Using an active placebo with broadly similar intoxication effects is possible and may reduce demand characteristics, though it is unclear what drug could be used to mimic cannabis, and experienced cannabis users are likely to recognise the differences in subjective effects.

Of note, I have primarily focused on behavioural data throughout this thesis, since my empirical chapters all describe behavioural studies. A number of studies have administered memory and response inhibition tasks to cannabis users, or following acute administration of cannabis, while

utilising neuroimaging techniques such as fMRI. Such studies have sometimes found no difference in behavioural task performance differences while also finding evidence of increased or altered BOLD response to task demands in cannabis users or following acute cannabis. However, to date no clear pattern of altered activity has been identified for similar tasks, with regions of increased response often varying across different studies. Nevertheless, such findings have implications for the interpretation of the data described in this thesis, and it may be that neural responses differed between adolescents and adults following cannabis.

Finally, since I have described a number of novel studies, with multiple statistical comparisons and limited or mixed evidence on which to base my prior hypotheses, it is important to treat these findings with caution. Indeed, a number of my statistical comparisons resulted in results 'on the cusp' of significance, reducing confidence in the reliability of these findings. The results from chapter 6 regarding anhedonia in particular should be treated as preliminary, given that the sample size was considerably smaller than I initially aimed for. Replications with larger sample sizes (which can now be determined according to effect sizes reported in this thesis) are required for all novel findings reported in this thesis, before strong conclusions can be drawn.

### 7.2.3 Lost in translation?

Given the lack of human literature on which to base the hypotheses for my acute studies with adolescents, many decisions about which domains to assess and the direction of hypotheses were based on animal literature. As described above, the preclinical findings have been inconsistent, likely resulting from typically small sample sizes, and hard to compare, given often poor reporting of methods and statistics. Moreover, there are a number of reasons to be cautious of extrapolating the cannabinoid administration findings in rodents to humans.

As described in chapter 1, the most robust finding in humans from acute and non-acute cannabis research is impairment to verbal learning and memory. Such a domain clearly cannot have a rodent analogue, preventing assessment of such effects in translational models. Moreover, adult rats typically find THC to be aversive, and will not self-administer it, while humans clearly have

the opposite reaction- recreational users find cannabis rewarding and of course choose to administer it. Intriguingly, there is some evidence to suggest that while adult rats develop conditioned place aversion (CPA) to THC, adolescent rats do not. However, interpretation of this age difference is difficult given that the adult rodent findings do not match that seen in adult humans.

In many of the animal studies I have referred to in this thesis, rats were administered highly potent full $CB_1R$ agonists, typically WIN, rather than THC or cannabis. The effects of such drugs are therefore likely to differ to that of THC, which is typically a partial agonist at $CB_1R$ but can also have antagonist properties under certain conditions. Significantly, human usage of synthetic cannabinoids, that are typically high-affinity and high-efficacy $CB_1R$ agonists, has been linked to medical emergencies including seizures, agitation and vomiting, while natural cannabis consumption rarely results in adverse physical health effects (Hermanns-Clausen, Kneisel, Szabo, & Auwärter, 2013; Seely, Lapoint, Moran, & Fattore, 2012). Relatedly, when THC is used in rat studies, the doses are often high compared to typical human consumption, and for repeated administration studies dosing regimens are often intensive and do not reflect human self-administration patterns. Of interest, a recent study demonstrated impaired spatial recognition memory following an experimenter administered dose of WIN to adolescent rats, but no impairment when the drug was self-administered (Kirschmann et al., 2016). Moreover, they found no effect of repeated adolescent self-administration on subsequent spatial recognition and working memory performance in adulthood. Given that most animal studies use experimenter administration, these recent findings to some extent question the validity of the translation of cognitive impairment findings following cannabinoid administration in animals to naturalistic human use. Such findings clearly also have implications for controlled administration studies in humans, and highlight the benefit of using naturalistic experiments alongside controlled paradigms such as those described in this thesis.

Finally, adolescence as a concept is difficult to define. Unlike puberty, which is characterised by a series of biological events, adolescence is marked instead more by the steady transition

between childhood dependency and adulthood independence. Indeed, in humans, adolescence has been defined in developmental research as the period of time that begins with puberty and ends when an individual takes on a stable, independent role in society (Steinberg, 2010). Others define adolescence by age, with UNICEF and the WHO defining adolescents as those aged 10-19 years. In this thesis I defined adolescence as those under the age of 18 years, given the significance of the age 18 in the UK, both legally and socially. Translation of the adolescent period to animals is therefore complex, and the concept of an adolescent period in rodents remains controversial. In an influential review, Spear (2000) defined the adolescent period in rats as PND 28 to 42, while also acknowledging the difficulties and limitations of defining such a period. In a personal communication, Professor Clare Stanford (Pharmacology, UCL) also highlighted the inherent difficulty in defining adolescence in rodents and other mammals due to the differing rates and times at which different physiological systems, including different brain regions, mature. Moreover, given that we do not fully understand such processes in humans, it is hard to validate such changes against human adolescent maturational processes.

Nevertheless, animal research with cannabis clearly has its strengths, not least that it can address causal hypotheses in ways which are ethically impossible with humans. A recently developed method of administrating cannabinoids via inhalation to rodents, using e-cigarette technology (Nguyen et al., 2016), could increase the ecological validity of rodent cannabinoid research, as would increased use of THC rather than more potent synthetic cannabinoids. To my mind, the best way to increase the translation and utility of rodent work in this field is to increase collaboration between human and animal research groups. Adopting a mixed methods approach from the outset of a project and developing closely matched study designs and drug protocols would increase comparability between species. Of note, an example of such an approach comes from a study utilising the Probabilistic Reward Task (PRT) as described in chapter 6, in which the PRT was administered to both humans and rats to assess the cross-species translation of the effects of nicotine withdrawal on reward responsivity (Pergadia et al., 2014).

### 7.2.4 Are there 'developmental windows' for cannabis-related harms?

An important consideration for the interpretation of the results in this thesis and previous animal findings, is developmental stage. Adolescent brain development is thought to start approximately at the onset of puberty, lasting until at least the mid-20's (Giedd et al., 1999), with white matter development thought to continue into the early-30s (Tamnes et al., 2010). Maturational processes in adolescence are complex and not fully understood, as is demonstrated by a recent paper tracking structural brain development throughout the lifespan (Mills et al., 2016). Combining data from four longitudinal adolescent cohorts, their findings questioned the common understanding that grey matter volumes peak around the onset of puberty before declining throughout adolescence, instead finding that the peak and onset of declining volume occurs in childhood. Moreover, maturation does not occur at similar rates throughout all regions of the brain. For instance, Shaw et al demonstrated that more complex growth trajectories are apparent in higher-order association cortices relative to regions with more simple laminar architecture including limbic regions (Shaw et al., 2008; Tamnes et al., 2013). In an influential early study Giedd et al (1999) demonstrated that different cortices showed variation in the age of peak grey matter volume and shape of trajectory. It has therefore been suggested that some brain areas and functions will be particularly sensitive to certain environmental inputs at different stages throughout adolescence (Fuhrmann, Knoll, & Blakemore, 2015).

As such, the adolescent brain may only be susceptible to harm from cannabis use during certain periods of development, or specific structures and functions may only be susceptible if exposure coincides within a certain developmental stage. In support of this, in adolescent rhesus monkeys, as described previously, Verrico and colleagues found that both acute (Verrico et al., 2012) and repeated (Verrico et al., 2014) doses of THC led to impaired spatial working memory but neither regime had any effect on the earlier developing object working memory. Moreover, repeated THC doses prevented the maturational improvement in spatial working memory that was seen over the course of the six-month administration period in the monkeys given placebo. The effects of cannabis both acutely and following repeated administration may therefore be

dependent upon the developmental stage of the cognitive function being measured. Though, to better test this explanation of Verrico and colleagues' findings, a similar study in which cannabis exposure is specifically timed to coincide with the period at which object working memory is maturing is now needed.

In humans, memory ability improves throughout childhood and adolescence. For instance, there is evidence that basic abilities such as spatial location recall maturing in late childhood (by age 11-12 years) while more complex abilities such as strategic organization of spatial information continue to improve until mid to late adolescence (age 16-17 years) (Luciana, Conklin, Hooper, & Yarger, 2005). Similarly, response inhibition improves throughout childhood and adolescence, with Rubia et al finding that adolescents aged 10-17 years made more inhibition errors on the go/no-go task than the adults aged 20-43 years, and performance positively correlated with age (Rubia et al., 2006). Although, another study found that performance on an antisaccade task of interference (an alternative index of inhibition) matched that of adults by age 14 years (Luna, Garver, Urban, Lazar, & Sweeney, 2004). Of interest, others have found evidence of increasing inhibition-specific neural responses continuing into adulthood (for review, see (Blakemore & Robbins, 2012)), suggesting that while in chapter 4 no age group differences in placebo performance were detected on the stop-signal task, related neural processes may have differed. Together these previous findings suggest that maturation of the abilities required for the completion of the N-back and stop-signal tasks as used in chapter 4 may already have been fully matured in the 16-17 year-olds. The tasks may therefore not be sensitive to age differences in the effects of cannabis on performance. Indeed, we saw no placebo session group differences in performance on any of the cognitive tasks. Had my adolescent participants been younger it is possible that heightened working memory deficits or lengthened stop-signal reaction times would have become apparent, and indeed the pattern of results across all studies may have differed.

Verrico and colleagues' (2014) findings are relevant also to my ALSPAC findings in chapter 3. I summarised cannabis exposure in one variable reflecting cumulative number of cannabis uses

in the participant's lifetime, as reported at one time-point at age 15. Cohort studies such as this, and studies relying on retrospective reporting of drug use in general, may not be sensitive enough to detect associations between outcome and cannabis use or age of cannabis use onset, if developmentally sensitive periods are short, or, more problematically, if there is considerable inter-individual variation in the age at which relevant maturational processes occur, as indeed the evidence described above suggests. Using chronological age of cannabis use onset as a predictor may therefore mask true relationships between exposure and outcome.

In an attempt to reduce the variance in brain maturational state between participants – given evidence of differing age of puberty onset and inconclusive evidence of differing brain development trajectories between sexes (Giedd et al., 1999) – I recruited only males for studies 2, 3 and 5. This therefore precludes generalisation of findings to teenage girls. Unfortunately, participants in cannabis research are predominantly male and gender effects have rarely been assessed, with inconsistent findings (Broyd et al., 2015). Some have shown heightened subjective (Cooper & Haney, 2014) and working memory (Makela et al., 2006) effects in women compared to men, though others have found no differences (Anderson, Rizzo, Block, Pearlson, & O'Leary, 2010). Recently it was demonstrated that younger age of cannabis use onset predicted poorer verbal memory in women but not men (Crane et al., 2015), suggesting that there may be age-dependent gender differences in the cognitive effects of cannabis. Moreover, there is mixed evidence to suggest sex differences in vulnerabilities to addiction, for instance some have found that women progress from drug initiation to addiction quicker than men (Tuchman, 2010). Given such findings, there is a clear evidence gap regarding the effects of cannabis in young women and girls.

### 7.3 Future directions

As described above, replication of my novel findings should be the next step, and given the potential age-dependent gender differences in the effects of cannabis, this should involve females as well as males. Determining the mechanisms by which the pattern of age-related

differences and similarities emerged is also key. A simple next step would be to address the rate of THC metabolism by taking repeated blood samples following cannabis administration and comparing the rate of breakdown of THC between different age groups.

Ideally, future acute and non-acute studies would also index developmental stage of the adolescent brain, though how this could be achieved is unclear. While pubertal stage may be a better index of maturational brain state than age, measurement of pubertal stage may be unreliable (Blakemore, Burnett, & Dahl, 2010; Desmangles, Lappe, Lipaczewski, & Haynatzki, 2006), and by age 16 years many boys and girls have reached pubertal maturation. Indeed, when planning my acute studies I considered including measures to index pubertal stage, such as self-reported Tanner staging (Marshall & Tanner, 1970) and salivary testosterone measurements, however the reliability of both has been questioned, and little variation on either measure would be apparent by age 16 (Desmangles et al., 2006; Granger, Shirtcliff, Booth, Kivlighan, & Schwartz, 2004; Marshall & Tanner, 1970). Moreover, the relationship between pubertal stage and brain and cognitive development is not well understood, so interpretation of this data would have been limited. Indeed, while it is thought that the hormonal changes that occur at the onset of puberty – including rising levels of oestrogen and testosterone – are likely to influence the onset of adolescent brain development processes, including synaptic pruning (Goddings et al., 2014), brain maturational processes continue for many years after the end of pubertal maturation, demonstrating some degree of independence between adolescent brain and pubertal development (Blakemore et al., 2010). Our knowledge of the ontogeny of adolescent brain development remains in its infancy, but as knowledge improves we will be better able to index region-specific developmental stage of the brain. If we were able to reliably index stages of adolescent hippocampal maturation, for instance, we could potentially identify specific sensitive periods at which the hippocampus may be more vulnerable to THC exposure. Ideally, future acute administration studies would also include younger participants. This would allow us to address the hypotheses regarding sensitive periods described in the previous section. However, in the UK under-16's are not able to provide informed consent, and it is questionable whether

parents or guardians would be willing to allow their children to participate in such research. Moreover, the ethical considerations of such a study would be complex and it is dubious whether it would be appropriate to administer cannabinoids to healthy children even if they were recreational users.

Naturalistic and self-administration studies with adolescent and adult cannabis users are also needed, to assess whether the pattern of age-related cannabis effects I found translates to more ecologically valid cannabis use contexts. Importantly, such studies could also identify whether adolescents do indeed tend to use greater quantities of cannabis per session, as I have predicted from my results. I am aware of no such data to either support or refute this hypothesis in relation to cannabis, but, as described earlier, there is some epidemiological evidence to suggest this pattern may be seen in adolescent alcohol use (Kuntsche et al., 2004; Naimi et al., 2003; Research & Evaluation, 2005). Tracking participants longitudinally following participation in these studies would allow us to investigate whether age-related sensitivities do indeed impact in the long-term on cannabis use patterns, cognition and mental health outcomes.

Finally, something that became very apparent in the course of reviewing the acute cannabis literature in chapter 1 and 2, is a considerable lack of methodological consistency across studies, in terms of doses, cannabinoids, route of administration, participant characteristics, and the specific tasks administered to index similar domains. Such discrepancies restrict meaningful comparisons of study findings. Similarly to the above point, one goal could be standardisation of cannabis user classification, for instance a strict definition of what constitutes a frequent versus infrequent user. Additionally, given the robustness of the verbal immediate recall deficit caused by cannabis, consistent assessment of this domain using a standard methodology across different studies and research groups would be quick to administer, and could provide an indicator of the comparability of studies with wisely varied methodologies.

## 7.4 Concluding remarks

In summary then, does adolescent cannabis use have greater potential for harm than adult use? In terms of non-acute effects, I found no difference in IQ and educational attainment for adolescent cannabis users and non-users after adjusting for potential confounding variables. Considering these findings alongside previous research, I would therefore argue that there is lacking evidence to date that adolescent cannabis use causally impairs intellectual and educational performance. In terms of the acute effects of cannabis, my findings suggest that adolescents do not experience greater memory impairment or psychotic-like symptoms acutely, though as discussed above the pattern of age group differences may have the effect of encouraging increased cannabis use in adolescent populations. Nevertheless, before strong conclusions can be drawn these novel findings must be replicated, and longitudinal studies must assess whether differential acute responses to cannabis do indeed affect future cannabis use patterns and other outcomes.

### 7.4.1 Making a hash of it

I often find myself wondering what I would do differently if I was to rerun my acute studies now. While as described above there are many directions in which future work could go, I have settled on three key improvements that I wish I had done differently in the first place. Firstly, given that for a number of my outcomes I found 'trend-level' effects, I would increase my sample size. It was perhaps unrealistic to expect a medium sized effect across the board for age group differences. Secondly, I would ensure that my groups were better matched on recent cannabis use, particularly on frequency of use, to address the frustrating possibility that the adolescents may have had greater tolerance to some of the effects of cannabis than the adults. Finally, I would have checked that my data files were saving correctly for the probabilistic reward task earlier, so that I didn't lose 35% of participants' data.

### 7.4.2 Gone to pot?

In the short four years that I have been working in the field of cannabis research, so much has changed in the world of cannabis. In December 2013, Uruguay became the first country to fully

legalise the production, sale and recreational use of cannabis. As of November 2016, there are now eight states in the USA that have voted to legalise possession and consumption of cannabis for recreational purposes. In the UK, public health bodies (Royal Society for Public Health and Faculty of Public Health, June 2016), national newspapers (The Times, June 2016) and major political parties (Liberal Democrats, March 2016 and Green Party, from at least 2006) have now supported decriminalisation of cannabis. On the day I write this, an editorial in the British Medical Journal has supported drugs policy reform focused on a move away from the criminalisation of drug users. Around the world there are increasing calls to legalise the production, sale and use of cannabis for medicinal purposes, and in September 2016, a cross-party group of MPs and peers recommended that medicinal cannabis use should be legalised in the UK. Of particular relevance to this thesis, there is ongoing debate about the likely impact of relaxed cannabis laws across the globe on adolescent cannabis consumption. While age restrictions have been imposed in the USA, as with alcohol and cigarettes, there is evidence that adolescent use is rising. For better or worse, this changing "cannabis climate" has made it an exciting time to work in this field.

