SCHIZOPHRENIA-LIKE COGNITIVE, TRAIT AND DNA MARKERS IN REGULAR CANNABIS USERS

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Rationale: Converging evidence suggests that cannabis use can induce psychosis and is a distinct risk factor for schizophrenia. Taken together with the effects of Tetrahydrocannabinol (THC) on neural systems, dopamine and endocannabinoids it is likely that cannabis use may also produce sub- clinical psychosis-linked changes in a much larger number of regular recreational users; observable in schizophrenia-sensitive assessments. Use of the drug by individuals with genetic risk factors for schizophrenia appears to magnify the chances of pathology, and so changes in recreational users with one or more of these genetic markers may be more evident or pronounced.

Method: 50 cannabis users and 50 non cannabis users were assessed in each of two studies. Study one assessed selective attention in the Latent Inhibition (LI) and Kamin Blocking (KB) paradigms and examined schizophrenia-linked traits using the short form of the Schizotypal Personality Questionnaire (the SPQ-B; Raine and Benishay, 1995). Study two assessed executive control (using an Anti-Saccade Test), decision-making (using the Iowa Gambling Task), and selective/sustained attention and inhibitory control (Continuous Performance Test). Study two included additional personality measures to explore paranoia, emotional processing, ambivalence and impulsivity. Across both studies, the relative contribution of seven genetic risk markers in five candidate genes for schizophrenia (DAOA, COMT, NRG1, FAAH and CNR1) were assessed.

Key Results: Cannabis use was associated with abolished latent inhibition and significantly riskier decision making, especially in those who used the drug more frequently. Cannabis users reported significantly higher scores for psychosis-linked personality traits and there was a dose-response effect with heavier users experiencing more of these schizotypal traits. Some key trends existed in the genotyping data for the cannabis group. The psychosis-risk C allele in the NRG1 gene was linked to higher SPQ-B scores and more errors on the AST; and was also associated with longer use of cannabis. Cannabis users without the protective three-way T-G-G haplotype COMT gene had higher scores for the SPQ-B disorganised thinking subscale than users with the protective haplotype.

Discussion: The data in this thesis suggests that cannabis users are showing differences in brain inhibitory function and decision-making akin to previous research with schizophrenic patients, their first degree relatives and high schizotypy scorers. Exposure to THC may contribute to changes in individuals by pushing them further along a schizophrenia-spectrum resulting in the display of more psychotic-like traits and cognitive dysfunction at sub-clinical levels. These preliminary findings need expansion and replication, particularly with regards to the COMT three-way haplotype.

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ANOVA	Analysis of Variance	**EMCDDA**	European Monitoring Centre for Drugs and Drug Addiction	**Ns**	Not statistically significant
AST	Anti-Saccade Task	**FAAH**	Fatty Acid Amide Hydrolase	**NS**	Non-sense
BCS	British Crime Survey	**FEF**	Frontal Eye-Field	**OCD**	Obsessive Compulsive Disorder
BIS	Barratts Impulsivity Scale	**FEP**	First Episode of a Psychosis	**OFC**	Orbito Prefrontal Cortex
BL	Blocking	**fMRI**	Functional Magnetic Reasonance Imaging	**PANSS**	Positive and Negative Symptoms in Schizophrenia
BPRS	Brief Psychiatric Rating Scale	**GABA**	Gamma-Aminobutyric Acid	**PCR**	Polymerase Chain Reaction
CAPE	Community Assessment of Psychic Experiences	**GHB**	Gamma-Hydroxybutyric Acid	**PE**	Pre-exposed
CBD	Cannabidiol	**GPTS**	Green et al Paranoid Thoughts Scale	**PV**	Predictor Variables
CIS-R	Clinical Interview Schedule - Revised	**GWAS**	Genetic Wide Association Studies	**RS#**	SNP given an (rs) identified # by dbSNP (which represents the largest public database of SNPs).
CNR1	Cannabinoid Receptor 1	**HWE**	Hardy Weinberger Equilibrium	**SAS**	Schizotypal Ambivalence Scale
CNS	Central Nervous System	**IGT**	Iowa Gambling Task	**SCRs**	Skin Conductance Responses
COMT	The Catechol-O-methyl transferase	**JPW**	Joints per Week	**SDS**	Severity of Dependency Scale
CPT	Continuous Performance Test	**KB**	Kamin Blocking	**SMH**	Somatic Marker Hypothesis
CS	Conditioned Stimulus	**LI**	Latent inhibition	**SNP**	Single Nucleotide Polymorphism
CV	Criterion Variable	**LSD**	Lysergic Acid Diethylamide	**SPQ-B**	Schizotypal Personality Questionnaire - brief
DA	Dopamine	**MANOVA**	Multiple Analysis of Variance	**SPQ-CP**	Schizotypal Personality Questionnaire-Cognitive Perceptual
DAOA	D-amino Acid Oxidase Activator	**MDMA**	Methylenedioxy-Methamphetamine	**SPQ-DT**	Schizotypal Personality Questionnaire-Disorganised thinking
DNA	Deoxy Ribonucleic Acid	**Mg**	Milligrams	**SPQ-IP**	Schizotypal Personality Questionnaire-Interpersonal
DSM	Diagnostic and Statistical Manual of Mental Disorders	**MRI**	Magnetic Resonance Imaging	**THC**	Delta-9-tetrahydrocannabinol
DTI	Diffusion tensor imaging	**NBL**	Non-blocking	**TMMS**	Trait-Meta Mood Scale
DTNPB1	Dysbindin 1	**NPE**	Non Pre exposed	**UCS**	Unconditioned Stimulus
EDSP	Early Developmental Stages of Psychopathology	**NRG1**	Neuregulin1	**WT**	Wild-type

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"Happiness does not come from doing easy work but from the afterglow of satisfaction that comes after the achievement of a difficult task that demanded our best". Theodore Isaac Rubin

Chapter 1: Background Literature review

1.1 Cannabis

Seventy-five million Europeans have reportedly used cannabis at least once in their lifetime, with an estimated twenty-million Europeans having used cannabis in the past year (Seshadri *et al*., 2011). Data taken from the 2008 national school survey reported that lifetime use among fifteen to sixteen year olds in the UK ranged from 26%-32% (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 2010). A recent report from the Crime Survey in England and Wales (CSEW, 2015) found a 21% rise in the number of 16-24 years reporting cannabis use in 2014-2015 compared to 2013-2014 data.

1.1.1 Basic psychopharmacology

Cannabis is derived from the plant *Cannabis sativa*, and is made up of over 400 naturally occurring compounds, with delta-9-tetrahydrocannabinol (THC) involved in creating the main psychoactive effect of the drug (Gaoni & Mechoulam, 1964). More recently another compound referred to as cannabidiol (CBD), also present in significant quantities, has been shown to have a role in moderating the impact of THC, in terms of reducing the psychoactive/psychological effects of the drug (Mechoulam *et al*, 2007; Morgan & Curran, 2008). The cannabis plant is either male or female, with THC more concentrated in the female version of the plant. Cannabis cultivated indoors, through the use of soils, light and hydroponics for shorter time frames (e.g. around four months) produces high THC levels at the expense of CBD, these are often referred to as the 'skunk' and stronger forms of cannabis (see Potter *et al*, 2008). There is emerging research on recreational use of the legal high 'Spice', which contains a mixture of highly potent synthetic cannabinoids (Vardakou *et al*, 2010).

The 'pleasurable' effect of cannabis use is named as one of the key reasons for using the drug (e.g. Chait & Perry, 1992). The influence of the drug on the mind is very subjective and it has a varied euphoriant effect (e.g. a feeling of being 'high' or stoned) depending on the dose, route of administration, expectations and other individual factors such as personality (Aston

et al, 1981). Users often report feeling relaxed, having a heightened sense of awareness, and that their senses are distorted; they experience profound thoughts and a distorted sense of time (Jaffe, 1985; Tart, 1970). It is thought that this distortion of time, with participants tending to focus on the present rather than on the past or the future, could amplify the drug experience and is linked to the rush in thinking and sensations experienced through the high (Melges *et al*., 1971). Negative effects of cannabis intoxication include making the user feel nauseous, dizzy, anxious, and the drug may induce panic attacks (Ashton, 2001) or psychotic-like symptoms (Arendt *et al*, 2005).

Smoking is the preferred method of administration, providing effective delivery to the brain. Thus the psychoactive effects start within seconds to a few minutes after smoking and the peak levels of THC are usually present after 30 minutes and can last up to several hours (Grotenhermen, 2003). Experienced cannabis users (e.g. minimum of two years) can regulate the dose required for the subjective high. Herning *et al*. (1986) found that when such users were given different quantities of THC via a cigarette without knowing the exact quantities, they adjusted their smoking behaviour to reach the same THC absorption, i.e., those with higher doses took smaller puffs from the cigarette and vice versa for the low doses. Another form of administration is oral absorption (e.g. cannabis oil or cannabis baked into a cake or other food products), but this method is less predictable for its psychoactive effect, as it is slow and can deliver inconsistent doses of THC with peak levels occurring anytime between 1-4 hours after consumption (Mason & Mc Bay, 1985).

An average joint contains about 10-20 milligrams (mg) of THC, with around 10-20% (i.e. 1-mg of THC) normally absorbed through the lungs, which then rapidly enters the blood stream and reaches the brain within minutes (for a review see Agurell *et al*, 1986 or Mayat, 1985). Elimination of cannabis compounds from the body is inconsistent; in some people it can be rapidly metabolised in days whereas in others it takes nearly one month (Huestis *et al*, 1996). It has been estimated that cannabis has a 3-5 day half-life but in some people it may persist for weeks (Agurell *et al*., 1986). Objective tests using urine or blood to assess for one of the major metabolites, 11-nor-carboxy-THC, can give positive results for days or weeks after consumption (Huestis *et al*., 1996).