As I finalise my thesis, Donald Trump has been elected president of the USA, while on the same day four more states voted for legalisation of recreational cannabis. The following day, myself and colleagues were awarded an MRC grant to take the research described in this thesis forward over the next four years. How the cannabis climate will change over the next four years – four years of Trump and four years of our grant – has yet to be seen.

**References**


Abel, E. L. (1971). Marihuana and memory: acquisition or retrieval? *Science, 173*(4001), 1038-1040.

Abrams, D. I., Vizoso, H. P., Shade, S. B., Jay, C., Kelly, M. E., & Benowitz, N. L. (2007). Vaporization as a smokeless cannabis delivery system: a pilot study. *Clinical Pharmacology & Therapeutics, 82*(5), 572-578.

Acheson, S. K., Moore, N. L., Kuhn, C. M., Wilson, W. A., & Swartzwelder, H. S. (2011). The synthetic cannabinoid WIN 55212-2 differentially modulates thigmotaxis but not spatial learning in adolescent and adult animals. *Neuroscience letters, 487*(3), 411-414.

Agrawal, A., Lynskey, M., Bucholz, K., Martin, N., Madden, P., & Heath, A. (2007). Contrasting models of genetic co-morbidity for cannabis and other illicit drugs in adult Australian twins. *Psychological Medicine, 37*(01), 49-60.

Anderson, B. M., Rizzo, M., Block, R. I., Pearlson, G. D., & O'Leary, D. S. (2010). Sex, drugs, and cognition: effects of marijuana. *Journal of psychoactive drugs, 42*(4), 413-424.

Andreasen, N. C. (1989). Scale for the Assessment of Negative Symptoms (SANS). *The British Journal of Psychiatry*.

Andréasson, S., Engström, A., Allebeck, P., & Rydberg, U. (1987). Cannabis and schizophrenia A longitudinal study of swedish conscripts. *The Lancet, 330*(8574), 1483-1486.

Angold, A., Costello, E. J., Messer, S. C., & Pickles, A. (1995). Development of a short questionnaire for use in epidemiological studies of depression in children and adolescents. *International journal of methods in psychiatric research*.

APA. (2013). *Diagnostic and statistical manual of mental disorders, (DSM-5®)*: American Psychiatric Pub.

Arseneault, L., Cannon, M., Poulton, R., Murray, R. M., Caspi, A., & Moffitt, T. E. (2002). Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. *BMJ, 325*(7374), 1212-1213.

Atakan, Z., Bhattacharyya, S., Allen, P., Martín-Santos, R., Crippa, J., Borgwardt, S., . . . Stahl, D. (2013). Cannabis affects people differently: inter-subject variation in the psychotogenic effects of Δ 9-tetrahydrocannabinol: a functional magnetic resonance imaging study with healthy volunteers. *Psychological medicine, 43*(06), 1255-1267.

Axelrod, B. N. (2002). Validity of the Wechsler abbreviated scale of intelligence and other very short forms of estimating intellectual functioning. *Assessment, 9*(1), 17-23.

Babor, T. F., Higgins-Biddle, J. C., Saunders, J. B., & Monteiro, M. G. (2001). Audit. *The Alcohol Use Disorders Identification Test (AUDIT): Guidelines for use in primary care*.

Baddeley, A. (1992). Working memory. *Science, 255*(5044), 556.

Barkus, E. J., & Lewis, S. (2008). Schizotypy and psychosis-like experiences from recreational cannabis in a non-clinical sample. *Psychological Medicine, 38*(09), 1267-1276.

Barkus, E. J., Stirling, J., Hopkins, R. S., & Lewis, S. (2006). Cannabis-induced psychosis-like experiences are associated with high schizotypy. *Psychopathology, 39*(4), 175-178.

Barnard, M., & McKeganey, N. (2004). The impact of parental problem drug use on children: what is the problem and what can be done to help? *Addiction, 99*(5), 552-559.

Batalla, A., Bhattacharyya, S., Yücel, M., Fusar-Poli, P., Crippa, J. A., Nogué, S., . . . Martin-Santos, R. (2013). Structural and functional imaging studies in chronic cannabis users: a systematic review of adolescent and adult findings. *PloS One, 8*(2), e55821.

Bava, S., Jacobus, J., Thayer, R. E., & Tapert, S. F. (2013). Longitudinal changes in white matter integrity among adolescent substance users. *Alcoholism: Clinical and Experimental Research, 37*(s1), E181-E189.

Bayer, T. A., Falkai, P., & Maier, W. (1999). Genetic and non-genetic vulnerability factors in schizophrenia: the basis of the "two hit hypothesis". *Journal of psychiatric research, 33*(6), 543-548.

Beavan, V., Read, J., & Cartwright, C. (2011). The prevalence of voice-hearers in the general population: a literature review. *Journal of Mental Health, 20*(3), 281-292.

Beck, A. T., Epstein, N., Brown, G., & Steer, R. A. (1988). An inventory for measuring clinical anxiety: psychometric properties. *Journal of consulting and clinical psychology, 56*(6), 893.

Beck, A. T., Steer, R. A., & Brown, G. K. (1996). Beck depression inventory-II. *San Antonio, TX: Psychological Corporation*, b9.

Behan, B., Connolly, C., Datwani, S., Doucet, M., Ivanovic, J., Morioka, R., . . . Garavan, H. (2014). Response inhibition and elevated parietal-cerebellar correlations in chronic adolescent cannabis users. *Neuropharmacology, 84*, 131-137.

Belmore, S. M., & Miller, L. L. (1980). Levels of processing and acute effects of marijuana on memory. *Pharmacology Biochemistry and Behavior, 13*(2), 199-203.

Bergamaschi, M. M., Queiroz, R. H. C., Chagas, M. H. N., de Oliveira, D. C. G., De Martinis, B. S., Kapczinski, F., . . . Nardi, A. E. (2011). Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naive social phobia patients. *Neuropsychopharmacology, 36*(6), 1219-1226.

Bhattacharyya, S., Atakan, Z., Martin-Santos, R., Crippa, J., Kambeitz, J., Malhi, S., . . . Rubia, K. (2015). Impairment of inhibitory control processing related to acute psychotomimetic effects of cannabis. *European Neuropsychopharmacology, 25*(1), 26-37.

Black, A., Coward, W., Cole, T., & Prentice, A. (1996). Human energy expenditure in affluent societies: an analysis of 574 doubly-labelled water measurements. *European journal of clinical nutrition, 50*(2), 72.

Blakemore, S.-J., Burnett, S., & Dahl, R. E. (2010). The role of puberty in the developing adolescent brain. *Human brain mapping, 31*(6), 926-933.

Blakemore, S.-J., & Robbins, T. W. (2012). Decision-making in the adolescent brain. *Nature neuroscience, 15*(9), 1184-1191.

Bloomfield, M., Mouchlianitis, E., Morgan, C., Freeman, T., Roiser, J., & Howes, O. (2016). Salience attribution and its relationship to cannabis-induced psychotic symptoms.

Bloomfield, M. A., Morgan, C. J., Kapur, S., Curran, H. V., & Howes, O. D. (2014). The link between dopamine function and apathy in cannabis users: an [18F]-DOPA PET imaging study. *Psychopharmacology, 231*(11), 2251-2259.

Bogdan, R., & Pizzagalli, D. A. (2006). Acute stress reduces reward responsiveness: implications for depression. *Biological psychiatry, 60*(10), 1147-1154.

Borgwardt, S. J., Allen, P., Bhattacharyya, S., Fusar-Poli, P., Crippa, J. A., Seal, M. L., . . . O'Carroll, C. (2008). Neural basis of Δ-9-tetrahydrocannabinol and cannabidiol: effects during response inhibition. *Biological psychiatry, 64*(11), 966-973.

Bossong, M. G., Jansma, J. M., Van Hell, H. H., Jager, G., Oudman, E., Saliasi, E., . . . Ramsey, N. F. (2012). Effects of δ9-tetrahydrocannabinol on human working memory function. *Biological psychiatry, 71*(8), 693-699.

Bossong, M. G., & Niesink, R. J. (2010). Adolescent brain maturation, the endogenous cannabinoid system and the neurobiology of cannabis-induced schizophrenia. *Progress in neurobiology, 92*(3), 370-385.

Bossong, M. G., van Berckel, B. N., Boellaard, R., Zuurman, L., Schuit, R. C., Windhorst, A. D., . . . Kahn, R. S. (2009). Δ9-tetrahydrocannabinol induces dopamine release in the human striatum. *Neuropsychopharmacology, 34*(3), 759-766.

Bovasso, G. B. (2001). Cannabis abuse as a risk factor for depressive symptoms. *American journal of Psychiatry*.

Boyd, A., Golding, J., Macleod, J., Lawlor, D. A., Fraser, A., Henderson, J., . . . Smith, G. D. (2012). Cohort profile: the 'children of the 90s'—the index offspring of the Avon Longitudinal Study of Parents and Children. *International journal of epidemiology*, dys064.

Braver, T. S., Cohen, J. D., Nystrom, L. E., Jonides, J., Smith, E. E., & Noll, D. C. (1997). A parametric study of prefrontal cortex involvement in human working memory. *Neuroimage, 5*(1), 49-62.

Bremner, J. D., Krystal, J. H., Putnam, F. W., Southwick, S. M., Marmar, C., Charney, D. S., & Mazure, C. M. (1998). Measurement of dissociative states with the clinician-

administered dissociative states scale (CADSS). *Journal of traumatic stress, 11*(1), 125-136.
Broyd, S. J., van Hell, H. H., Beale, C., Yücel, M., & Solowij, N. (2015). Acute and chronic effects of cannabinoids on human cognition–A systematic review. *Biological psychiatry*.
Buckner, J. D., & Carroll, K. M. (2010). Effect of anxiety on treatment presentation and outcome: Results from the Marijuana Treatment Project. *Psychiatry Research, 178*(3), 493-500.
Buckner, J. D., Schmidt, N. B., Lang, A. R., Small, J. W., Schlauch, R. C., & Lewinsohn, P. M. (2008). Specificity of social anxiety disorder as a risk factor for alcohol and cannabis dependence. *Journal of psychiatric research, 42*(3), 230-239.
Carlin, A. S., Bakker, C. B., Halpern, L., & Post, R. D. (1972). Social facilitation of marijuana intoxication: Impact of social set and pharmacological activity. *Journal of Abnormal Psychology, 80*(2), 132.
Carvalho, A., Reyes, B., Ramalhosa, F., Sousa, N., & Van Bockstaele, E. (2016). Repeated administration of a synthetic cannabinoid receptor agonist differentially affects cortical and accumbal neuronal morphology in adolescent and adult rats. *Brain Structure and Function, 221*(1), 407-419.
Casey, B. J., Jones, R. M., & Hare, T. A. (2008). The adolescent brain. *Annals of the New York Academy of Sciences, 1124*(1), 111-126.
Caspi, A., Moffitt, T. E., Cannon, M., McClay, J., Murray, R. M., Harrington, H., . . . Braithwaite, A. (2005). Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. *Biological psychiatry, 57*(10), 1117-1127.
Cass, D. K., Flores-Barrera, E., Thomases, D. R., Vital, W. F., Caballero, A., & Tseng, K. Y. (2014). CB1 cannabinoid receptor stimulation during adolescence impairs the maturation of GABA function in the adult rat prefrontal cortex. *Molecular psychiatry, 19*(5), 536.
Catalan, A., Simons, C. J., Bustamante, S., Drukker, M., Madrazo, A., de Artaza, M. G., . . . Gonzalez-Torres, M. A. (2014). Novel evidence that attributing affectively salient signal to random noise is associated with psychosis. *PLoS One, 9*(7), e102520.
Cha, Y. M., Jones, K. H., Kuhn, C. M., Wilson, W. A., & Swartzwelder, H. S. (2007). Sex differences in the effects of Δ9-tetrahydrocannabinol on spatial learning in adolescent and adult rats. *Behavioural pharmacology, 18*(5-6), 563-569.
Cha, Y. M., White, A. M., Kuhn, C. M., Wilson, W. A., & Swartzwelder, H. (2006). Differential effects of delta 9-THC on learning in adolescent and adult rats. *Pharmacology Biochemistry and Behavior, 83*(3), 448-455.
Chait, L., & Zacny, J. P. (1992). Reinforcing and subjective effects of oral Δ9-THC and smoked marijuana in humans. *Psychopharmacology, 107*(2-3), 255-262.
Chamberlain, S. R., Odlaug, B. L., Schreiber, L., & Grant, J. E. (2012). Association between tobacco smoking and cognitive functioning in young adults. *The American Journal on Addictions, 21*(s1), S14-S19.
Chapman, L. J., Chapman, J. P., & Raulin, M. L. (1976). Scales for physical and social anhedonia. *Journal of Abnormal Psychology, 85*(4), 374.
Cheetham, A., Allen, N. B., Whittle, S., Simmons, J. G., Yücel, M., & Lubman, D. I. (2012). Orbitofrontal volumes in early adolescence predict initiation of cannabis use: a 4-year longitudinal and prospective study. *Biological psychiatry, 71*(8), 684-692.
Chen, C. Y., & Anthony, J. C. (2003). Possible age-associated bias in reporting of clinical features of drug dependence: epidemiological evidence on adolescent-onset marijuana use. *Addiction, 98*(1), 71-82.
Chen, C. Y., O'Brien, M. S., & Anthony, J. C. (2005). Who becomes cannabis dependent soon after onset of use? Epidemiological evidence from the United States: 2000–2001. *Drug and alcohol dependence, 79*(1), 11-22.

Christensen, R., Kristensen, P. K., Bartels, E. M., Bliddal, H., & Astrup, A. (2007). Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. *The Lancet, 370*(9600), 1706-1713.

Clark, D. B., Chung, T., Thatcher, D. L., Pajtek, S., & Long, E. C. (2012). Psychological dysregulation, white matter disorganization and substance use disorders in adolescence. *Addiction, 107*(1), 206-214.

Compton, M. T., Furman, A. C., & Kaslow, N. J. (2004). Lower negative symptom scores among cannabis-dependent patients with schizophrenia-spectrum disorders: preliminary evidence from an African American first-episode sample. *Schizophrenia research, 71*(1), 61-64.

Congdon, E., Mumford, J. A., Cohen, J. R., Galvan, A., Canli, T., & Poldrack, R. A. (2012). Measurement and reliability of response inhibition. *Frontiers in psychology, 3*(37), 1-10.

Cooper, Z. D., & Haney, M. (2014). Investigation of sex-dependent effects of cannabis in daily cannabis smokers. *Drug and alcohol dependence, 136*, 85-91.

Corlett, P., Honey, G., & Fletcher, P. C. (2007). From prediction error to psychosis: ketamine as a pharmacological model of delusions. *Journal of psychopharmacology, 21*(3), 238-252.

Crane, N. A., Schuster, R. M., Fusar-Poli, P., & Gonzalez, R. (2013). Effects of cannabis on neurocognitive functioning: recent advances, neurodevelopmental influences, and sex differences. *Neuropsychology review, 23*(2), 117-137.

Crane, N. A., Schuster, R. M., Mermelstein, R. J., & Gonzalez, R. (2015). Neuropsychological sex differences associated with age of initiated use among young adult cannabis users. *Journal of clinical and experimental neuropsychology*(ahead-of-print), 1-13.

Crean, R. D., Crane, N. A., & Mason, B. J. (2011). An evidence based review of acute and long-term effects of cannabis use on executive cognitive functions. *Journal of addiction medicine, 5*(1), 1.

Crown, S., & Crisp, A. H. (1979). *Crown-crisp Experiential Index: Specimen Set*: Hodder and Stoughton.

Curran, H. V., Brignell, C., Fletcher, S., Middleton, P., & Henry, J. (2002). Cognitive and subjective dose-response effects of acute oral Δ9-tetrahydrocannabinol (THC) in infrequent cannabis users. *Psychopharmacology, 164*(1), 61-70.

Curran, H. V., Freeman, T. P., Mokrysz, C., Lewis, D. A., Morgan, C. J., & Parsons, L. H. (2016). Keep off the grass? Cannabis, cognition and addiction. *Nature Reviews Neuroscience, 17*(5), 293-306.

Cyders, M. A., Littlefield, A. K., Coffey, S., & Karyadi, K. A. (2014). Examination of a short English version of the UPPS-P Impulsive Behavior Scale. *Addictive behaviors, 39*(9), 1372-1376.

D'Souza, D. C., Braley, G., Blaise, R., Vendetti, M., Oliver, S., Pittman, B., & Perry, E. (2008). Effects of haloperidol on the behavioral, subjective, cognitive, motor, and neuroendocrine effects of Δ-9-tetrahydrocannabinol in humans. *Psychopharmacology, 198*(4), 587-603.

D'Souza, D. C., Cortes-Briones, J. A., Ranganathan, M., Thurnauer, H., Creatura, G., Surti, T., . . . Normandin, M. D. (2016). Rapid Changes in Cannabinoid 1 Receptor Availability in Cannabis-Dependent Male Subjects After Abstinence From Cannabis. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging, 1*(1), 60-67.

D'Souza, D. C., Perry, E., MacDougall, L., Ammerman, Y., Cooper, T., Wu, Y.-t., . . . Krystal, J. H. (2004). The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology, 29*(8), 1558-1572.

D'Souza, D. C., Ranganathan, M., Braley, G., Gueorguieva, R., Zimolo, Z., Cooper, T., . . . Krystal, J. (2008). Blunted psychotomimetic and amnestic effects of Δ-9-tetrahydrocannabinol in frequent users of cannabis. *Neuropsychopharmacology, 33*(10), 2505-2516.