In animal models, tolerance to THC can be seen even after very modest doses, but is very apparent after large doses greater than 5mg/kg. When animals are given 20 mg injections per day they become insensitive to any further treatment with THC (Hutchenson *et al*, 1998). In humans, it has been predicted that around 10% of cannabis users will become dependent on the drug (Hall & Solowij, 1997). A survey conducted in Australia of more than 10,000 participants indicated that 20% of cannabis users met DSM-IV (APA, 1994) criteria for substance dependence (Swift *et al*., 2001). However, there is a contentious issue as to whether people become dependent or addicted to cannabis. Animal research has shown rats become physically dependent on THC, with withdrawal symptoms such as convulsive shaking, compulsive grooming, spasms, head shakes, arched back (De Fonseca *et al*, 1997). In humans, when cannabis is suddenly stopped withdrawal is not typically seen and this may be due to THC's long half-life. However, controlled studies using a CB_1 antagonist upon cessation of cannabis has shown withdrawal symptoms, such as weight loss, poor sleep, decreased appetite, anger, aggression and increased irritability (Budney *et al*., 2001).

1.1.2 Neuropharmacology of cannabis

THC is currently viewed as acting as a partial agonist at two endocannabinoid receptors; CB_1 (Devane *et al*, 1988) and CB_2 (Munro *et al*, 1993). CB_1 receptors are distributed across numerous brain loci, including the cerebral cortex, limbic areas (including the hippocampus and amygdala), the basal ganglia, the cerebellum, the thalamus and to a lesser degree in the brainstem (Herkenham, 1990; 1995). CB_1 receptor regions are highly implicated in producing the psychological effects associated with smoking cannabis. Huestis *et al*. (2001) looked at the effects of taking rimonabant (a CB_1 antagonist) or placebo in those who smoked a THC cigarette or a placebo THC cigarette in 63 cannabis users. Rimonabant blocked the acute psychological effects of THC in the cannabis cigarette. However, research in this area of inhibition of neurotransmitter release is only emerging and has not been clearly established as to how action of cannabis on CB_1 receptors leads to the psychoactive effects of THC. What is clear is that cannabinoids have generally been implicated in the inhibition of neurotransmitter release, and have been involved in reducing the release of the amino acid y-aminobutyric acid (GABA) and the amines noradrenaline and acetylcholine (Szabo & Schlicker, 2005). Reducing the release of GABA inhibits other neurotransmitters and it

could be implicated in two important effects of cannabinoids: one would be that the administration of THC leads to a release of dopamine, and the second effect would be that they increase endorphin levels (Iverson, 2003; Pertwee, 2005).

1.2 Cannabis and Cognitive research

There is growing evidence for cognitive dysfunction associated with long term and heavy use of cannabis which runs parallel with the endophenotypes of schizophrenia. These are seen to represent a risk for vulnerability to developing schizophrenia or psychotic disorders (Solowij & Michie, 2007); to which we turn in more detail in the next section. Problems with learning, memory, decision-making and attention are common in people that misuse cannabis (Millsap *et al*., 1994; Tapert *et al*., 2002; Fried *et al*., 2002; Whitlow *et al*, 2003; 2004). These deficits may persist for weeks after abstinence from the drug (e.g. Schwartz *et al*., 1989). It is argued that regular use of cannabis is linked to cognitive brain dysfunction, specifically in areas of the brain which are rich in CB_1 receptors (Solowij & Michie, 2007), due to the links between CB_1 receptors areas and higher level cognitive processing (Miller *et al*, 2002).

1.2.1 Effects on decision-making

Decision making deficits have been observed amongst groups of cannabis users and are seen as one of the key reasons why people continue to consume the drug even when there is potential for negative physical, psychological, social and legal consequences (Whitlow *et al*, 2003; 2004). Decision making can be defined as the ability to select the most adaptable behaviour which has the best outcome when faced with a range of alternative outcomes (see Bechara *et al*., 2001). Volkow & Fowler (2000) conducted a study scanning the brains of cannabis users whilst they also performed a decision making task. The researchers reported that the right orbital frontal cortex (OFC) is less efficient in regular cannabis users; a brain area linked to decision-making. Similarly, Bolla *et al* (2005) also reported that cannabis users had greater activation in the cerebellum, and less activation in the right lateral OFC, and that there is a dose-response effect, with heavy use of cannabis linked to less efficient OFC activation in decision-making. Whitlow *et al* (2003) found that short term withdrawal from cannabis was associated with poorer outcomes on the Iowa Gambling decision making task

(IGT). Bolla *et al* (1995) found deficits on the IGT in a group of cannabis users even after a 28-day abstinence period. Verdejo-Gracia *et al* (2007) found decision making deficits in cannabis users after a 25-day abstinence period and a dose response effect, with heavier use being linked to poorer outcomes on the IGT. However, some researchers have not found these effects in cannabis users who abstained for seven days (Quednow *et al*, 2007).