D'Souza, D. C., Abi-Saab, W. M., Madonick, S., Forselius-Bielen, K., Doersch, A., Braley, G., . . . Krystal, J. H. (2005). Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. *Biological Psychiatry, 57*(6), 594-608.

Daalman, K., Boks, M. P., Diederen, K. M., de Weijer, A. D., Blom, J. D., Kahn, R. S., & Sommer, I. E. (2011). The same or different? A phenomenological comparison of auditory verbal hallucinations in healthy and psychotic individuals. *The Journal of clinical psychiatry, 72*(3), 320-325.

Daalman, K., Verkooijen, S., Derks, E. M., Aleman, A., & Sommer, I. E. (2012). The influence of semantic top-down processing in auditory verbal hallucinations. *Schizophrenia research, 139*(1), 82-86.

Darley, C., Tinklenberg, J., Roth, W., & Atkinson, R. (1974). The nature of storage deficits and state-dependent retrieval under marihuana. *Psychopharmacologia, 37*(2), 139-149.

Darley, C., Tinklenberg, J., Roth, W., Hollister, L., & Atkinson, R. (1973). Influence of marihuana on storage and retrieval processe in memory. *Memory & cognition, 1*(2), 196-200.

Davidow, J. Y., Foerde, K., Galván, A., & Shohamy, D. (2016). An Upside to Reward Sensitivity: The Hippocampus Supports Enhanced Reinforcement Learning in Adolescence. *Neuron, 92*(1), 93-99.

Davison, K., & Wilson, C. H. (1972). Psychosis associated with cannabis smoking. *British Journal of Addiction to Alcohol & Other Drugs, 67*(3), 225-228.

Dawes, G. M., Sitharthan, T., Conigrave, K. M., Phung, N., & Weltman, M. (2011). Patients admitted for inpatient cannabis detoxification: withdrawal symptoms and impacts of common comorbidities. *Journal of substance use, 16*(5), 392-405.

De Wit, H. (2009). Impulsivity as a determinant and consequence of drug use: a review of underlying processes. *Addiction biology, 14*(1), 22-31.

Degenhardt, L., Chiu, W.-T., Sampson, N., Kessler, R. C., Anthony, J. C., Angermeyer, M., . . . Huang, Y. (2008). Toward a global view of alcohol, tobacco, cannabis, and cocaine use: findings from the WHO World Mental Health Surveys. *PLoS Med, 5*(7), e141.

Degenhardt, L., & Hall, W. (2012). Extent of illicit drug use and dependence, and their contribution to the global burden of disease. *The Lancet, 379*(9810), 55-70.

Degenhardt, L., Hall, W., & Lynskey, M. (2003a). Exploring the association between cannabis use and depression. *Addiction, 98*(11), 1493-1504.

Degenhardt, L., Hall, W., & Lynskey, M. (2003b). Testing hypotheses about the relationship between cannabis use and psychosis. *Drug and alcohol dependence, 71*(1), 37-48.

Degenhardt, L., Stockings, E., Patton, G., Hall, W. D., & Lynskey, M. (2016). The increasing global health priority of substance use in young people. *The Lancet Psychiatry, 3*(3), 251-264.

Desmangles, J.-C., Lappe, J. M., Lipaczewski, G., & Haynatzki, G. (2006). Accuracy of pubertal Tanner staging self-reporting. *Journal of Pediatric Endocrinology and Metabolism, 19*(3), 213-222.

DeWit, D. J., Adlaf, E. M., Offord, D. R., & Ogborne, A. C. (2000). Age at first alcohol use: a risk factor for the development of alcohol disorders. *American journal of Psychiatry, 157*(5), 745-750.

Di Forti, M., Iyegbe, C., Sallis, H., Kolliakou, A., Falcone, M. A., Paparelli, A., . . . Murray, R. M. (2012). Confirmation that the AKT1 (rs2494732) genotype influences the risk of psychosis in cannabis users. *Biol Psychiatry, 72*(10), 811-816. doi:10.1016/j.biopsych.2012.06.020

Di Forti, M., Marconi, A., Carra, E., Fraietta, S., Trotta, A., Bonomo, M., . . . Russo, M. (2015). Proportion of patients in south London with first-episode psychosis attributable to use of high potency cannabis: a case-control study. *The Lancet Psychiatry, 2*(3), 233-238.

Di Forti, M., Morgan, C., Dazzan, P., Pariante, C., Mondelli, V., Marques, T. R., . . . Paparelli, A. (2009). High-potency cannabis and the risk of psychosis. *The British Journal of Psychiatry, 195*(6), 488-491.

Di Forti, M., Sallis, H., Allegri, F., Trotta, A., Ferraro, L., Stilo, S. A., . . . Murray, R. M. (2014). Daily use, especially of high-potency cannabis, drives the earlier onset of psychosis in cannabis users. *Schizophr Bull, 40*(6), 1509-1517. doi:10.1093/schbul/sbt181

Di Marzo, V., Berrendero, F., Bisogno, T., Gonzalez, S., Cavaliere, P., Romero, J., . . . Fernández-Ruiz, J. (2000). Enhancement of anandamide formation in the limbic forebrain and reduction of endocannabinoid contents in the striatum of Δ9-tetrahydrocannabinol-tolerant rats. *Journal of neurochemistry, 74*(4), 1627-1635.

Dolgov, I., & McBeath, M. K. (2005). A signal-detection-theory representation of normal and hallucinatory perception. *Behavioral and Brain Sciences, 28*(06), 761-762.

Dorard, G., Berthoz, S., Phan, O., Corcos, M., & Bungener, C. (2008). Affect dysregulation in cannabis abusers. *European child & adolescent psychiatry, 17*(5), 274-282.

Dornbush, R. L. (1974). Marijuana and memory: Effects of smoking on storage. *Transactions of the New York Academy of Sciences, 36*(1 Series II), 94-100.

Dougherty, D. M., Mathias, C. W., Dawes, M. A., Furr, R. M., Charles, N. E., Liguori, A., . . . Acheson, A. (2013). Impulsivity, attention, memory, and decision-making among adolescent marijuana users. *Psychopharmacology, 226*(2), 307-319.

Eagle, D. M., Bari, A., & Robbins, T. W. (2008). The neuropsychopharmacology of action inhibition: cross-species translation of the stop-signal and go/no-go tasks. *Psychopharmacology, 199*(3), 439-456.

Eagle, D. M., & Baunez, C. (2010). Is there an inhibitory-response-control system in the rat? Evidence from anatomical and pharmacological studies of behavioral inhibition. *Neuroscience & Biobehavioral Reviews, 34*(1), 50-72.

Ellgren, M., Artmann, A., Tkalych, O., Gupta, A., Hansen, H. S., Hansen, S., . . . Hurd, Y. (2008). Dynamic changes of the endogenous cannabinoid and opioid mesocorticolimbic systems during adolescence: THC effects. *European Neuropsychopharmacology, 18*(11), 826-834.

Englund, A., Morrison, P. D., Nottage, J., Hague, D., Kane, F., Bonaccorso, S., . . . Holt, D. (2013). Cannabidiol inhibits THC-elicited paranoid symptoms and hippocampal-dependent memory impairment. *Journal of Psychopharmacology, 27*(1), 19-27.

Epstein, K. A., & Kumra, S. (2015). White matter fractional anisotropy over two time points in early onset schizophrenia and adolescent cannabis use disorder: A naturalistic diffusion tensor imaging study. *Psychiatry Research: Neuroimaging, 232*(1), 34-41.

*European Drug Report 2016: Trends and Developments.* (2016). Retrieved from

Fergusson, D. M., Boden, J. M., & Horwood, L. J. (2006). Cannabis use and other illicit drug use: testing the cannabis gateway hypothesis. *Addiction, 101*(4), 556-569.

Fergusson, D. M., & Horwood, L. (1997). Early onset cannabis use and psychosocial adjustment in young adults. *Addiction, 92*(3), 279-296.

Fergusson, D. M., Horwood, L., & Lawton, J. (1990). Vulnerability to childhood problems and family social background. *Journal of Child Psychology and Psychiatry, 31*(7), 1145-1160.

Fergusson, D. M., Horwood, L., & Swain-Campbell, N. (2003). Cannabis dependence and psychotic symptoms in young people. *Psychological Medicine, 33*(01), 15-21.

Fergusson, D. M., & Horwood, L. J. (2000). Does cannabis use encourage other forms of illicit drug use? *Addiction, 95*(4), 505-520.

Fergusson, D. M., Horwood, L. J., & Beautrais, A. L. (2003). Cannabis and educational achievement. *Addiction, 98*(12), 1681-1692.

Flórez-Salamanca, L., Secades-Villa, R., Budney, A. J., García-Rodríguez, O., Wang, S., & Blanco, C. (2013). Probability and predictors of cannabis use disorders relapse: results of the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC). *Drug and alcohol dependence, 132*(1), 127-133.

Fontes, M. A., Bolla, K. I., Cunha, P. J., Almeida, P. P., Jungerman, F., Laranjeira, R. R., . . . Lacerda, A. L. (2011). Cannabis use before age 15 and subsequent executive functioning. *The British Journal of Psychiatry, 198*(6), 442-447.

Forbes, G. B., & Reina, J. C. (1970). Adult lean body mass declines with age: some longitudinal observations. *Metabolism, 19*(9), 653-663.
Foussias, G., & Remington, G. (2010). Negative symptoms in schizophrenia: avolition and Occam's razor. *Schizophr Bull, 36*(2), 359-369.
Fox, K. M., Sterling, R. C., & Van Bockstaele, E. J. (2009). Cannabinoids and novelty investigation: Influence of age and duration of exposure. *Behavioural brain research, 196*(2), 248-253.
Freeman, D., Dunn, G., Murray, R. M., Evans, N., Lister, R., Antley, A., . . . Williams, J. (2014). How cannabis causes paranoia: using the intravenous administration ofΔ 9-tetrahydrocannabinol (THC) to identify key cognitive mechanisms leading to paranoia. *Schizophr Bull*, sbu098.
Freeman, T., & Curran, H. (in prep.). Psychotomietic States Inventory: a meta-analysis of the effects of cannabis, ketamine and sensory deprivation.
Freeman, T., Pope, R., & Curran, H. V. (in prep.). Cannabis increases wanting but not liking of music and dampens its rewarding effects on the brain.
Freeman, T. P., Morgan, C. J., Hindocha, C., Schafer, G., Das, R. K., & Curran, H. V. (2014). Just say 'know': how do cannabinoid concentrations influence users' estimates of cannabis potency and the amount they roll in joints? *Addiction*.
Freeman, T. P., Morgan, C. J., Vaughn-Jones, J., Hussain, N., Karimi, K., & Curran, H. V. (2012). Cognitive and subjective effects of mephedrone and factors influencing use of a 'new legal high'. *Addiction, 107*(4), 792-800.
French, E. D., Dillon, K., & Wu, X. (1997). Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. *Neuroreport, 8*(3), 649-652.
Fried, P., Watkinson, B., & Gray, R. (2005). Neurocognitive consequences of marihuana—a comparison with pre-drug performance. *Neurotoxicology and teratology, 27*(2), 231-239.
Fuhrmann, D., Knoll, L. J., & Blakemore, S.-J. (2015). Adolescence as a sensitive period of brain development. *Trends in cognitive sciences, 19*(10), 558-566.
Gage, S. H., Hickman, M., Heron, J., Munafò, M., Lewis, G., Macleod, J., & Zammit, S. (2014). Associations of cannabis and cigarette use with psychotic experiences at age 18: findings from the Avon Longitudinal Study of Parents and Children. *Psychological Medicine, 44*(16), 3435-3444.
Gage, S. H., Hickman, M., Heron, J., Munafò, M. R., Lewis, G., Macleod, J., & Zammit, S. (2015). Associations of cannabis and cigarette use with depression and anxiety at age 18: findings from the Avon Longitudinal Study of Parents and Children. *PLoS One, 10*(4), e0122896.
Gage, S. H., Hickman, M., & Zammit, S. (2016). Association between cannabis and psychosis: epidemiologic evidence. *Biological psychiatry, 79*(7), 549-556.
Galdos, M., Simons, C., Fernandez-Rivas, A., Wichers, M., Peralta, C., Lataster, T., . . . Gonzalez-Torres, M. A. (2011). Affectively salient meaning in random noise: a task sensitive to psychosis liability. *Schizophr Bull, 37*(6), 1179-1186.
Gard, D. E., Gard, M. G., Kring, A. M., & John, O. P. (2006). Anticipatory and consummatory components of the experience of pleasure: a scale development study. *Journal of Research in Personality, 40*(6), 1086-1102.
Giedd, J. N., Blumenthal, J., Jeffries, N. O., Castellanos, F. X., Liu, H., Zijdenbos, A., . . . Rapoport, J. L. (1999). Brain development during childhood and adolescence: a longitudinal MRI study. *Nature neuroscience, 2*(10), 861-863.
Ginovart, N., Tournier, B. B., Moulin-Sallanon, M., Steimer, T., Ibanez, V., & Millet, P. (2012). Chronic Δ9-tetrahydrocannabinol exposure induces a sensitization of dopamine D2/3 receptors in the mesoaccumbens and nigrostriatal systems. *Neuropsychopharmacology, 37*(11), 2355-2367.
Goddings, A.-L., Mills, K. L., Clasen, L. S., Giedd, J. N., Viner, R. M., & Blakemore, S.-J. (2014). The influence of puberty on subcortical brain development. *Neuroimage, 88*, 242-251.

Gold, J. M., Waltz, J. A., Prentice, K. J., Morris, S. E., & Heerey, E. A. (2008). Reward processing in schizophrenia: a deficit in the representation of value. *Schizophr Bull, 34*(5), 835-847.

Gonzalez, R. (2007). Acute and non-acute effects of cannabis on brain functioning and neuropsychological performance. *Neuropsychology review, 17*(3), 347-361.

Gonzalez, R., Schuster, R. M., Mermelstein, R. J., Vassileva, J., Martin, E. M., & Diviak, K. R. (2012). Performance of young adult cannabis users on neurocognitive measures of impulsive behavior and their relationship to symptoms of cannabis use disorders. *Journal of clinical and experimental neuropsychology, 34*(9), 962-976.

González, S., Fernández-Ruiz, J., Di Marzo, V., Hernández, M., Arévalo, C., Nicanor, C., . . . Ramos, J. A. (2004). Behavioral and molecular changes elicited by acute administration of SR141716 to Δ 9-tetrahydrocannabinol-tolerant rats: an experimental model of cannabinoid abstinence. *Drug and alcohol dependence, 74*(2), 159-170.

Goodman, R. (2001). Psychometric properties of the strengths and difficulties questionnaire. *Journal of the American Academy of Child & Adolescent Psychiatry, 40*(11), 1337-1345.

Granger, D. A., Shirtcliff, E. A., Booth, A., Kivlighan, K. T., & Schwartz, E. B. (2004). The "trouble" with salivary testosterone. *Psychoneuroendocrinology, 29*(10), 1229-1240.

Grant, B. F., & Dawson, D. A. (1997). Age at onset of alcohol use and its association with DSM-IV alcohol abuse and dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. *Journal of substance abuse, 9*, 103-110.

Grant, I., Gonzalez, R., Carey, C. L., Natarajan, L., & Wolfson, T. (2003). Non-acute (residual) neurocognitive effects of cannabis use: a meta-analytic study. *Journal of the International Neuropsychological Society, 9*(05), 679-689.

Grant, J. D., Scherrer, J. F., Lynskey, M. T., Agrawal, A., Duncan, A. E., Haber, J. R., . . . Bucholz, K. K. (2012). Associations of Alcohol, Nicotine, Cannabis, and Drug Use/Dependence with Educational Attainment: Evidence from Cotwin-Control Analyses. *Alcoholism: Clinical and Experimental Research, 36*(8), 1412-1420.

Green, B., Kavanagh, D., & Young, R. (2003). Being stoned: a review of self-reported cannabis effects. *Drug and alcohol review, 22*(4), 453-460.

Gruber, S. A., Dahlgren, M. K., Sagar, K. A., Gönenç, A., & Lukas, S. E. (2014). Worth the wait: effects of age of onset of marijuana use on white matter and impulsivity. *Psychopharmacology, 231*(8), 1455-1465.

Gruber, S. A., Sagar, K. A., Dahlgren, M. K., Racine, M., & Lukas, S. E. (2012). Age of onset of marijuana use and executive function. *Psychology of Addictive Behaviors, 26*(3), 496.

Gruber, S. A., & Yurgelun-Todd, D. A. (2005). Neuroimaging of marijuana smokers during inhibitory processing: a pilot investigation. *Cognitive Brain Research, 23*(1), 107-118.

Grucza, R. A., Agrawal, A., Krauss, M. J., Bongu, J., Plunk, A. D., Cavazos-Rehg, P. A., & Bierut, L. J. (2016). Declining Prevalence of Marijuana Use Disorders Among Adolescents in the United States, 2002 to 2013. *Journal of the American Academy of Child & Adolescent Psychiatry*.

Guo, S. S., Chumlea, W. C., Roche, A. F., & Siervogel, R. M. (1998). Age-and maturity-related changes in body composition during adolescence into adulthood: the Fels Longitudinal Study. *Applied radiation and isotopes, 49*(5), 581-585.

Gurillo, P., Jauhar, S., Murray, R. M., & MacCabe, J. H. (2015). Does tobacco use cause psychosis? Systematic review and meta-analysis. *The Lancet Psychiatry, 2*(8), 718-725.