It is worth noting here that OFC regions are also associated with decision making dysfunction in schizophrenia (Convit *et al*., 2001; Goldstein *et al*., 1999; Pantelis *et al*., 2003) and people diagnosed with schizophrenia perform badly on neurocognitive decision making tasks (see review: Moburg *et al*, 1999). The OFC seems to be very important for complex decision making processes as well as emotional and social decision making in people with acquired damage to that brain region (Rolls, 1999). Damasio (1996) postulated that damage to the OFC causes poorer decision making as a result of the inability to 'somatically mark' an internal representation of a given situation with either a positive or negative valance based on their previous experiences. Poor decision-making in the cannabis group is seen as either being less responsive to bad outcomes on the decision-making task or alternatively are being driven by immediate higher rewards which leads to poorer decision making overall. Chapter 3 reviews the decision-making literature in cannabis users, particularly for outcomes on the IGT.

1.2.2 Effects on Associative learning

Human learning is underpinned by a fundamental need to form relationships between concepts, of which 'associative learning' is a term used to describe this process (Gallistel, 1990). The working memory system plays a key role along with the executive system in providing the ability to learn new skills to form associations (Tanji & Hoshi, 2001). Some research has revealed associative learning deficits in cannabis users. Jager *et al* (2006) tested 20 frequent cannabis users versus 20 non-users, using an associative memory task and functional magnetic resonance imaging (fMRI) techniques. It was found that cannabis users displayed lower activation in brain regions involved with associative learning, particularly the (para) hippocampal region implicated in memory functioning and the dorsolateral pre frontal

cortex (associated with higher level cognitive functions), despite no differences found between the groups on associative learning in overall task performance.

Skosnik *et al* (2008) examined the effect of chronic cannabis use on classical eyeblink conditioning (EBC), which is an associative learning task. They tested 14 cannabis users and 10 non users, on EBC where there is a paired association between a conditioned stimulus (CS, e.g 400ms tone) and a corneal airpuff, the unconditioned stimulus (US), which is a puff of air to the eye at 50ms. Both of these stimuli paired together results in a conditioned response of an eyeblink. Cannabis users exhibited fewer and more poorly timed conditioned responses (CR) compared to the non-cannabis group. Interestingly, no differences were found in the unconditioned response between the groups (i.e. learning the paired associated without any previous conditioning of the white noise). Further to this, no differences were found in EEG responding to the conditioned stimulus. The authors therefore argued that the findings in the cannabis group represented specific problems relating to conditioned response acquisition.

In a later study, Skosnik *et al* (2012) assessed 10 cannabis users, 10 ex users, and 10 non-cannabis users on EBC and found that cannabis users exhibited more errors in the acquisition and timing of the conditioned responses compared to non-users. The ex-users had intact conditioned response acquisition but were impaired in having shorter conditioned response latencies (e.g. timing of the CR during each block of trials). The authors argued that cannabis is seen to disrupt acquisition of conditioned responses and this may improve upon complete cessation of the drug, although ex-users still demonstrated difficulty in correctly timing their response on the EBC task. CB_1 receptors have their highest density in the cerebellum and a previous animal study indicated that CB_1 knockout mice also highlight severe impairment using a version of the EBC task (Kishimoto & Kano, 2006). Therefore it could be argued that cannabis use may be a risk factor for functional deficits in the cerebellum which controls for some aspects of associative learning (Skosnik *et al*, 2008; 2012).

The above findings run parallel to research in people diagnosed with schizophrenia who cannot filter out irrelevant information, as demonstrated by the selective attention tasks,

namely Latent Inhibition (Lubow, Weiner & Feldon, 1982; Serra *et al*, 2001) and Kamin Blocking (Serra *et al*, 2001; Jones *et al*, 2002); these tasks will be further explored in Chapter 2. The inability to filter out what appears to be irrelevant information, is typically detected in the earlier stages of schizophrenia, and has been linked to explaining more of the positive symptomology e.g., delusions, hallucinations and so forth (Hemsley, 1993).

1.2.3 Effects on executive control

Response inhibition is an important executive function that allows for the suppression of actions and resistance to interference from irrelevant stimuli (Bjorklund & Harnishfeger, 1995). This key executive function is also seen as controlling complex cognition and behaviours, which is essential for effective interaction with our environment (Burke *et al*., 1991). Response inhibition has also been characterised as a level of control over a voluntary response, and has been demonstrated in a range of standardised tasks. For example, the Stroop task has been used most often for assessing inhibitory function in cannabis users, with mixed results (Pope *et al*, 1996; 2001; Solowij *et al*, 2002). However, most of these studies generally find that frequency and duration of cannabis use interact with IQ; with longer duration of use, increased joints per week and low IQ appearing all to contribute to greater impairments (Solowij & Michie, 2007). Further to this, imaging studies have shown that altered activation in the dorsolateral pre-frontal cortex (DLPFC) and anterior cingulate are seen during the interference component of the Stroop task, despite lack of differences in performance between cannabis users and non-cannabis users (Gruber & Yurgelun-Todd, 2005). Neuroimaging studies have reported that increased activity of bilateral PFC and right premotor cortex and attenuation of left cerebellar activity can be found in response selection and inhibition, as demonstrated by the Go/No-go task in people prenatally exposed to cannabis (Smith *et al*, 2004). Administration of THC to healthy controls increased impulsiveness on the Stop signal but not the Go/No-go task (Mc Donald *et al*, 2003); with THC intoxication related to premature responding and poorer control of inhibitory responses (Hart *et al*, 2001). Ploner *et al* (1998; 1999) assessed the acute effects of THC on humans and found that this substance affected spatial accuracy, volitional saccades and inhibition of inappropriate saccades for eye movement control. Executive control in cannabis users is further explored in Chapter 3.

1.2.4 Effects on attention

Cannabis users have been shown to demonstrate impaired attention, on tasks assessing either sustained (maintaining a consistent level of attention) or selective (selecting what is relevant and irrelevant) attention (Hall & Solowij, 1998). Similar effects were also found using EEG, as selective attention for selecting relevant from irrelevant information was disrupted in cannabis users and linked to duration of use. For example, the speed of processing for the P300 response (an indicator of frontal brain activity), was slower in people using cannabis and worsened in those who used cannabis for longer durations (Solowij *et al*, 2002). Age of onset of cannabis (e.g. before 16 years old) is also seen as one of the strongest predictors of attentional dysfunction in adults for visual searching (Ehrenreich *et al*., 1999). Attentional problems are also seen in light users of cannabis (e.g. once a week) as demonstrated by a negative priming study, where cannabis users showed deficits in trying to select the relevant from irrelevant material when compared to non-cannabis users (Skosnik *et al*, 2001). Other studies assessing levels of attention in cannabis users, reported a disruption in sustained attention as a result of acute cannabis (e.g. naturally smoked), particularly in regards to shifting and sustaining attention (Pope & Yurgelun-Todd, 1996). Selective attention in cannabis users is further explored in Chapter 2 and selective/sustained attention is also covered in Chapter 3.

The decision-making, attention, and executive control assessments described above are together linked by the component of behavioural and trait impulsivity (Giel *et al*, 2013; Swann *et al*, 2010, Upton *et al*, 2011). Impulsivity is defined as actions which are poorly conceived, prematurely expressed, unduly risky, or inappropriate to the situation and may results in negative consequences (Wrege *et al*, 2014). Impulsivity is linked to a broad spectrum of psychiatric disorders, including schizophrenia (Ouzir, 2013) and is a core deficit in addictive disorders and substance misuse problems (Crews & Boettinger, 2009). Impulsive behaviour is a pre-existing personality that may motivate the initiation of drug use, whereas the consumption of cannabis may result in other behavioural changes which include alterations of impulsivity (Wrege *et al*, 2014).

1.2.5 Effects on other brain areas linked to cognition

Studies indicate that brain structure may change as a result of cannabis use. For example, Arnone *et al*. (2008) assessed prolonged and heavy use in 11 participants versus 11 controls of non-cannabis users, using a method called diffusion tensor imaging (DTI) which looks at white matter tracts. The researchers reported the axonal connectivity was impaired in regions in the hippocampus and corpus callosum in the heavy cannabis users relative to the control sample. However, these findings were not replicated in people diagnosed with schizophrenia and in those who started using cannabis during adolescence (Dekker *et al*., 2010). Yucel *et al*. (2008) assessed people who reported use of more than 5 joints daily for more than 10 years, compared with a control group of non-users and found that heavy use was associated with bilateral reduced hippocampal and amygdala volumes. Deficits were pronounced in left hemisphere hippocampal volume and exposure to cannabis was associated with greater positive psychotic symptoms. This finding runs parallel to research conducted on people diagnosed with schizophrenia as having more left hemispheric hippocampal deficits (Petty, 1999). Further to this, Rais *et al*. (2008) followed up people, diagnosed with a first episode of a psychosis, for a period of over 5 years and found that those people who continued to use cannabis had more grey matter loss, compared to non-users and healthy controls. Regular use of cannabis during adolescence was also associated with gyrification abnormalities in the cortex (e.g. a process of cortical folding), which suggests that pre-exposure to cannabis in early adolescence may affect normal brain development (Mati *et al*., 2008). Neuroimaging studies looking at the acute effects of cannabis on brain function reported that resting global, prefrontal and anterior cingulate cortex blood flow seem to be lower in cannabis users relative to non-cannabis using controls (Martino-Santos *et al*., 2010). This is supported by research on acute administration of THC or cannabis cigarettes which show increased prefrontal, insular and anterior cingulate activity during rest state and also during cognitive testing. For example, Bhattacharyya *et al*. (2009) administered THC and placebo to healthy volunteers and assessed them on a verbal learning tasks performed under fMRI scanning. The researchers found that THC was associated with an increase in blood flow in the mediotemporal and anterior cingulate (key areas in the brain linked to decision-making) and in the medioprefrontal cortex (which plays a central role in memory relational binding) during the learning phase of the task, and THC also appeared to induce psychotic symptoms in the healthy volunteers.