Hall, W., & Degenhardt, L. (2007). Prevalence and correlates of cannabis use in developed and developing countries. *Current opinion in psychiatry, 20*(4), 393-397.

Hampshire, A., Chamberlain, S. R., Monti, M. M., Duncan, J., & Owen, A. M. (2010). The role of the right inferior frontal gyrus: inhibition and attentional control. *Neuroimage, 50*(3), 1313-1319.

Hanson, K. L., Winward, J. L., Schweinsburg, A. D., Medina, K. L., Brown, S. A., & Tapert, S. F. (2010). Longitudinal study of cognition among adolescent marijuana users over three weeks of abstinence. *Addictive behaviors, 35*(11), 970-976.

Harley, M., Kelleher, I., Clarke, M., Lynch, F., Arseneault, L., Connor, D., . . . Cannon, M. (2010). Cannabis use and childhood trauma interact additively to increase the risk of psychotic symptoms in adolescence. *Psychological Medicine, 40*(10), 1627-1634.

Hart, C. L., Ilan, A. B., Gevins, A., Gunderson, E. W., Role, K., Colley, J., & Foltin, R. W. (2010). Neurophysiological and cognitive effects of smoked marijuana in frequent users. *Pharmacology Biochemistry and Behavior, 96*(3), 333-341.

Hart, C. L., Ward, A. S., Haney, M., Comer, S. D., Foltin, R. W., & Fischman, M. W. (2002). Comparison of smoked marijuana and oral Δ9-tetrahydrocannabinol in humans. *Psychopharmacology, 164*(4), 407-415.

Harvey, M. A., Harvey, M. A., Sellman, J. D., Harvey, M. A., Sellman, J. D., Porter, R. J., . . . Frampton, C. M. (2007). The relationship between non-acute adolescent cannabis use and cognition. *Drug and alcohol review, 26*(3), 309-319.

Hawkins, J. D., Catalano, R. F., & Miller, J. Y. (1992). Risk and protective factors for alcohol and other drug problems in adolescence and early adulthood: implications for substance abuse prevention. *Psychological bulletin, 112*(1), 64.

Hayatbakhsh, M. R., Najman, J. M., Jamrozik, K., Mamun, A. A., Alati, R., & Bor, W. (2007). Cannabis and anxiety and depression in young adults: a large prospective study. *Journal of the American Academy of Child & Adolescent Psychiatry, 46*(3), 408-417.

Hazekamp, A., Ruhaak, R., Zuurman, L., van Gerven, J., & Verpoorte, R. (2006). Evaluation of a vaporizing device (Volcano®) for the pulmonary administration of tetrahydrocannabinol. *Journal of pharmaceutical sciences, 95*(6), 1308-1317.

Heatherton, T. F., Kozlowski, L. T., Frecker, R. C., & Fagerstrom, K. O. (1991). The Fagerström test for nicotine dependence: a revision of the Fagerstrom Tolerance Questionnaire. *British journal of addiction, 86*(9), 1119-1127.

Heerey, E. A., & Gold, J. M. (2007). Patients with schizophrenia demonstrate dissociation between affective experience and motivated behavior. *Journal of Abnormal Psychology, 116*(2), 268.

Heishman, S. J., Kleykamp, B. A., & Singleton, E. G. (2010). Meta-analysis of the acute effects of nicotine and smoking on human performance. *Psychopharmacology, 210*(4), 453-469.

Henquet, C., Di Forti, M., Morrison, P., Kuepper, R., & Murray, R. M. (2008). Gene-environment interplay between cannabis and psychosis. *Schizophr Bull, 34*(6), 1111-1121.

Henquet, C., van Os, J., Kuepper, R., Delespaul, P., Smits, M., à Campo, J., & Myin-Germeys, I. (2010). Psychosis reactivity to cannabis use in daily life: an experience sampling study. *The British Journal of Psychiatry, 196*(6), 447-453.

Hermanns-Clausen, M., Kneisel, S., Szabo, B., & Auwärter, V. (2013). Acute toxicity due to the confirmed consumption of synthetic cannabinoids: clinical and laboratory findings. *Addiction, 108*(3), 534-544.

Heron, J., Barker, E. D., Joinson, C., Lewis, G., Hickman, M., Munafò, M., & Macleod, J. (2013). Childhood conduct disorder trajectories, prior risk factors and cannabis use at age 16: birth cohort study. *Addiction, 108*(12), 2129-2138.

Hester, R., Nestor, L., & Garavan, H. (2009). Impaired error awareness and anterior cingulate cortex hypoactivity in chronic cannabis users. *Neuropsychopharmacology, 34*(11), 2450-2458.

Hibell, B., Guttormsson, U., Ahlström, S., Balakireva, O., Bjarnason, T., Kokkevi, A., & Kraus, L. (2012). The 2011 ESPAD report. *Substance use among students in, 36*.

Hillard, C. J. (2015). Chapter One-The Endocannabinoid Signaling System in the CNS: A Primer. *International review of neurobiology, 125*, 1-47.

Hindocha, C., Freeman, T. P., Ferris, J. A., Lynskey, M. T., & Winstock, A. R. (2016). No Smoke without tobacco: A global overview of cannabis and tobacco routes of administration and their association with intention to quit. *Frontiers in psychiatry, 7*, 104.

Hindocha, C., Freeman, T. P., Schafer, G., Gardener, C., Das, R. K., Morgan, C. J., & Curran, H. V. (2014). Acute effects of delta-9-tetrahydrocannabinol, cannabidiol and their

combination on facial emotion recognition: A randomised, double-blind, placebo-controlled study in cannabis users. *European Neuropsychopharmacology*.
Hindocha, C., Freeman, T. P., Schafer, G., Gardener, C., Das, R. K., Morgan, C. J., & Curran, H. V. (2015). Acute effects of delta-9-tetrahydrocannabinol, cannabidiol and their combination on facial emotion recognition: A randomised, double-blind, placebo-controlled study in cannabis users. *European Neuropsychopharmacology, 25*(3), 325-334. doi:10.1016/j.euroneuro.2014.11.014
Hindocha, C., Shaban, N. D., Freeman, T. P., Das, R. K., Gale, G., Schafer, G., . . . Curran, H. V. (2015). Associations between cigarette smoking and cannabis dependence: a longitudinal study of young cannabis users in the United Kingdom. *Drug and alcohol dependence*.
Hines, L. A., Morley, K. I., Strang, J., Agrawal, A., Nelson, E. C., Statham, D., . . . Lynskey, M. T. (2015). The association between speed of transition from initiation to subsequent use of cannabis and later problematic cannabis use, abuse and dependence. *Addiction, 110*(8), 1311-1320.
Hirvonen, J., Goodwin, R., Li, C.-T., Terry, G., Zoghbi, S., Morse, C., . . . Innis, R. (2012). Reversible and regionally selective downregulation of brain cannabinoid CB1 receptors in chronic daily cannabis smokers. *Molecular psychiatry, 17*(6), 642-649.
Hoffman, R. E., Woods, S. W., Hawkins, K. A., Pittman, B., Tohen, M., Preda, A., . . . Perkins, D. O. (2007). Extracting spurious messages from noise and risk of schizophrenia-spectrum disorders in a prodromal population. *The British Journal of Psychiatry, 191*(4), 355-356.
Holdnack, H. (2001). Wechsler test of adult reading: WTAR. *San Antonio. The Psychological Corporation*.
Holt, D. J., Titone, D., Long, L. S., Goff, D. C., Cather, C., Rauch, S. L., . . . Kuperberg, G. R. (2006). The misattribution of salience in delusional patients with schizophrenia. *Schizophrenia research, 83*(2), 247-256.
Hooper, S. R., Woolley, D., & De Bellis, M. D. (2014). Intellectual, neurocognitive, and academic achievement in abstinent adolescents with cannabis use disorder. *Psychopharmacology, 231*(8), 1467-1477.
Horwood, J., Salvi, G., Thomas, K., Duffy, L., Gunnell, D., Hollis, C., . . . Wolke, D. (2008). IQ and non-clinical psychotic symptoms in 12-year-olds: results from the ALSPAC birth cohort. *The British Journal of Psychiatry, 193*(3), 185-191.
Houston, J. E., Murphy, J., Adamson, G., Stringer, M., & Shevlin, M. (2008). Childhood sexual abuse, early cannabis use, and psychosis: testing an interaction model based on the National Comorbidity Survey. *Schizophr Bull, 34*(3), 580-585.
Huddy, V., Clark, L., Harrison, I., Ron, M., Moutoussis, M., Barnes, T., & Joyce, E. (2013). Reflection impulsivity and response inhibition in first-episode psychosis: relationship to cannabis use. *Psychological Medicine, 43*(10), 2097-2107.
Hunault, C. C., Mensinga, T. T., Böcker, K. B., Schipper, C. M. A., Kruidenier, M., Leenders, M. E., . . . Meulenbelt, J. (2009). Cognitive and psychomotor effects in males after smoking a combination of tobacco and cannabis containing up to 69 mg delta-9-tetrahydrocannabinol (THC). *Psychopharmacology, 204*(1), 85-94.
Ilan, A. B., Smith, M. E., & Gevins, A. (2004). Effects of marijuana on neurophysiological signals of working and episodic memory. *Psychopharmacology, 176*(2), 214-222.
Jackson, N. J., Isen, J. D., Khoddam, R., Irons, D., Tuvblad, C., Iacono, W. G., . . . Baker, L. A. (2016). Impact of adolescent marijuana use on intelligence: Results from two longitudinal twin studies. *Proceedings of the National Academy of Sciences, 113*(5), E500-E508.
Jacobsen, L. K., Pugh, K. R., Constable, R. T., Westerveld, M., & Mencl, W. E. (2007). Functional correlates of verbal memory deficits emerging during nicotine withdrawal in abstinent adolescent cannabis users. *Biological psychiatry, 61*(1), 31-40.
Jacobus, J., McQueeny, T., Bava, S., Schweinsburg, B., Frank, L., Yang, T. e., . . . Tapert, S. F. (2009). White matter integrity in adolescents with histories of marijuana use and binge drinking. *Neurotoxicology and teratology, 31*(6), 349-355.

Jacobus, J., Squeglia, L. M., Infante, M. A., Bava, S., & Tapert, S. F. (2013). White matter integrity pre-and post marijuana and alcohol initiation in adolescence. *Brain sciences, 3*(1), 396-414.

Jacobus, J., & Tapert, S. F. (2014). Effects of cannabis on the adolescent brain. *Current pharmaceutical design, 20*(13), 2186-2193.

Jager, G., Block, R. I., Luijten, M., & Ramsey, N. F. (2010). Cannabis use and memory brain function in adolescent boys: a cross-sectional multicenter functional magnetic resonance imaging study. *Journal of the American Academy of Child & Adolescent Psychiatry, 49*(6), 561-572. e563.

Johnson, K. A., Bonn-Miller, M. O., Leyro, T. M., & Zvolensky, M. J. (2009). Anxious arousal and anhedonic depression symptoms and the frequency of current marijuana use: Testing the mediating role of marijuana-use coping motives among active users. *Journal of Studies on Alcohol and Drugs, 70*(4), 543.

Jutras-Aswad, D., Jacobs, M. M., Yiannoulos, G., Roussos, P., Bitsios, P., Nomura, Y., . . . Hurd, Y. L. (2012). Cannabis-dependence risk relates to synergism between neuroticism and proenkephalin SNPs associated with amygdala gene expression: case-control study. *PLoS One, 7*(6), e39243.

Kay, S. R., Flszbein, A., & Opfer, L. A. (1987). The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull, 13*(2), 261.

Kessler, R. C., Amminger, G. P., Aguilar-Gaxiola, S., Alonso, J., Lee, S., & Ustun, T. B. (2007). Age of onset of mental disorders: a review of recent literature. *Current opinion in psychiatry, 20*(4), 359.

Kirschmann, E. K., Pollock, M. W., Nagarajan, V., & Torregrossa, M. M. (2016). Effects of Adolescent Cannabinoid Self-Administration in Rats on Addiction-Related Behaviors and Working Memory. *Neuropsychopharmacology*.

Kollins, S. H., Schoenfelder, E. N., English, J. S., Holdaway, A., Van Voorhees, E., O'Brien, B. R., . . . Chrisman, A. K. (2015). An exploratory study of the combined effects of orally administered methylphenidate and delta-9-tetrahydrocannabinol (THC) on cardiovascular function, subjective effects, and performance in healthy adults. *Journal of substance abuse treatment, 48*(1), 96-103.

Kråkvik, B., Larøi, F., Kalhovde, A. M., Hugdahl, K., Kompus, K., Salvesen, Ø., . . . Vedul-Kjelsås, E. (2015). Prevalence of auditory verbal hallucinations in a general population: A group comparison study. *Scandinavian Journal of Psychology, 56*(5), 508-515.

Kuntsche, E., Rehm, J., & Gmel, G. (2004). Characteristics of binge drinkers in Europe. *Social science & medicine, 59*(1), 113-127.

Lane, S. D., & Cherek, D. R. (2002). Marijuana effects on sensitivity to reinforcement in humans.

Lane, S. D., Cherek, D. R., Pietras, C. J., & Steinberg, J. L. (2005). Performance of heavy marijuana-smoking adolescents on a laboratory measure of motivation. *Addictive behaviors, 30*(4), 815-828.

Lanz, C., Mattsson, J., Soydaner, U., & Brenneisen, R. (2016). Medicinal Cannabis: In Vitro Validation of Vaporizers for the Smoke-Free Inhalation of Cannabis. *PLoS One, 11*(1).

Large, M., Sharma, S., Compton, M. T., Slade, T., & Nielssen, O. (2011). Cannabis use and earlier onset of psychosis: a systematic meta-analysis. *Arch Gen Psychiatry, 68*(6), 555-561. doi:10.1001/archgenpsychiatry.2011.5

Larøi, F., Sommer, I. E., Blom, J. D., Fernyhough, C., Hugdahl, K., Johns, L. C., . . . Slotema, C. W. (2012). The characteristic features of auditory verbal hallucinations in clinical and nonclinical groups: state-of-the-art overview and future directions. *Schizophrenia Bulletin, 38*(4), 724-733.

Lawn, W., Freeman, T. P., Pope, R. A., Joye, A., Harvey, L., Hindocha, C., . . . Bloomfield, M. A. (2016). Acute and chronic effects of cannabinoids on effort-related decision-making and reward learning: an evaluation of the cannabis 'amotivational'hypotheses. *Psychopharmacology, 233*(19-20), 3537-3552.

Lawn, W., Freeman, T. P., Pope, R. A., Joye, A., Harvey, L., Hindocha, C., . . . Curran, H. V. (2016). Acute and chronic effects of cannabinoids on effort-related decision-making

and reward learning: an evaluation of the cannabis 'amotivational' hypotheses. *Psychopharmacology*, 1-16. doi:10.1007/s00213-016-4383-x
Legleye, S., Karila, L., Beck, F., & Reynaud, M. (2007). Validation of the CAST, a general population Cannabis Abuse Screening Test. *Journal of substance use, 12*(4), 233-242.
Leweke, F., Piomelli, D., Pahlisch, F., Muhl, D., Gerth, C., Hoyer, C., . . . Koethe, D. (2012). Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. *Translational psychiatry, 2*(3), e94.
Liem-Moolenaar, M., Te Beek, E. T., de Kam, M. L., Franson, K. L., Kahn, R. S., Hijman, R., . . . van Gerven, J. M. (2010). Central nervous system effects of haloperidol on THC in healthy male volunteers. *Journal of psychopharmacology, 24*(11), 1697-1708.
Lisdahl, K. M., Gilbart, E. R., Wright, N. E., & Shollenbarger, S. (2013). Dare to delay? The impacts of adolescent alcohol and marijuana use onset on cognition, brain structure, and function. *Frontiers in psychiatry, 4*.
Lisdahl, K. M., & Price, J. S. (2012). Increased marijuana use and gender predict poorer cognitive functioning in adolescents and emerging adults. *Journal of the International Neuropsychological Society, 18*(04), 678-688.
Logan, G. D., & Cowan, W. B. (1984). On the ability to inhibit thought and action: A theory of an act of control. *Psychological review, 91*(3), 295.
Lorenzetti, V., Solowij, N., Fornito, A., Ian Lubman, D., & Yucel, M. (2014). The association between regular cannabis exposure and alterations of human brain morphology: an updated review of the literature. *Current pharmaceutical design, 20*(13), 2138-2167.
Lubman, D. I., Cheetham, A., & Yücel, M. (2015). Cannabis and adolescent brain development. *Pharmacology & therapeutics, 148*, 1-16.
Luciana, M., Conklin, H. M., Hooper, C. J., & Yarger, R. S. (2005). The development of nonverbal working memory and executive control processes in adolescents. *Child development, 76*(3), 697-712.
Luna, B., Garver, K. E., Urban, T. A., Lazar, N. A., & Sweeney, J. A. (2004). Maturation of cognitive processes from late childhood to adulthood. *Child Development, 75*(5), 1357-1372.
Lynam, D. (2013). Development of a short form of the UPPS-P Impulsive Behavior Scale. *Unpublished Technical Report*.
Lynskey, M. T., Coffey, C., Degenhardt, L., Carlin, J. B., & Patton, G. (2003). A longitudinal study of the effects of adolescent cannabis use on high school completion. *Addiction, 98*(5), 685-692.
Lynskey, M. T., & Fergusson, D. M. (1995). Childhood conduct problems, attention deficit behaviors, and adolescent alcohol, tobacco, and illicit drug use. *Journal of abnormal child psychology, 23*(3), 281-302.
Lynskey, M. T., & Hall, W. (2000). The effects of adolescent cannabis use on educational attainment: a review. *Addiction, 95*(11), 1621-1630.
Lynskey, M. T., Vink, J. M., & Boomsma, D. I. (2006). Early onset cannabis use and progression to other drug use in a sample of Dutch twins. *Behavior genetics, 36*(2), 195-200.
Macmillan, N. A., & Kaplan, H. L. (1985). Detection theory analysis of group data: estimating sensitivity from average hit and false-alarm rates. *Psychological bulletin, 98*(1), 185.
Mahmood, O. M., Jacobus, J., Bava, S., Scarlett, A., & Tapert, S. F. (2010). Learning and Memory Performances in Adolescent Users of Alcohol and Marijuana: Interactive Effects*. *Journal of Studies on Alcohol and Drugs, 71*(6), 885-894.
Makela, P., Wakeley, J., Gijsman, H., Robson, P. J., Bhagwagar, Z., & Rogers, R. D. (2006). Low doses of Δ-9 tetrahydrocannabinol (THC) have divergent effects on short-term spatial memory in young, healthy adults. *Neuropsychopharmacology, 31*(2), 462-470.
Manini, T. M. (2010). Energy expenditure and aging. *Ageing research reviews, 9*(1), 1-11.
Maremmani, I., Lazzeri, A., Pacini, M., Lovrecic, M., Placidi, G. F., & Perugi, G. (2004). Diagnostic and symptomatological features in chronic psychotic patients according to cannabis use status. *Journal of psychoactive drugs, 36*(2), 235-241.