The research presented here in section 1.2 indicates that cognitive disruption and changes in brain activity and structures linked to key cognitive processes appear to be commonplace in cannabis users, and that those that use the drug more frequently demonstrate more cognitive deficits. Importantly, in the context of this thesis, these cognitive data in cannabis users reflect similar results to those found in persons diagnosed with schizophrenia.

1.3 Schizophrenia and schizotypy

1.3.1 Schizophrenia

Schizophrenia belongs to a group of disorders that are called 'psychosis', usually representing a detachment from reality. The term schizophrenia which literally means 'splitting of psychic functions' was first described by Eugen Bleuler in 1908, to describe patients with the four As: symptoms of loosening of associations, ambivalence, autism, and affective problems. The splitting of the mind referred to the loss of unity in the person's personality (as cited in Kuhn, 2004). Schizophrenia today is seen as a heterogeneous disorder which is linked to a range of symptoms, but each individual's experience is unique. Clinicians have attempted to classify these clusters of symptoms into the DSM III (American Psychiatric Association (APA), 1987) and revised DSM-IV (APA, 2000); DSM-V (APA, 2013) as a way of diagnosing this disorder. Schizophrenia is classified into three main factors: positive symptoms (e.g. hallucinations), and negative symptoms (e.g. flat affect, blunted emotional responses) and disorganised thinking and behaviours in relation to odd speech, odd associations (Liddle, 1987) as well as cognitive disruption (Bilder et al, 1996; Heinrichs & Zakzanis, 1998).

About 1 in a 100 people are at risk of developing schizophrenia during the course of their lifetime (DeLisi, 1992), increasing to around 10 in 100 if it runs in the family (Rosenthal *et al*., 1980). The concordance rates of schizophrenia being diagnosed in identical twins is 45.5 in 100 compared to 10 in 100 in non-identical twins at (Holzman & Matthyse, 1990). That schizophrenia is not concordant to 100% in identical twins indicates that environmental causes play a major role in its aetiology, and include factors such as infections, toxins, traumatic injury, and psychosocial stress (Leask, 2004).

In the 1950s, there was the discovery of the first anti-schizophrenic drug called chlorpromazine, which alleviated symptoms in people diagnosed with schizophrenia (for a review see Ban *et al*, 2006). Carlsson and Lindqvist (1963) found differential effects of two antipsychotic drugs by antagonising levels of dopamine, chlorpromazine and reserpine, with the former binding to dopamine receptors and the latter depleting the brain of dopamine. Traditionally the formulation of the dopamine theory of schizophrenia was linked to the excessive transmission at dopamine receptors (Matthysse, 1973; Snyder, 1976). The dopamine theory was supported by research on the clinical effectiveness of anti-psychotic drugs linked to their affinity for dopamine receptors (Seeman & Lee, 1975; Creese *et al*, 1976; Seeman *et al*, 1976). The revised the dopamine theory of schizophrenia is that rather than high levels of dopamine, it was due to high levels of activity at the receptors (Davis *et al*, 1991), but modern research has moved also towards a genetic level and neurodevelopmental understanding (Howes & Kapur, 2009).

1.3.2 Schizophrenia Genes

Schizophrenia has multiple causes and on-going research is investigating the link between candidate genes and risk for developing the disorder (Plomin *et al*, 1994). Genetic wide association studies (GWAS) have been conducted to look for a genetic link in Schizophrenia. GWAS look at the density of genetic markers and the extent of linkage disequilibrium (e.g. non-random association between the alleles at different sites in the genome) in a population, to make sure it is sufficient enough to capture variation amongst these groups. Manolio *et al* (2010) carried out some GWAS and documented at least 5 candidate genes that have been implicated as potential risk markers for developing schizophrenia: NRG1 (Neuregulin 1); DISC1 (Disrupted in Schizophrenia); DTNBP1 (Dysbindin); DAAO (D-aminoacid oxidase); COMT (Cathecol-O-methyltransferase).

Chapter 4, section 4.3 reports a study conducted with the Dunedin cohort investigated whether specific genes increase the risks associated with early cannabis use (Caspi *et al*, 2005). The researchers examined the role of the catechol-*O*-methyltransferase (COMT) gene, which is responsible for the breakdown of dopamine. A functional polymorphism of this gene, Val158Met, has been shown to slow the metabolism of dopamine, which

potentially increases the risk of psychosis (Lachman *et al*., 1996; Bilder et 2004). It was found that those with Val/Val or Val/Met genotypes and adolescent cannabis use were at increased risk for schizophreniform disorder, whereas individuals with Met/Met genotypes were not. This finding therefore implicates genetic factors as important distinct risk factor to the cannabis-psychosis link. Chapter 4 provides a more detailed account of risk genes for schizophrenia, but it is clear that there are several distinct genes and gene markers which may link to schizophrenia, symptoms and/or underlying risk.

1.3.3 Schizotypy

Schizotypy is characterised by symptoms similar to schizophrenia but are seen as less severe. Some people could represent an at-risk group and do not have a diagnosis of schizophrenia, but may score highly on one or more aspects of schizotypy measures and such traits are often referred to as schizotypal traits. There is some debate over whether such 'schizotypy' as a personality trait, differs in nature from aspects of schizophrenia and so may be a distinct characteristic, which are distinct from schizotypal personality disorder (Claridge, 1997). On one hand, there is the quasi-dimensional approach that views schizotypy on a dimension but its presence is indicative of risk for future psychopathology (Eckblad & Chapman, 1983). Others view schizotypy as a personality dimension and rather than being associated with psychosis itself (e.g. meeting a diagnosis for schizotypal personality disorder or schizophrenia) it exists on a continuum across which we are all dispersed (McCreery & Claridge, 1995). The latter assumption uses a model of risk for psychosis of interacting variables such as schizotypy, stressors and social support (e.g. Claridge & Beech, 1995). Schizotypy has been conceptualised in many ways, with Rado (1953) and Meehl (1962) first describing it as a genetic predisposition to schizophrenia. The traditional view (e.g. quasi dimensional) by (Meehl, 1962; 1990) posits a categorical view that these traits (or symptoms) are dichotomous, either people have these or do not, and measures have been created to assess for these traits using Yes/No response measures (e.g. the Schizotypal Personality Questionnaire (Benishay & Raine, 1995). Whereas, other researchers (e.g. Claridge, 1994; Eysenck & Eysenck, 1976; Claridge, 1994; Chapman, Chapman and Miller, 1982) posit the continuum view, where these traits are represented along a continuum and these have been measured using a likert-scale responding for personality traits associated with schizophrenia (e.g. Schizotypal Personality Scale, Jackson & Claridge, 1991). As a result of this, there has

been varying theoretical and empirical origins of schizotypy (Lenzenweger, 1994), however, most researchers are now in agreement for a factor structure of schizotypy and the two approaches are converging to look at psychosis-proneness within each of these measures (Claridge *et al*, 1996). What is clear is that people scoring high on schizotypy traits demonstrate some of the clinical features of schizophrenia, in that they share similar performance deficits on certain cognitive, neuropsychological and psychophysiological assessments (Lenzenweger, 1998; Claridge, 1994; Raine, Sheard, Reynolds & Lencz, 1992; Rawlings & Claridge, 1984). Further to this, relatives of those diagnosed with schizophrenia are more likely to show higher scores on schizotypy measures and performance decrements on schizophrenia sensitive tests/traits relative to those in the general population (Kendler *et al*., 1995; Vollema & Postma, 2002).

Meehl (1990) postulated a model for schizotypy to a gene defect which causes a generalised dysfunction throughout the brain, which he coined as 'hypokrisia', a Greek word to describe insufficiency of separation, differentiation or discrimination. The integrative failure is at the sub-cellular level which produces a neurochemical deficiency leading to Schizotaxia. Schizotaxia causes a person to be socially avoidant and misinterpret social relationships and predisposes an individual to schizophrenia, and this is acted upon by epigenetics (social or physiological) and leads to degrees or levels of schizotypy. However, Meehl argues that only a small minority with the CNS defect are diagnosable by DSM criteria. Manifestations of the gene defect are linked to personality traits, social learning history and unpredictable life events. Schizotypy and such traits may be produced as changes happen to structures which are involved in schizophrenia.

Schizophrenic-like tendencies have been explained in terms of existing on a continuum, at one end those being classified with very low (or no) psychotic traits or behaviours within the general population compared to those at the other end being classified as having schizophrenia. Those 'at risk' or with higher schizotypal traits would be seen somewhere higher up the scale or in the middle (i.e., relatives of those with schizophrenia and have a high number of psychotic-like traits and behaviours but do not have a full blown disorder (Claridges & Broks, 1984; Eysenck & Eysenck, 1975; Claridges & Beech, 1995). There is some debate over 'schizotypy' being seen as a personality trait, which is distinct from

schizotypal personality disorder (Claridge, 1997). One view is that presence is indicative of risk for future psychopathology (Eckblad & Chapman, 1983). Others view schizotypy as a personality dimension and rather than being associated with psychosis itself (e.g. meeting a diagnosis for schizotypal personality disorder or schizophrenia), it exists on a continuum in which all people vary (McCreery & Claridge, 1995). What is clear is that people scoring high on schizotypy traits demonstrate some of the clinical features of schizophrenia for performance deficits on certain cognitive, neuropsychological and psychophysiological assessments (e.g. Lenzenweger, 1998; Claridge, 1994; Raine, Sheard, Reynolds & Lencz, 1992; Rawlings & Claridge, 1984). Further to this, relatives of those diagnosed with schizophrenia are more likely to show higher scores on schizotypy measures, and performance decrements on schizophrenia sensitive tests relative to those in the general population (Kendler *et al*., 1995; Vollema & Postma, 2002).