Marin, R. S., Biedrzycki, R. C., & Firinciogullari, S. (1991). Reliability and validity of the Apathy Evaluation Scale. *Psychiatry research, 38*(2), 143-162.
Marshall, W. A., & Tanner, J. M. (1970). Variations in the pattern of pubertal changes in boys. *Archives of disease in childhood, 45*(239), 13-23.
Martin, G., Copeland, J., Gates, P., & Gilmour, S. (2006). The Severity of Dependence Scale (SDS) in an adolescent population of cannabis users: reliability, validity and diagnostic cut-off. *Drug and alcohol dependence, 83*(1), 90-93.
Mason, O. J., Morgan, C., Dhiman, S., Patel, A., Parti, N., Patel, A., & Curran, H. (2009). Acute cannabis use causes increased psychotomimetic experiences in individuals prone to psychosis. *Psychological Medicine, 39*(6), 951.
Mason, O. J., Morgan, C. J., Stefanovic, A., & Curran, H. V. (2008). The psychotomimetic states inventory (PSI): measuring psychotic-type experiences from ketamine and cannabis. *Schizophrenia research, 103*(1), 138-142.
Maynard, T. M., Sikich, L., Lieberman, J. A., & LaMantia, A.-S. (2001). Neural development, cell-cell signaling, and the "two-hit" hypothesis of schizophrenia. *Schizophr Bull, 27*(3), 457-476.
McCaffrey, D. F., Liccardo Pacula, R., Han, B., & Ellickson, P. (2010). Marijuana use and high school dropout: the influence of unobservables. *Health economics, 19*(11), 1281-1299.
McDonald, J., Schleifer, L., Richards, J. B., & de Wit, H. (2003). Effects of THC on behavioral measures of impulsivity in humans. *Neuropsychopharmacology*.
McGrath, J., Welham, J., Scott, J., Varghese, D., Degenhardt, L., Hayatbakhsh, M. R., . . . Najman, J. M. (2010). Association between cannabis use and psychosis-related outcomes using sibling pair analysis in a cohort of young adults. *Arch Gen Psychiatry, 67*(5), 440-447.
Mechoulam, R., Hanuš, L. O., Pertwee, R., & Howlett, A. C. (2014). Early phytocannabinoid chemistry to endocannabinoids and beyond. *Nature Reviews Neuroscience, 15*(11), 757-764.
Medina, K. L., Hanson, K. L., Schweinsburg, A. D., Cohen-Zion, M., Nagel, B. J., & Tapert, S. F. (2007). Neuropsychological functioning in adolescent marijuana users: subtle deficits detectable after a month of abstinence. *Journal of the International Neuropsychological Society, 13*(05), 807-820.
Meier, M. H., Caspi, A., Ambler, A., Harrington, H., Houts, R., Keefe, R. S., . . . Moffitt, T. E. (2012). Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proceedings of the National Academy of Sciences, 109*(40), E2657-E2664.
Mello, N. K., & Mendelson, J. H. (1985). Operant acquisition of marihuana by women. *Journal of Pharmacology and Experimental Therapeutics, 235*(1), 162-171.
Mendelson, J. H., Kuehnle, J. C., Greenberg, I., & Mello, N. K. (1976). Operant acquisition of marihuana in man. *Journal of Pharmacology and Experimental Therapeutics, 198*(1), 42-53.
Merckelbach, H., & van de Ven, V. (2001). Another White Christmas: fantasy proneness and reports of 'hallucinatory experiences' in undergraduate students. *Journal of Behavior Therapy and Experimental Psychiatry, 32*(3), 137-144.
Metrik, J., Kahler, C. W., Reynolds, B., McGeary, J. E., Monti, P. M., Haney, M., . . . Rohsenow, D. J. (2012). Balanced placebo design with marijuana: pharmacological and expectancy effects on impulsivity and risk taking. *Psychopharmacology, 223*(4), 489-499.
Miech, R. A., Johnston, L., O'Malley, P. M., Bachman, J. G., Schulenberg, J., & Patrick, M. E. (2015). Trends in use of marijuana and attitudes toward marijuana among youth before and after decriminalization: The case of California 2007–2013. *International Journal of Drug Policy, 26*(4), 336-344.
Miettunen, J., Törmänen, S., Murray, G. K., Jones, P. B., Mäki, P., Ebeling, H., . . . Joukamaa, M. (2008). Association of cannabis use with prodromal symptoms of psychosis in adolescence. *The British Journal of Psychiatry, 192*(6), 470-471.

Mills, K. L., Goddings, A.-L., Herting, M. M., Meuwese, R., Blakemore, S.-J., Crone, E. A., . . . Sowell, E. R. (2016). Structural brain development between childhood and adulthood: Convergence across four longitudinal samples. *Neuroimage, 141*, 273-281.

Mokrysz, C., Landy, R., Gage, S., Munafò, M., Roiser, J., & Curran, H. (2016). Are IQ and educational outcomes in teenagers related to their cannabis use? A prospective cohort study. *Journal of psychopharmacology, 30*(2), 159-168.

Moore, N. L., Greenleaf, A. L., Acheson, S. K., Wilson, W. A., Swartzwelder, H. S., & Kuhn, C. M. (2010). Role of cannabinoid receptor type 1 desensitization in greater tetrahydrocannabinol impairment of memory in adolescent rats. *Journal of Pharmacology and Experimental Therapeutics, 335*(2), 294-301.

Moore, T. H., Zammit, S., Lingford-Hughes, A., Barnes, T. R., Jones, P. B., Burke, M., & Lewis, G. (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet, 370*(9584), 319-328. doi:10.1016/S0140-6736(07)61162-3

Moreira, F. A., & Wotjak, C. T. (2010). Cannabinoids and anxiety *Behavioral Neurobiology of Anxiety and Its Treatment* (pp. 429-450): Springer.

Moreno, M., Estevez, A. F., Zaldivar, F., Montes, J. M. G., Gutiérrez-Ferre, V. E., Esteban, L., . . . Flores, P. (2012). Impulsivity differences in recreational cannabis users and binge drinkers in a university population. *Drug and alcohol dependence, 124*(3), 355-362.

Morgan, C. J., & Curran, H. V. (2008). Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. *The British Journal of Psychiatry, 192*(4), 306-307.

Morgan, C. J., Freeman, T., Powell, J., & Curran, H. (2016). AKT1 genotype moderates the acute psychotomimetic effects of naturalistically smoked cannabis in young cannabis smokers. *Translational psychiatry, 6*(2), e738.

Morgan, C. J., Gardener, C., Schafer, G., Swan, S., Demarchi, C., Freeman, T., . . . Tan, N. (2012). Sub-chronic impact of cannabinoids in street cannabis on cognition, psychotic-like symptoms and psychological well-being. *Psychological Medicine, 42*(02), 391-400.

Morgan, C. J., Page, E., Schaefer, C., Chatten, K., Manocha, A., Gulati, S., . . . Leweke, F. M. (2013). Cerebrospinal fluid anandamide levels, cannabis use and psychotic-like symptoms. *The British Journal of Psychiatry, 202*(5), 381-382.

Morgan, C. J., Schafer, G., Freeman, T. P., & Curran, H. V. (2010). Impact of cannabidiol on the acute memory and psychotomimetic effects of smoked cannabis: naturalistic study. *The British Journal of Psychiatry, 197*(4), 285-290.

Morrison, P., & Stone, J. (2011). Synthetic delta-9-tetrahydrocannabinol elicits schizophrenia-like negative symptoms which are distinct from sedation. *Human Psychopharmacology: Clinical and Experimental, 26*(1), 77-80.

Morrison, P., Zois, V., McKeown, D., Lee, T., Holt, D., Powell, J., . . . Murray, R. (2009). The acute effects of synthetic intravenous Δ 9-tetrahydrocannabinol on psychosis, mood and cognitive functioning. *Psychological Medicine, 39*(10), 1607-1616.

Muniyappa, R., Sable, S., Ouwerkerk, R., Mari, A., Gharib, A. M., Walter, M., . . . Volkow, N. D. (2013). Metabolic effects of chronic cannabis smoking. *Diabetes Care, 36*(8), 2415-2422.

Murray, R. M., & Di Forti, M. (2016). Cannabis and psychosis: what degree of proof do we require? *Biological psychiatry, 79*(7), 514-515.

Murray, R. M., Morrison, P. D., Henquet, C., & Di Forti, M. (2007). Cannabis, the mind and society: the hash realities. *Nature Reviews Neuroscience, 8*(11), 885-895.

Naimi, T. S., Brewer, R. D., Mokdad, A., Denny, C., Serdula, M. K., & Marks, J. S. (2003). Binge drinking among US adults. *Jama, 289*(1), 70-75.

Nakonezny, P. A., Carmody, T. J., Morris, D. W., Kurian, B. T., & Trivedi, M. H. (2010). Psychometric evaluation of the Snaith–Hamilton Pleasure Scale (SHAPS) in adult outpatients with major depressive disorder. *International clinical psychopharmacology, 25*(6), 328.

NDTMS. (2014). Adult Drug Statistics from the National Drug Treatment Monitoring System (NDTMS). Public Health England.

Nestor, L., Hester, R., & Garavan, H. (2010). Increased ventral striatal BOLD activity during non-drug reward anticipation in cannabis users. *Neuroimage, 49*(1), 1133-1143.
Nguyen, J. D., Aarde, S. M., Vandewater, S. A., Grant, Y., Stouffer, D. G., Parsons, L. H., . . . Taffe, M. A. (2016). Inhaled delivery of Δ 9-tetrahydrocannabinol (THC) to rats by e-cigarette vapor technology. *Neuropharmacology*.
Nigg, J. T., Wong, M. M., Martel, M. M., Jester, J. M., Puttler, L. I., Glass, J. M., . . . Zucker, R. A. (2006). Poor response inhibition as a predictor of problem drinking and illicit drug use in adolescents at risk for alcoholism and other substance use disorders. *Journal of the American Academy of Child & Adolescent Psychiatry, 45*(4), 468-475.
Nissen, S. E., Nicholls, S. J., Wolski, K., Rodés-Cabau, J., Cannon, C. P., Deanfield, J. E., . . . Kapadia, S. (2008). Effect of rimonabant on progression of atherosclerosis in patients with abdominal obesity and coronary artery disease: the STRADIVARIUS randomized controlled trial. *JAMA, 299*(13), 1547-1560.
Norman, A. L., Pulido, C., Squeglia, L. M., Spadoni, A. D., Paulus, M. P., & Tapert, S. F. (2011). Neural activation during inhibition predicts initiation of substance use in adolescence. *Drug and alcohol dependence, 119*(3), 216-223.
O'Shea, M., Singh, M. E., McGregor, I. S., & Mallet, P. E. (2004). Chronic cannabinoid exposure produces lasting memory impairment and increased anxiety in adolescent but not adult rats. *Journal of Psychopharmacology, 18*(4), 502-508.
Ohlsson, A., Lindgren, J., Wahlen, A., Agurell, S., Hollister, L., & Gillespie, H. (1981). Plasma Levels of Tetrahydrocannabinol After Intravenous, Oral, and Smoke Administration. *Problems of drug dependence*, 250.
Padula, C. B., Schweinsburg, A. D., & Tapert, S. F. (2007). Spatial working memory performance and fMRI activation interaction in abstinent adolescent marijuana users. *Psychology of Addictive Behaviors, 21*(4), 478.
Pattij, T., Janssen, M. C., Schepers, I., González-Cuevas, G., De Vries, T. J., & Schoffelmeer, A. N. (2007). Effects of the cannabinoid CB1 receptor antagonist rimonabant on distinct measures of impulsive behavior in rats. *Psychopharmacology, 193*(1), 85-96.
Patton, G. C., Coffey, C., Carlin, J. B., Degenhardt, L., Lynskey, M., & Hall, W. (2002). Cannabis use and mental health in young people: cohort study. *BMJ, 325*(7374), 1195-1198.
Patton, G. C., Coffey, C., Carlin, J. B., Sawyer, S. M., & Lynskey, M. (2005). Reverse gateways? Frequent cannabis use as a predictor of tobacco initiation and nicotine dependence. *Addiction, 100*(10), 1518-1525.
Paus, T., Keshavan, M., & Giedd, J. N. (2008). Why do many psychiatric disorders emerge during adolescence? *Nature Reviews Neuroscience, 9*(12), 947-957.
Peralta, V., & Cuesta, M. J. (1992). Influence of cannabis abuse on schizophrenic psychopathology. *Acta Psychiatrica Scandinavica, 85*(2), 127-130.
Pergadia, M. L., Der-Avakian, A., D'Souza, M. S., Madden, P. A., Heath, A. C., Shiffman, S., . . . Pizzagalli, D. A. (2014). Association between nicotine withdrawal and reward responsiveness in humans and rats. *JAMA psychiatry, 71*(11), 1238-1245.
Pertwee, R. G. (2005). Pharmacological actions of cannabinoids *Cannabinoids* (pp. 1-51): Springer.
Pertwee, R. G. (2008). The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: Δ9-tetrahydrocannabinol, cannabidiol and Δ9-tetrahydrocannabivarin. *Br J Pharmacol, 153*(2), 199-215.
Pertwee, R. G. (2014). *Handbook of cannabis*: Oxford University Press, USA.
Peters, B. D., Szeszko, P. R., Radua, J., Ikuta, T., Gruner, P., DeRosse, P., . . . Tapert, S. F. (2012). White matter development in adolescence: diffusion tensor imaging and meta-analytic results. *Schizophr Bull, 38*(6), 1308-1317.
Pizzagalli, D. A., Goetz, E., Ostacher, M., Iosifescu, D. V., & Perlis, R. H. (2008). Euthymic patients with bipolar disorder show decreased reward learning in a probabilistic reward task. *Biological psychiatry, 64*(2), 162-168.

Pizzagalli, D. A., Iosifescu, D., Hallett, L. A., Ratner, K. G., & Fava, M. (2008). Reduced hedonic capacity in major depressive disorder: evidence from a probabilistic reward task. *Journal of psychiatric research, 43*(1), 76-87.

Pizzagalli, D. A., Jahn, A. L., & O'Shea, J. P. (2005). Toward an objective characterization of an anhedonic phenotype: a signal-detection approach. *Biological psychiatry, 57*(4), 319-327.

Pol, P., Liebregts, N., Brunt, T., Amsterdam, J., Graaf, R., Korf, D. J., . . . Laar, M. (2014). Cross-sectional and prospective relation of cannabis potency, dosing and smoking behaviour with cannabis dependence: an ecological study. *Addiction*.

Pol, P., Liebregts, N., Graaf, R., Have, M., Korf, D. J., Brink, W., & Laar, M. (2013). Mental health differences between frequent cannabis users with and without dependence and the general population. *Addiction, 108*(8), 1459-1469.

Pope, H. G., Gruber, A. J., Hudson, J. I., Cohane, G., Huestis, M. A., & Yurgelun-Todd, D. (2003). Early-onset cannabis use and cognitive deficits: what is the nature of the association? *Drug and alcohol dependence, 69*(3), 303-310.

Price, J. S., McQueeny, T., Shollenbarger, S., Browning, E. L., Wieser, J., & Lisdahl, K. M. (2015). Effects of marijuana use on prefrontal and parietal volumes and cognition in emerging adults. *Psychopharmacology, 232*, 2939-2950.

Quinn, H. R., Matsumoto, I., Callaghan, P. D., Long, L. E., Arnold, J. C., Gunasekaran, N., . . . Kashem, M. A. (2008). Adolescent rats find repeated Δ9-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. *Neuropsychopharmacology, 33*(5), 1113-1126.

Raine, A. (1991). The SPQ: a scale for the assessment of schizotypal personality based on DSM-III-R criteria. *Schizophr Bull, 17*(4), 555.

Ramaekers, J. G., Kauert, G., Theunissen, E., Toennes, S. W., & Moeller, M. (2008). Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. *Journal of psychopharmacology*.

Ramaekers, J. G., Kauert, G., van Ruitenbeek, P., Theunissen, E. L., Schneider, E., & Moeller, M. R. (2006). High-potency marijuana impairs executive function and inhibitory motor control. *Neuropsychopharmacology, 31*(10), 2296-2303.

Ramaekers, J. G., Theunissen, E. L., de Brouwer, M., Toennes, S. W., Moeller, M. R., & Kauert, G. (2011). Tolerance and cross-tolerance to neurocognitive effects of THC and alcohol in heavy cannabis users. *Psychopharmacology, 214*(2), 391-401.

Ramaekers, J. G., van Wel, J., Spronk, D., Toennes, S., Kuypers, K., Theunissen, E., & Verkes, R. (2016). Cannabis and tolerance: acute drug impairment as a function of cannabis use history. *Scientific reports, 6*.

Ranganathan, M., Carbuto, M., Braley, G., Elander, J., Perry, E., Pittman, B., . . . D'Souza, D. C. (2012). Naltrexone does not attenuate the effects of intravenous Δ9-tetrahydrocannabinol in healthy humans. *International Journal of Neuropsychopharmacology, 15*(9), 1251-1264.

Ranganathan, M., & D'souza, D. C. (2006). The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology, 188*(4), 425-444.

Ranganathan, M., Skosnik, P. D., & D'Souza, D. C. (2016). Marijuana and Madness: Associations Between Cannabinoids and Psychosis. *Biological psychiatry, 79*(7), 511.

Raznahan, A., Shaw, P., Lalonde, F., Stockman, M., Wallace, G. L., Greenstein, D., . . . Giedd, J. N. (2011). How does your cortex grow? *The Journal of Neuroscience, 31*(19), 7174-7177.

Realini, N., Rubino, T., & Parolaro, D. (2009). Neurobiological alterations at adult age triggered by adolescent exposure to cannabinoids. *Pharmacological research, 60*(2), 132-138.

Research, P. I. f., & Evaluation. (2005). Drinking In America: Myths, Realities, and Prevention Policy.

Rimvall, M., Clemmensen, L., Munkholm, A., Rask, C., Larsen, J., Skovgaard, A., . . . Jeppesen, P. (2016). Introducing the White Noise task in childhood: associations between speech illusions and psychosis vulnerability. *Psychological Medicine*, 1-10.

Rob, M., Reynolds, I., & Finlayson, P. F. (1990). Adolescent marijuana use: Risk factors and implications. *Australian and New Zealand Journal of Psychiatry, 24*(1), 47-56.
Rogeberg, O. (2013). Correlations between cannabis use and IQ change in the Dunedin cohort are consistent with confounding from socioeconomic status. *Proceedings of the National Academy of Sciences, 110*(11), 4251-4254.
Romero, J., Garcia-Palomero, E., Berrendero, F., Garcia-Gil, L., Hernandez, M., Ramos, J., & Fernandez-Ruiz, J. (1997). Atypical location of cannabinoid receptors in white matter areas during rat brain development. *Synapse, 26*(3), 317-323.
Rubia, K., Smith, A. B., Woolley, J., Nosarti, C., Heyman, I., Taylor, E., & Brammer, M. (2006). Progressive increase of frontostriatal brain activation from childhood to adulthood during event-related tasks of cognitive control. *Human Brain Mapping, 27*(12), 973-993.
Rubino, T., & Parolaro, D. (2015). The Impact of Exposure to Cannabinoids in Adolescence: Insights from Animal Models. *Biological psychiatry*.
Rubino, T., Prini, P., Piscitelli, F., Zamberletti, E., Trusel, M., Melis, M., . . . Di Marzo, V. (2015). Adolescent exposure to THC in female rats disrupts developmental changes in the prefrontal cortex. *Neurobiology of disease, 73*, 60-69.
Rubino, T., Realini, N., Braida, D., Alberio, T., Capurro, V., Vigano, D., . . . Parolaro, D. (2009). The depressive phenotype induced in adult female rats by adolescent exposure to THC is associated with cognitive impairment and altered neuroplasticity in the prefrontal cortex. *Neurotoxicity research, 15*(4), 291-302.
Rubino, T., Realini, N., Braida, D., Guidi, S., Capurro, V., Vigano, D., . . . Bartesaghi, R. (2009). Changes in hippocampal morphology and neuroplasticity induced by adolescent THC treatment are associated with cognitive impairment in adulthood. *Hippocampus, 19*(8), 763-772.
Sagar, K. A., Dahlgren, M. K., Gönenç, A., Racine, M. T., Dreman, M. W., & Gruber, S. A. (2015). The impact of initiation: Early onset marijuana smokers demonstrate altered Stroop performance and brain activation. *Developmental cognitive neuroscience, 16*, 84-92.
Saha, S., Chant, D., Welham, J., & McGrath, J. (2005). A systematic review of the prevalence of schizophrenia. *PLoS Med, 2*(5), e141.
Schneider, M., & Koch, M. (2003). Chronic pubertal, but not adult chronic cannabinoid treatment impairs sensorimotor gating, recognition memory, and the performance in a progressive ratio task in adult rats. *Neuropsychopharmacology*.
Schneider, M., Schömig, E., & Leweke, F. M. (2008). PRECLINICAL STUDY: acute and chronic cannabinoid treatment differentially affects recognition memory and social behavior in pubertal and adult rats. *Addiction biology, 13*(3-4), 345-357.
Schoedel, K. A., Chen, N., Hilliard, A., White, L., Stott, C., Russo, E., . . . Sellers, E. M. (2011). A randomized, double-blind, placebo-controlled, crossover study to evaluate the subjective abuse potential and cognitive effects of nabiximols oromucosal spray in subjects with a history of recreational cannabis use. *Human Psychopharmacology: Clinical and Experimental, 26*(3), 224-236.
Schoeler, T., Kambeitz, J., Behlke, I., Murray, R., & Bhattacharyya, S. (2016). The effects of cannabis on memory function in users with and without a psychotic disorder: findings from a combined meta-analysis. *Psychological Medicine, 46*(01), 177-188.
Schoeler, T., Monk, A., Sami, M. B., Klamerus, E., Foglia, E., Brown, R., . . . Bhattacharyya, S. (2016). Continued versus discontinued cannabis use in patients with psychosis: a systematic review and meta-analysis. *The Lancet Psychiatry, 3*(3), 215-225.
Schramm-Sapyta, N. L., Cha, Y. M., Chaudhry, S., Wilson, W. A., Swartzwelder, H. S., & Kuhn, C. M. (2007). Differential anxiogenic, aversive, and locomotor effects of THC in adolescent and adult rats. *Psychopharmacology, 191*(4), 867-877.
Schreiner, A. M., & Dunn, M. E. (2012). Residual effects of cannabis use on neurocognitive performance after prolonged abstinence: A meta-analysis. *Experimental and clinical psychopharmacology, 20*(5), 420.

Schuster, R. M., Crane, N. A., Mermelstein, R., & Gonzalez, R. (2015). Tobacco May Mask Poorer Episodic Memory Among Young Adult Cannabis Users.
Schuster, R. M., Mermelstein, R. J., & Hedeker, D. (2016). Ecological momentary assessment of working memory under conditions of simultaneous marijuana and tobacco use. *Addiction*.
Schweinsburg, A. D., Nagel, B. J., Schweinsburg, B. C., Park, A., Theilmann, R. J., & Tapert, S. F. (2008). Abstinent adolescent marijuana users show altered fMRI response during spatial working memory. *Psychiatry Research: Neuroimaging, 163*(1), 40-51.
Schweinsburg, A. D., Schweinsburg, B. C., Medina, K. L., McQueeny, T., Brown, S. A., & Tapert, S. F. (2010). The influence of recency of use on fMRI response during spatial working memory in adolescent marijuana users. *Journal of psychoactive drugs, 42*(3), 401-412.
Seely, K. A., Lapoint, J., Moran, J. H., & Fattore, L. (2012). Spice drugs are more than harmless herbal blends: a review of the pharmacology and toxicology of synthetic cannabinoids. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 39*(2), 234-243.
Shaw, P., Kabani, N. J., Lerch, J. P., Eckstrand, K., Lenroot, R., Gogtay, N., . . . Rapoport, J. L. (2008). Neurodevelopmental trajectories of the human cerebral cortex. *The Journal of Neuroscience, 28*(14), 3586-3594.
Shedler, J., & Block, J. (1990). Adolescent drug use and psychological health: A longitudinal inquiry. *American psychologist, 45*(5), 612.
Sherif, M., Radhakrishnan, R., D'Souza, D. C., & Ranganathan, M. (2016). Human laboratory studies on cannabinoids and psychosis. *Biological psychiatry, 79*(7), 526-538.
Sidhpura, N., & Parsons, L. H. (2011). Endocannabinoid-mediated synaptic plasticity and addiction-related behavior. *Neuropharmacology, 61*(7), 1070-1087.
Silins, E., Horwood, L. J., Patton, G. C., Fergusson, D. M., Olsson, C. A., Hutchinson, D. M., . . . Swift, W. (2014). Young adult sequelae of adolescent cannabis use: an integrative analysis. *The Lancet Psychiatry, 1*(4), 286-293.
Smith, J. L., Mattick, R. P., Jamadar, S. D., & Iredale, J. M. (2014). Deficits in behavioural inhibition in substance abuse and addiction: a meta-analysis. *Drug and alcohol dependence, 145*, 1-33.
Snaith, R., Hamilton, M., Morley, S., Humayan, A., Hargreaves, D., & Trigwell, P. (1995). A scale for the assessment of hedonic tone the Snaith-Hamilton Pleasure Scale. *The British Journal of Psychiatry, 167*(1), 99-103.
Solowij, N., Jones, K. A., Rozman, M. E., Davis, S. M., Ciarrochi, J., Heaven, P. C., . . . Yücel, M. (2011). Verbal learning and memory in adolescent cannabis users, alcohol users and non-users. *Psychopharmacology, 216*(1), 131-144.
Solowij, N., Stephens, R. S., Roffman, R. A., Babor, T., Kadden, R., Miller, M., . . . Vendetti, J. (2002). Cognitive functioning of long-term heavy cannabis users seeking treatment. *Jama, 287*(9), 1123-1131.
Solowij, N., Yücel, M., Respondek, C., Whittle, S., Lindsay, E., Pantelis, C., & Lubman, D. (2011). Cerebellar white-matter changes in cannabis users with and without schizophrenia. *Psychological Medicine, 41*(11), 2349-2359.
Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neuroscience & Biobehavioral Reviews, 24*(4), 417-463.
Spear, L. P. (2016). Alcohol Consumption in Adolescence: a Translational Perspective. *Current addiction reports, 3*(1), 50-61.
Spencer, D. (1970). Cannabis induced psychosis. *British Journal of Addiction to Alcohol & Other Drugs, 65*(4), 369-372.
Stanislaw, H., & Todorov, N. (1999). Calculation of signal detection theory measures. *Behavior research methods, instruments, & computers, 31*(1), 137-149.
Stefanis, N., Delespaul, P., Henquet, C., Bakoula, C., Stefanis, C., & Van Os, J. (2004). Early adolescent cannabis exposure and positive and negative dimensions of psychosis. *Addiction, 99*(10), 1333-1341.
Steinberg, L. (2010). *Adolescence* (9th ed.).

Stiby, A. I., Hickman, M., Munafò, M. R., Heron, J., Yip, V. L., & Macleod, J. (2014). Adolescent Cannabis and Tobacco use and Educational Outcomes at Age 16: Birth Cohort Study. *Addiction*.

Stokes, P. R., Mehta, M. A., Curran, H. V., Breen, G., & Grasby, P. M. (2009). Can recreational doses of THC produce significant dopamine release in the human striatum? *Neuroimage, 48*(1), 186-190.

Sutton, R. (2012). Adult anthropometric measures, overweight and obesity. *Health Survey for England–2011, Health, Social Care and Lifestyles*, 325-362.

Tait, R. J., Mackinnon, A., & Christensen, H. (2011). Cannabis use and cognitive function: 8-year trajectory in a young adult cohort. *Addiction, 106*(12), 2195-2203.

Tamm, L., Epstein, J. N., Lisdahl, K. M., Molina, B., Tapert, S., Hinshaw, S. P., . . . Swanson, J. M. (2013). Impact of ADHD and cannabis use on executive functioning in young adults. *Drug and alcohol dependence, 133*(2), 607-614.

Tamnes, C. K., Østby, Y., Fjell, A. M., Westlye, L. T., Due-Tønnessen, P., & Walhovd, K. B. (2010). Brain maturation in adolescence and young adulthood: regional age-related changes in cortical thickness and white matter volume and microstructure. *Cerebral Cortex, 20*(3), 534-548.

Tamnes, C. K., Walhovd, K. B., Dale, A. M., Østby, Y., Grydeland, H., Richardson, G., . . . Due-Tønnessen, P. (2013). Brain development and aging: overlapping and unique patterns of change. *Neuroimage, 68*, 63-74.

Tapert, S. F., Schweinsburg, A. D., Drummond, S. P., Paulus, M. P., Brown, S. A., Yang, T. T., & Frank, L. R. (2007). Functional MRI of inhibitory processing in abstinent adolescent marijuana users. *Psychopharmacology, 194*(2), 173-183.

Temple, E. C., Brown, R. F., & Hine, D. W. (2011). The 'grass ceiling': limitations in the literature hinder our understanding of cannabis use and its consequences. *Addiction, 106*(2), 238-244.

Thanki, D., Matias, J., Griffiths, P., Noor, A., Olszewski, D., Simon, R., & Vicente, J. (2013). Prevalence of daily cannabis use in the European Union and Norway. *Lisbon, Portugal: European Monitoring Centre for Drugs and Drug Addiction*.

Theunissen, E., Heckman, P., Perna, E. d. S. F., Kuypers, K., Sambeth, A., Blokland, A., . . . Ramaekers, J. (2015). Rivastigmine but not vardenafil reverses cannabis-induced impairment of verbal memory in healthy humans. *Psychopharmacology, 232*(2), 343-353.

Tool, S. (1988). Brief psychiatric rating scale. *Psychopharmacology bulletin, 24*, 97-99.

Townsend, L., Flisher, A. J., & King, G. (2007). A systematic review of the relationship between high school dropout and substance use. *Clinical Child and Family Psychology Review, 10*(4), 295-317.

Treadway, M. T., Buckholtz, J. W., Schwartzman, A. N., Lambert, W. E., & Zald, D. H. (2009). Worth the 'EEfRT'? The effort expenditure for rewards task as an objective measure of motivation and anhedonia. *PLoS One, 4*(8), e6598.

Treadway, M. T., & Zald, D. H. (2011). Reconsidering anhedonia in depression: lessons from translational neuroscience. *Neuroscience & Biobehavioral Reviews, 35*(3), 537-555.

Treffert, D. A. (1978). Marihuana use in schizophrenia: A clear hazard. *The American journal of psychiatry*.

Tuchman, E. (2010). Women and addiction: the importance of gender issues in substance abuse research. *Journal of addictive diseases, 29*(2), 127-138.

Valverde, O., & Torrens, M. (2012). CB1 receptor-deficient mice as a model for depression. *Neuroscience, 204*, 193-206. doi:10.1016/j.neuroscience.2011.09.031

Van Buuren, S., Boshuizen, H. C., & Knook, D. L. (1999). Multiple imputation of missing blood pressure covariates in survival analysis. *Statistics in medicine, 18*(6), 681-694.

Van Dam, N. T., Bedi, G., & Earleywine, M. (2012). Characteristics of clinically anxious versus non-anxious regular, heavy marijuana users. *Addictive Behaviors, 37*(11), 1217-1223.

van den Bree, M. B., & Pickworth, W. B. (2005). Risk factors predicting changes in marijuana involvement in teenagers. *Arch Gen Psychiatry, 62*(3), 311-319.

van der Pol, P., Liebregts, N., Brunt, T., Amsterdam, J., Graaf, R., Korf, D. J., . . . Laar, M. (2014). Cross-sectional and prospective relation of cannabis potency, dosing and smoking behaviour with cannabis dependence: an ecological study. *Addiction*.
van Hell, H. H., Vink, M., Ossewaarde, L., Jager, G., Kahn, R. S., & Ramsey, N. F. (2010). Chronic effects of cannabis use on the human reward system: an fMRI study. *European Neuropsychopharmacology, 20*(3), 153-163.
Van Os, J., Hanssen, M., Bijl, R. V., & Ravelli, A. (2000). Strauss (1969) revisited: a psychosis continuum in the general population? *Schizophrenia research, 45*(1), 11-20.
Van Wel, J., Kuypers, K., Theunissen, E., Toennes, S., Spronk, D., Verkes, R., & Ramaekers, J. (2013). Single doses of THC and cocaine decrease proficiency of impulse control in heavy cannabis users. *Br J Pharmacol, 170*(7), 1410.
van Winkel, R., van Beveren, N. J., & Simons, C. (2011). AKT1 moderation of cannabis-induced cognitive alterations in psychotic disorder. *Neuropsychopharmacology, 36*(12), 2529-2537. doi:10.1038/npp.2011.141
Vandrey, R., Stitzer, M. L., Mintzer, M. Z., Huestis, M. A., Murray, J. A., & Lee, D. (2013). The dose effects of short-term dronabinol (oral THC) maintenance in daily cannabis users. *Drug and alcohol dependence, 128*(1), 64-70.
Verbruggen, F., Logan, G. D., & Stevens, M. A. (2008). STOP-IT: Windows executable software for the stop-signal paradigm. *Behavior research methods, 40*(2), 479-483.
Vercammen, A., & Aleman, A. (2010). Semantic expectations can induce false perceptions in hallucination-prone individuals. *Schizophr Bull, 36*(1), 151-156.
Vercammen, A., De Haan, E., & Aleman, A. (2008). Hearing a voice in the noise: auditory hallucinations and speech perception. *Psychological Medicine, 38*(08), 1177-1184.
Verdurand, M., Nguyen, V., Stark, D., Zahra, D., Gregoire, M.-C., Greguric, I., & Zavitsanou, K. (2011). Comparison of Cannabinoid CB1 Receptor Binding in Adolescent and Adult Rats: A Positron Emission Tomography Study Using [18F] MK-9470. *International journal of molecular imaging, 2011*.
Verrico, C. D., Gu, H., Peterson, M. L., Sampson, A. R., & Lewis, D. A. (2014). Repeated Δ9-tetrahydrocannabinol exposure in adolescent monkeys: persistent effects selective for spatial working memory.
Verrico, C. D., Liu, S., Bitler, E. J., Gu, H., Sampson, A. R., Bradberry, C. W., & Lewis, D. A. (2012). Delay-and dose-dependent effects of Δ9-tetrahydrocannabinol administration on spatial and object working memory tasks in adolescent rhesus monkeys. *Neuropsychopharmacology, 37*(6), 1357-1366.
Verweij, K. J., Huizink, A. C., Agrawal, A., Martin, N. G., & Lynskey, M. T. (2013). Is the relationship between early-onset cannabis use and educational attainment causal or due to common liability? *Drug and alcohol dependence, 133*(2), 580-586.
Vollema, M. G., & Hoijtink, H. (2000). The multidimensionality of self-report schizotypy in a psychiatric population: an analysis using multidimensional Rasch models. *Schizophr Bull, 26*(3), 565-575.
von Sydow, K., Lieb, R., Pfister, H., Höfler, M., & Wittchen, H.-U. (2002). What predicts incident use of cannabis and progression to abuse and dependence?: A 4-year prospective examination of risk factors in a community sample of adolescents and young adults. *Drug and alcohol dependence, 68*(1), 49-64.
Wagner, D., Becker, B., Gouzoulis-Mayfrank, E., & Daumann, J. (2010). Interactions between specific parameters of cannabis use and verbal memory. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 34*(6), 871-876.
Wechsler, D. (1991). Manual for the Wechsler intelligence scale for Children—Third edition (WISC-III). *San Antonio, TX: Psychological Corporation*.
Wechsler, D. (1999). The Psychological Corporation; San Antonio, TX: 1999. *Wechsler abbreviated scale of intelligence*.
Weiser, M., Zarka, S., Werbeloff, N., Kravitz, E., & Lubin, G. (2010). Cognitive test scores in male adolescent cigarette smokers compared to non-smokers: a population-based study. *Addiction, 105*(2), 358-363.

Wesnes, K., Annas, P., Edgar, C., Deeprose, C., Karlsten, R., Philipp, A., . . . Segerdahl, M. (2009). Nabilone produces marked impairments to cognitive function and changes in subjective state in healthy volunteers. *Journal of Psychopharmacology*.

Whalley, L. J., Fox, H. C., Deary, I. J., & Starr, J. M. (2005). Childhood IQ, smoking, and cognitive change from age 11 to 64 years. *Addictive behaviors, 30*(1), 77-88.

Whitlow, C. T., Liguori, A., Livengood, L. B., Hart, S. L., Mussat-Whitlow, B. J., Lamborn, C. M., . . . Porrino, L. J. (2004). Long-term heavy marijuana users make costly decisions on a gambling task. *Drug and alcohol dependence, 76*(1), 107-111.

Wilson, B., Cockburn, J., Baddeley, A., & Hiorns, R. (1989). The development and validation of a test battery for detecting and monitoring everyday memory problems. *Journal of clinical and experimental neuropsychology, 11*(6), 855-870.

Winward, J. L., Hanson, K. L., Tapert, S. F., & Brown, S. A. (2014). Heavy alcohol use, marijuana use, and concomitant use by adolescents are associated with unique and shared cognitive decrements. *Journal of the International Neuropsychological Society, 20*(08), 784-795.

Wittchen, H.-U., Jacobi, F., Rehm, J., Gustavsson, A., Svensson, M., Jönsson, B., . . . Faravelli, C. (2011). The size and burden of mental disorders and other disorders of the brain in Europe 2010. *European Neuropsychopharmacology, 21*(9), 655-679.

Zammit, S. (2004). An investigation into the use of cannabis and tobacco as risk factors for schizophrenia. *PhD thesis, Cardiff University*.

Zammit, S., Allebeck, P., Andreasson, S., Lundberg, I., & Lewis, G. (2002). Self reported cannabis use as a risk factor for schizophrenia in Swedish conscripts of 1969: historical cohort study. *BMJ, 325*(7374), 1199.

Zammit, S., Spurlock, G., Williams, H., Norton, N., Williams, N., O'DONOVAN, M. C., & Owen, M. J. (2007). Genotype effects of CHRNA7, CNR1 and COMT in schizophrenia: interactions with tobacco and cannabis use. *The British Journal of Psychiatry, 191*(5), 402-407.

Zanelati, T., Biojone, C., Moreira, F., Guimaraes, F., & Joca, S. (2010). Antidepressant-like effects of cannabidiol in mice: possible involvement of 5-HT1A receptors. *Br J Pharmacol, 159*(1), 122-128.

Zhu, P. J. (2006). Endocannabinoid signaling and synaptic plasticity in the brain. *Critical Reviews™ in Neurobiology, 18*(1-2).

## Appendix 1: ALSPAC data application, study 1.

**ALSPAC RESEARCH PROPOSAL FORM**

**Collaborator's outline proposal of a project to use existing ALSPAC data and/or biological samples or for the collection of new data.**

**1. Applicants:**

| | | |
|---|---|---|
| Principal applicant: | Name: | Dr Jonathan Roiser |
| | Affiliation: | UCL |
| | Email: | j.roiser@ucl.ac.uk |
| | Telephone: | +44 (207) 679 1170 (Internal 21170) |
| | Address: | UCL Institute of Cognitive Neuroscience<br>17 Queen Square<br>London<br>WC1N 3AR |
| Co-applicants: | Names: | Professor Valerie Curran Professor Marcus Munafò<br>Ms Claire Mokrysz Ms Suzanne Gage Dr Jon Heron<br>Professor Matt Hickman Dr Rebecca Landy |

**2. Project title** *(no more than 120 characters with spaces)* The long-term effects of adolescent cannabis use on cognitive functioning

| | |
|---|---|
| Start date: | 01/07/2013 |
| End date: | 01/07/2014 |

**3. Funding:**

Has the project been or will it be peer reviewed? Yes ☐ No ☒

| | |
|---|---|
| If so, by what organisation? | |
| Funding: *(If not specified above)* | |

Has funding been sought? * Yes ☐ No ☒

What is the deadline for application to the funder?

***Please note that applications for funding must be reviewed PRIOR to submission to a funding body and should be received AT LEAST two weeks before the deadline for submission.***

**4. Variables requested:**

Please check the variables that are requested/required for this proposal & give full details in your scientific outline:

| | |
|---|---|
| Existing questionnaire data: | ☒ |
| Existing data from direct assessment: | ☒ |
| Existing data from biological samples: | ☐ |
| Existing genetic data | ☐ |
| New questionnaire data: | ☒ |
| New data from direct assessment: | ☒ |
| *New data from biological samples: | ☐ |
| New genetic data: | ☐ |

*Please note we have minimal amounts of mothers antenatal blood and cord blood samples left and are now keeping these for high throughput proteomics and metabanomic analysis. The Executive are therefore unable to approve projects which ask to analyse a small number of analytes in these samples.

Data from external administrative data sources e.g. school records/NHS health data ☒

For biological sample (including genetic) data you are advised to discuss your requirements with the laboratory staff PRIOR to submission of this form (Contact: alspac-lab@bristol.ac.uk to discuss requirements/feasibility).

5. **Justification:**
Please state below the rationale for using ALSPAC data for this study, including consideration of other study methods considered (e.g. case-control):

Previous cross-sectional research has been unable to consider whether differences in pre-exposure cognitive functioning may be driving associations reported between cannabis use and cognitive deficits. ALSPAC is ideal to provide the longitudinal data needed to provide a better idea of this relationship and to exclude alternative explanations.

6. **Ethical approval:**
Does the study have ethical approval from a recognised Institutional Review Board/Ethics Committee? Yes ☐ No ☐ NA ☒
*For analysis of existing data and samples, generic ALSPAC Ethical approval will operate and the NA box above may be checked.*
**If Yes**, please append a copy of the approval.
**If No**, please specify arrangements for obtaining appropriate approvals:

n/a

7. **Laboratory Analysis:**
If the study involves analysis of biological samples (including DNA) please give details of the laboratory where analysis will be carried out. Also please provide list of all proposed genotypes and sample analysis in appendix.
Laboratory:

n/a

Contact person in your laboratory:

n/a

Is your laboratory covered by a Human Tissue Authority licence? Yes ☐ No ☐

**If Yes**, please give contact details of HTA Designated Individual.

n/a

8. **Scientific outline:**
Please provide a **1-2 page outline (see Project Outline on page 5)** of your proposal, highlighting the specific requirements of the project for the ALSPAC data specified above. Please make sure you include the following sections in this outline: aims, hypotheses, exposure variable(s), outcome variable(s) and confounding variable(s). Please ensure that for studies involving biological samples (including DNA) that you specify the material requested and the volume required in your outline.

As these details will become part of the data transfer agreement, please be as specific as possible about any phenotype data required. It is not necessary at this stage to list ALSPAC variable codes, but we do require a list of the data required complete with information on measures, measurement occasions, data source (questionnaire/clinic) and the type of measure (child-response/child-based/mother/partner/school/medical records).

Example of what we need:

| Concept | Specific measure | Person | Source | Time point(s) |
|---|---|---|---|---|
| Depression | EPDS | Mother | Questionnaire | Birth to 5 years |
| Concept | Specific measure | Partner | Questionnaire | Birth to 5 years |

**ALSPAC data required:**

| Concept | Specific measure | Person | Source | Time point(s) | Variables not available *FOR ALSPAC USE ONLY* | Date available *FOR ALSPAC USE ONLY* |
|---|---|---|---|---|---|---|
| Cannabis use | CCR-CCT, TF1-TF4 | Child | Questionnaire/ Clinic | Age 14 to present | | |
| IQ | F08, TF3 | Child | Clinic | Age 8 & 16 | | |
| Cognitive tasks | F08, F10-F11, TF2-TF4 | Child | Clinic | Age 8 to present | | |
| Psychosis | CCK, CCN, CCR, CCS, TF1, TF4 | Child | Questionnaire/ Clinic | Age 11 to present | | |
| Conduct Disorder | KQ-KW, TA-TC | Child-based | Questionnaire/ Clinic | Age 7 to present | | |
| Temperament and behaviour | SABC, SEFG, KL, KN | Child-based | Questionnaire | Age 4 to 11 | | |
| Demographics | A-XA, PA-PXA | Parent | Questionnaire | Age 0 to present | | |
| Parental IQ | TF3A | Parent | Clinic | Age 15/16 | | |
| Academic performance | SABC, SEFG | Child-based | Questionnaire | School years 3 & 6 | | |
| Personality | TF2 (IPIP) | Child | Clinic (Computer) | Age 13 | | |
| Depression | F10-F11, KR-KW, TA-TC, CCR-CCT, TF1-4 | Child/ Child-based | Questionnaire/Clinic | Age 7 to present | | |
| Parental drug use | B-P, PA-PM | Parent | Questionnaire | Prenatal | | |

**Appendix 1a: Project outline (please include the following sections – aims, hypotheses, exposure variable(s), outcome variable(s) and confounding variable(s):**

Aims

The aims of this project are to assess the long-term effects of adolescent cannabis use on cognitive functioning. Previous research has suggested heavy cannabis use in the teenage years may lead to persistent neuropsychological deficits; however much of the research to date has been cross-sectional and therefore unable to assess whether pre-exposure group differences are driving the association. A recent longitudinal study found chronic heavy cannabis use was associated with a decline in IQ in those with adolescent-onset cannabis use, but not in those with adult-onset use (Meier et al, 2012). However this study had a number of important limitations, including small group sizes and failure to adequately account for a number of potential confounding variables. The present study would address these issues with a new and larger sample, and by assessing other factors that may contribute to the relationship.

Research Questions

1. Is heavy cannabis use in adolescence associated with a decline in IQ?
2. Does the neuropsychological functioning of different cannabis-exposure groups differ in childhood, i.e. before onset of drug use? Do the exposure groups differ in other ways (e.g. early environment, socioeconomic status) that might influence neuropsychological functioning?
3. Does adolescent cannabis use affect cognitive function once pre-exposure functioning and other potential confounders (e.g. mental health issues or socioeconomic status) have been accounted for? If so what is the size of this effect, and does this also reflected in academic acheivement?
4. Are any deficits seen globally across all neuropsychological functioning, or are they specific to certain domains (e.g. attention or decision making)?

Exposure variables

1. Cannabis use. This measure will be defined according to lifetime reported usage between ages 13-18 years.
2. Cannabis dependence. This measure will be defined according to criteria for cannabis dependence between ages 16-18 years.

Outcome variables

1. IQ change from childhood to adolescence. This outcome will be defined based on performance on IQ assessments completed at ages 8 and 16.
2. Neuropsychological functioning. This outcome will be defined by performance on cognitive tasks completed between ages 16-18 years.
3. Academic acheivement. This outcome will be defined by performance on academic assessments completed at school year 11 (Key Stage 4).

Confounding variables

1. Pre-exposure neuropsychological functioning. Pre-existing differences or deficits in cognitive and academic ability will be related to post-exposure functioning.
2. Pre-exposure academic performance. Childhood problems at school may influence cognitive development and may also be related to initiation of cannabis use
3. Childhood temperament and behaviour. Childhood traits may influence cognitive development and may also be related to initiation of cannabis use
4. Mental health issues (including depression, psychosis and conduct disorder) will likely influence neuropsychological functioning and task performance
5. Sex, socioeconomic status (e.g. parental years of education) and early environment/ parental factors (e.g. parental IQ) will likely influence neuropsychological functioning
6. Other substance use and dependence (including alcohol and tobacco) may impact on neuropsychological functioning

7. Recent cannabis exposure at testing will likely affect performance on neuropsychological assessment due to acute (use in the past 24 hours) and residual (use in the past month) effects of the drug

Reference

Meier, M.H., Caspi, A., Ambler, A., Harrington, H., Houts, R., Keefe, R. S. E., McDonald, K., Ward, A., Poulton, R., & Moffitt, T. E. (2012) Persistent cannabis users show neuropsychological decline from childhood to midlife. PNAS, 109, 1-8.

**Appendix 1b: Genotypes or other sample analysis:**

## Appendix 2: ALSPAC study approval, study 1.

**From:** R Doerner [mailto:R.Doerner@bristol.ac.uk]

**Sent:** 23 July 2013 15:41

**To:** Roiser, Jonathan

**Subject:** ALSPAC Project B2031

Dear Jonathan,

The Executive Committee met yesterday and are pleased to approve your proposal. However, they have asked me to double-check with you that you correctly ticked the 'new data collection' box on the proposal form.

The reference number is B2031 (please quote this on all correspondence). Please note that due to the Wellcome Trust's open access policy, you will be responsible for making any publications open access. For further clarification, please visit the below link:-

<http://www.wellcome.ac.uk/About-us/Policy/Spotlight-issues/Open-access/index.htm>

I have copied in Kate Northstone who will be in touch to assign a data buddy to help with the data. This proposal will incur a Data Buddy Fee, which is a set amount of £896.00. Please could you provide me with a name and address to send the invoice to?

Please also note that I will be monitoring the proposals process and I would therefore appreciate any updates regarding the project.

The approved project will be listed on ALSPAC's website.

Best wishes

Barb.

## Appendix 3: Ethical approval, studies 2, 3 and 5.

UCL RESEARCH ETHICS COMMITTEE
ACADEMIC SERVICES

Professor Valerie Curran
Research Department of Clinical, Educational and Health Psychology
UCL

7 July 2014

Dear Professor Curran

**Notification of Ethical Approval**
**Project ID: 5929/001: How does THC affect adolescent and adult cannabis users?**

I am pleased to confirm that your study has been approved by the UCL Research Ethics Committee for the duration of the project i.e. **until July 2015.**

Approval is subject to the following conditions:

1. You must seek Chair's approval for proposed amendments to the research for which this approval has been given. Ethical approval is specific to this project and must not be treated as applicable to research of a similar nature. Each research project is reviewed separately and if there are significant changes to the research protocol you should seek confirmation of continued ethical approval by completing the 'Amendment Approval Request Form'.

The form identified above can be accessed by logging on to the ethics website at: http://ethics.grad.ucl.ac.uk/ and clicking on the button marked 'Responsibilities Following Approval'.

2. It is your responsibility to report to the Committee any unanticipated problems or adverse events involving risks to participants or others. Both non-serious and serious adverse events must be reported.

   **Reporting Non-Serious Adverse Events**
   For non-serious adverse events you will need to inform Helen Dougal, Ethics Committee Administrator (ethics@ucl.ac.uk), within ten days of an adverse incident occurring and provide a full written report that should include any amendments to the participant information sheet and study protocol. The Chair or Vice-Chair of the Ethics Committee will confirm that the incident is non-serious and report to the Committee at the next meeting. The final view of the Committee will be communicated to you.

   **Reporting Serious Adverse Events**
   The Ethics Committee should be notified of all serious adverse events via the Ethics Committee Administrator immediately the incident occurs. Where the adverse incident is unexpected and serious, the Chair or Vice-Chair will decide whether the study should be terminated pending the opinion of an independent expert. The adverse event will be considered at the next Committee meeting and a decision will be made on the need to change the information leaflet and/or study protocol.

On completion of the research you must submit a brief report (a maximum of two sides of A4) of your findings/concluding comments to the Committee, which includes in particular issues relating to the ethical implications of the research.

With best wishes for the research.

Yours sincerely

[redacted]

**Professor John Foreman**
**Chair of the UCL Research Ethics Committee**

Cc:
Claire Mokrysz, Applicant
Professor Peter Fonagy, Head of Department

Academic Service, 2 Taviton Street,
University College London Gower Street London WC1E 6BT
Tel: +44 (0)20 3108 4312
Email: ethics@ucl.ac.uk
http://ethics.grad.ucl.ac.uk/

## Appendix 4: Ethical amendment approval, studies 2, 3 and 5.

UCL RESEARCH ETHICS COMMITTEE

UCL

### Amendment Approval Request Form

| | | |
|---|---|---|
| 1 | **Project ID Number:** 5929/001 | **Name and Address of Principal Investigator:**<br>Prof. H Valerie Curran, Department of Clinical, Educational and Health Psychology, Gower Street, London, WC1E 6BT |
| 2 | **Project Title:** How does Δ9-tetrahydrocannabinol (THC) affect adolescent and adult cannabis users? | |
| 3 | **Type of Amendment/s (tick as appropriate)**<br>☒ Research procedure/protocol (including research instruments)<br>☐ Participant group<br>☐ Sponsorship/collaborators<br>☐ Extension to approval needed (extensions are given for one year)<br>☒ Information Sheet/s<br>☐ Consent form/s<br>☐ Other recruitment documents<br>☐ Principal researcher/medical supervisor*<br>☐ Other *<br>*Additions to the research team other than the principal researcher, student supervisor and medical supervisor do not need to be submitted as amendments but a complete list should be available upon request. | |
| 4 | **Justification** (give the reasons why the amendment/s are needed)<br>For consistency with recent acute cannabis work in our research group, and to build directly onto those findings, we now wish to administer the stipulated dose of THC via natural, medical cannabis instead of as synthetic Δ9-tetrahydrocannabinol (THC). Cannabis plant material contains at least 100 unique ingredient, so administration of cannabis rather than synthetic THC will provide a more ecologically valid picture of the acute effects of cannabis. However since THC is the main psychoactive component of cannabis we expect drug effects to be broadly similar. | |
| 5 | **Details of Amendments** (provide full details of each amendment requested, state where the changes have been made and attach all amended and new documentation)<br>We will now administer medical cannabis rather than synthetic THC. To control for body weight differences between adults and adolescents we will also adjust dose according to individual body weight. We will therefore administer cannabis material containing 0.07mg/kg THC equivalent. This dose is equivalent to that used in previous studies by our group. The medical cannabis will be supplied by the government approved producer in the Netherlands (Bedrocan). The same product from this same supplier has been used in our recent fMRI study of cannnabis (UCL Ethics ID number: 3325/002). The information sheet has been amended to reflect this change (please see new version attached). Drug administration will remain exactly as stated previously- inhalation of vaporised cannabis plant material. | |
| 6 | **Ethical Considerations** (insert details of any ethical issues raised by the proposed amendment/s)<br>The ethical considerations related to THC administration in medicinal plant cannabis are the same as previously covered for synthetic THC administration and the doses are the same. This dosage is similar or lower than is typically taken recreationally in street cannabis. | |
| 7 | **Other Information** (provide any other information which you believe should be taken into account during ethical review of the proposed changes) | |

**Declaration** (to be signed by the Principal Researcher)

- I confirm that the information in this form is accurate to the best of my knowledge and I take full responsibility for it.
- I consider that it would be reasonable for the proposed amendments to be implemented.
- For student projects I confirm that my supervisor has approved my proposed modifications.

Signature: [redacted]

Date: 09/01/15

FOR OFFICE USE ONLY:

Amendments to the proposed protocol have been approved by the Research Ethics Committee.

Signature of the REC Chair, Professor John Foreman: [redacted]

Date: 24/1/2015

## Appendix 5: Information sheet, studies 2, 3 and 5.

**VOLUNTEER INFORMATION SHEET**
Version 5 05/01/15

# How does Δ9-tetrahydrocannabinol (THC) affect adolescent and adult cannabis users?

**What is this study?**

You are being invited to participate in a research study. This study aims to increase our understanding of how cannabis affects the brain, psychological wellbeing and memory.

This study is being conducted by researchers from the Clinical Psychopharmacology Unit at University College London. Before we describe the study and its purpose we would like to make it clear that this is a completely voluntary study and that you will be free to pull out at any time.

**Why are we doing this study?**

Cannabis contains about 80 chemicals which are unique to the plant and are called 'cannabinoids'. One of these cannabinoids is Δ9-tetrahydrocannabinol (THC), the part of cannabis that is associated with the feeling of being 'high'. Some research suggests that adolescent users may be more at risk of aversive effects of THC than adult users, so we want to see how cannabis affects cannabis users of different ages.

The present study is a controlled laboratory study of cannabis. It aims to investigate the acute effects of cannabis on participants' memory, psychological well-being and subjective experiences.

**Who can participate in this study?**

We are inviting people aged 16-26 who have regularly used cannabis voluntarily without adverse consequences. You must be able to inhale a substance as the treatment is administered via inhalation from a balloon-like device. All volunteers should be healthy and not receiving treatment for any mental health problem. Volunteers must also have good spoken English and basic literacy skills, as well as good vision, no colour blindness and no history of psychosis either personally or in their immediate family (i.e. mother, father, siblings). If you are pregnant or are at risk of becoming pregnant you will not be able to take part.

**What is involved?**

The study will involve two separate testing days, which will each be at least 7 days apart. All testing sessions will be arranged outside of school/ college/ work commitments. All volunteers must agree to not use any recreational drug (including cannabis) or alcohol for 24 hours prior to each test day, and this will be confirmed with a urine drug screen at the start of each session. You will be sent a text message to remind you of this.

On each test day you will be asked to fill out questionnaires about your mood and mental state. You will then be asked to inhale one of 2 treatments via a balloon-like device. The 2 treatments are cannabis and placebo. The placebo is an inactive substance, with no drug effects. You will only receive one treatment on each of the 2 days. You will then be asked to fill out some further questionnaires about your mood and mental state and do some computer tasks.

Blood pressure will be monitored during each session, as THC can increase heart rate and blood pressure. Saliva samples will be taken twice during each session, to assess levels of the stress hormone cortisol and cannabinoids in your saliva. The samples will be sent to testing laboratories for processing promptly after collection. Urine samples are taken only for drug screening purposes, and will be disposed of immediately.

Each session will last for about two hours. Most people find the tests quite straightforward and fun to do. Neither you nor the researcher will know on which day you will receive each treatment.

If you agree to take part you will also be asked whether you are happy to be contacted about participation in future related studies. Your participation in the present study will not be affected should you choose to be re-contacted or not.

**What are the risks of taking part in this study?**

As participants are all experienced cannabis users, no risks are envisaged from the administration of THC, as they will be in similar or lower quantities than those commonly found in street cannabis. You will be familiar with its effects, which usually include ‘stoned’ feelings, anxiety, psychosis like effects, increased appetite, drowsiness, euphoria, and increased heart rate.

A doctor will be available for medical cover on the test days.

**What are the benefits to me?**

You will leave with the knowledge that you have contributed to our understanding of the effects of cannabis and further progress in medical and psychological research. In addition, you will be given a one page summary of results when the study has finished and an information leaflet containing advice for stopping cannabis use.

**Will I receive compensation for giving my time?**

You will be given a small payment of £7.50 per hour to compensate you for your time. You will also be reimbursed for travel on both test days (a standard day travel card; please provide your receipts to the researcher).

**How will my data be kept?**

Your data from this study will be stored electronically using a numbered code so that you cannot be personally identified. Only researchers directly involved in the study have access to the data. All data will be collected and stored in accordance with the Data Protection Act (1998).

**Who can I contact for further information?**

If you have any further questions please contact:

Ms Claire Mokrysz 020 7679 1231 c.mokrysz.12@ucl.ac.uk

Prof Val Curran 020 7679 1898 v.curran@ucl.ac.uk

It is up to you to decide whether to take part or not; choosing not to take part will not disadvantage you in any way. If you do decide to take part you are still free to withdraw at any time and without giving a reason.

All research projects are reviewed by an ethics committee. This proposal was reviewed and approved by the UCL Research Ethics Committee. All data will be collected and stored in accordance with the Data Protection Act 1998.

Please discuss the information above with others if you wish or ask us if there is anything that is not clear or if you would like more information.

## Appendix 6: Ethical approval, study 4.

UCL RESEARCH ETHICS COMMITTEE
GRADUATE SCHOOL OFFICE

Professor Valerie Curran
Research Department of Clinical, Educational and Health Psychology
UCL

12 November 2013

Dear Professor Curran

Notification of Ethical Approval
Project ID: 3325/002: How do different types of cannabis affect users' memory, well-being and experiences of the drug?

Further to receipt of the confirmation from the MHRA that your study is not a CTIMP, I am pleased to confirm that your project has been granted ethical approval by the UCL Research Ethics Committee for the duration of the study i.e. **until September 2016.**

Approval is subject to the following conditions:

1. You must seek Chair's approval for proposed amendments to the study for which this approval has been given. Ethical approval is specific to this project and must not be treated as applicable to research of a similar nature. Each research project is reviewed separately and if there are significant changes to the research protocol you should seek confirmation of continued ethical approval by completing the 'Amendment Approval Request Form'.

The form identified above can be accessed by logging on to the ethics website homepage: http://www.grad.ucl.ac.uk/ethics/ and clicking on the button marked 'Key Responsibilities of the Researcher Following Approval'.

2. It is your responsibility to report to the Committee any unanticipated problems or adverse events involving risks to participants or others. Both non-serious and serious adverse events must be reported.

   Reporting Non-Serious Adverse Events
   For non-serious adverse events you will need to inform Helen Dougal, Ethics Committee Administrator (ethics@ucl.ac.uk), within ten days of an adverse incident occurring and provide a full written report that should include any amendments to the participant information sheet and study protocol. The Chair or Vice-Chair of the Ethics Committee will confirm that the incident is non-serious and report to the Committee at the next meeting. The final view of the Committee will be communicated to you.

   Reporting Serious Adverse Events
   The Ethics Committee should be notified of all serious adverse events via the Ethics Committee Administrator immediately the incident occurs. Where the adverse incident is unexpected and serious, the Chair or Vice-Chair will decide whether the study should be terminated pending the opinion of an independent expert. The adverse event will be considered at the next Committee meeting and a decision will be made on the need to change the information leaflet and/or study protocol.

## Appendix 7: Information sheet, study 4.

*Appendix 11: Information sheet (study 1 reported in chapter 5)*

CLINICAL PSYCHOPHARMACOLOGY UNIT
CLINICAL PSYCHOLOGY

UCL

**VOLUNTEER INFORMATION SHEET**
Version 4: 01/05/14

**How do different types of cannabis affect users' memory, well-being and experiences of the drug?**

**What is this study?**

You are being invited to participate in a research study. This study aims to increase our understanding of why some types of cannabis may have different effects on the brain, psychological wellbeing and memory.

This study is being conducted by researchers from the Clinical Psychopharmacology Unit at University College London. Before we describe the study and its purpose we would like to make it clear that this is a completely voluntary study and that you will be free to pull out at any time.

**Why are we doing this study?**

Cannabis contains about 80 chemicals which are unique to the plant and are called 'cannabinoids'. The two most abundant cannabinoids in cannabis are Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD). High levels of THC are found in strains of cannabis such as 'skunk', which is now the most common type of cannabis available in the UK. Our recent research has found that different types of cannabis may have different effects on people's experience of the drug.

The present study is a controlled laboratory study of the above-mentioned cannabinoids. It aims to investigate how different types of cannabis affect users' brain function, memory, well-being and experiences of the drug.

**Who can participate in this study?**

We are inviting people aged 18-70 who have used cannabis voluntarily at least twice before without adverse consequences. You must be able to inhale a substance as that is how treatment is being administered. All volunteers should be healthy and not receiving treatment for any mental health problem. Volunteers must also have good spoken English and basic literacy skills, as well as good vision, no colour blindness and no history of psychosis either personally or in their immediate family (i.e. mother, father, siblings). If you are pregnant or are at risk of becoming pregnant you will not be able to take part. If you are afraid of small closed spaces or loud noises you may not be suitable for this study.

**What is involved?**

The study will involve four sessions. The first of these will be a baseline session (45-60 minutes) in which you will practice some of the tasks and fill out some questionnaires. Next, you will be asked to come for three separate testing days, at least 7 days apart. All volunteers must agree to not use any recreational drug (including cannabis) or alcohol for 24 hours prior to each test day, which will be tested with a urine sample. You will be sent a text message to remind you of this. Females will also be tested for pregnancy from a urine sample on each test day. If your test results suggest that you have used recreational drugs in the last 24 hours, or that you are might be pregnant, you will not be permitted to take part.

On each test day you will be asked to fill out questionnaires about your mood and mental state. You will then be asked to inhale one of 3 treatments via a balloon-like device. The 3 treatments are medical cannabis containing THC, medical cannabis containing THC+CBD, and placebo (cannabis with the active ingredients removed). You will receive two treatments on each of the 3 days. First you will be given a 133.3mg dose of cannabis/placebo, and 90 minutes later you

will be given a second dose of 66.7mg. You will be asked to fill out some further questionnaires about your mood and mental state and do some computer tasks. Some of the tasks will be given to you while you are in an fMRI scanner. On each test day we will collect blood pressure, heart rate, and samples of saliva and urine. These samples will be labelled anonymously and stored securely at -80°C. They will be sent for analysis as soon as possible. After analysis (for levels THC and CBD) all samples will be destroyed.

Each test day will last for about four hours. Most people find the tests quite straightforward and fun to do. Neither you nor the researcher will know on which day you will receive each treatment. You should not drive or operate machinery after each of the test days.

If you agree to take part you will also be asked whether you are happy to be contacted about participation in future related studies. Your participation in the present study will not be affected should you choose to be re-contacted or not.

**What are the risks of taking part in this study?**

As participants are all experienced cannabis users, no risks are envisaged from the administration of either type of medical cannabis because the THC and/or CBD content will be in similar or lower quantities than those commonly found in street cannabis. You will be familiar with its effects, which include 'stoned' feelings, anxiety, psychosis like effects, increased appetite, drowsiness, euphoria, and increased heart rate.

There are no known effects from exposure to magnetic fields (MRI). However, because MRI involves being placed in a strong magnetic field, there are times when it is not safe to be scanned. For example, in the first three months of pregnancy, or when there are surgical clips inside the brain, or if you have a heart pacemaker fitted. We have a safety questionnaire that you will fill in on the interview / screening day for the study, so that we can be sure that it is completely safe for you to be scanned. In case you have any questions, we will be happy to discuss this with you. We will also check that you are safe to be scanned on the day you come for the scan.

Some individuals undergoing fMRI become anxious being in a confined space, and some people do not like the sound of the scanner when it is in operation. If these reactions happen to you at any time during the procedure, the experiment will be stopped as soon as you tell us that you are uncomfortable.

We will be taking pictures of your brain, and occasionally we will have unexpected findings that none of us suspected. The pictures are reviewed by experienced doctors, called neuroradiologists who specialise in looking at pictures of brain and spine. If there are any unexpected findings that need further tests, he/she will write to your GP in the first instance. The GP will then contact you if further tests are required. This is why your GP details are required in the safety check form.

A doctor will be available for medical cover on the test days.

**What are the benefits to me?**

You will leave with the knowledge that you have contributed to our understanding of the effects of cannabis and further progress in medical and psychological research. In addition, you will be given a one page summary of results when the study has finished and an information leaflet containing advice for stopping cannabis use.

**Will I receive compensation for giving my time?**

You will be given a small honorarium of £10 per hour (maximum £130) to compensate you for your time. You will also be given the opportunity to win additional money (maximum £4 extra per testing session).

**How will my data be kept?**

Your data from this study will be stored electronically using a numbered code so that you cannot be personally identified. Only researchers directly involved in the study have access to the data. All data will be collected and stored in accordance with the Data Protection Act (1998).

**Who can I contact for further information?**

If you have any further questions please contact:

Dr Rebecca Pope 020 7679 5938 r.pope@ucl.ac.uk

Prof Val Curran 020 7679 1898 v.curran@ucl.ac.uk

It is up to you to decide whether to take part or not; choosing not to take part will not disadvantage you in any way. If you do decide to take part you are still free to withdraw at any time and without giving a reason.

All research projects are reviewed by an ethics committee. This proposal was reviewed and approved by the UCL Research Ethics Committee.

Please discuss the information above with others if you wish or ask us if there is anything that is not clear or if you would like more information.