In essence, there are two ways to assess schizotypy and cannabis effects:

1. There is variation between individuals on this schizotypy factor (with each individual somewhere on the continuum), which might also indicate who is more or less at risk of any issues with cannabis.

2. Cannabis use causes changes centrally over time, which push the brain to function in a more schizophrenia-like fashion – with changes in cognitive functioning and increased schizotypy, as a marker of this change.

1.4 Drugs and Psychosis

Drug induced psychosis is a term that has been used since the 1930s to describe the symptoms/behaviours linked to a psychotic disorder, which can be induced through heavy drug use. Young and Scovell (1938) first reported a case of psychosis following use of amphetamine; with these symptoms reduced following 2 weeks of abstinence. Amphetamine is a highly addictive drug which act directly on the mesolimbic dopaminergic reward system by inducing release of dopamine, and to some extent norepinephrine (Robinson & Berridge, 2000) and it is also commonly used amongst psychiatric patients (Gonzales *et al.*, 2008; Katz *et al.*, 2008). Use of amphetamine can cause acute psychotic symptoms and may also

contribute to persistent psychotic conditions such as schizophrenia (see Bramness *et al,* 2012). There is overwhelming evidence that patients with psychotic disorders have an increased vulnerability to compulsively use drugs (Cantor-Graae *et al.*, 2001; Regier *et al.*, 1990). There may be several causal explanations for this increased co morbidity, but the most convincing evidence comes from animal studies arguing that this is due to shared vulnerabilities for both psychosis and drug use disorders due to reduced inhibitory control over dopamine mediated behaviour which regulates drug reward and reinforcement. More specifically, abnormalities in hippocampal formation and the frontal cortex facilitates the positive reinforcement from drug use and reduces inhibitory control over drug-seeking behaviours (see: Chambers *et al.*, 2001). Since then many published studies have explored the link between certain other drugs and psychosis, such as alcohol (Ringen *et al,* 2008); methamphetamine (Medhus et al, 2012) cocaine (Brady et al, 1996) and cannabis (Degenhart, 1993). Interestingly, in the drug induced psychosis research most (if not all) of the people using stimulant drugs such as amphetamine and cocaine also use cannabis, whereas not all cannabis users are poly drug users.

1.4.1 Cannabis and schizophrenia

It is estimated that rates of co-occurring cannabis use in people diagnosed with schizophrenia ranges between 12%-42% (Chambers *et al*., 2001). One of the first studies to document a link between cannabis and schizophrenia was Chopra and Smith (1974) who reported that 200 people who were admitted to a psychiatric unit in Calcutta over a period of five years, had psychotic symptoms (which included sudden onset of confusion, hallucinations, depersonalisation, and paranoia) following heavy cannabis use. A third of these cases had no previous history of psychopathology, and there was an association between heavier use of cannabis and developing psychotic symptoms in the shortest period of use. These findings on heavier cannabis use and risk for developing psychotic symptoms have been supported globally in the Caribbean (Spencer, 1971), New Zealand (Eva, 1992), Scotland (Wylie *et al*., 1995), the USA (Talbott & Teague, 1969) and here in the UK (Carney *et al*., 1984; Drummon, 1986). Both the International Classification of Diseases (ICD-10; WHO, 1992) and the Diagnostic and Statistical Manual for Mental Disorders (DSM-IV; APA, 1994), include a specific diagnosis for cannabis-induced psychosis. However, some researchers are critical of this evidence based on the lack of information about cannabis use, lack of

accounting for genetic vulnerability and heterogeneity in terms of defining a specific cannabis psychosis (Gruber & Pope, 1994). However, the strongest evidence seems to come from the research on heavy consumption of the drug leading onto the person developing a specific cannabis-psychosis (Hall & Degenhardt, 2009) and heavy use is linked to higher risk of developing a first episode in psychosis (De Forti *et al*, 2015). Further to this, cannabis intoxication can induce brief episodes of psychotic symptoms, and it can exacerbate or bring about the reoccurrence of pre-existing psychotic symptoms (Negrete *et al*., 1986; Thornicroft, 1990; Mathers and Ghodse, 1992).

Thacore and Skukla (1976) published a case control study of people diagnosed with cannabis psychosis and 25 people with a diagnosis of schizophrenia (paranoid subtype) but no history of cannabis use. There was a direct correlation between prolonged use of cannabis and the onset of psychotic symptoms. Further to this, those diagnosed with cannabis-induced psychosis responded more quickly to anti-psychotic treatment. In contrast to this, other researchers have found no such associations between people with cannabis-induced psychosis and those with schizophrenia (Imade & Ebie, 1991). Rottanburg et al. (1982) compared 20 patients diagnosed with schizophrenia with cannabinoids in their urine with 20 schizophrenia patients without cannabinoids in their urine. Patients with more cannabinoids in their urine had higher rates of hypomania and agitation symptoms, but less auditory hallucinations, flattened affect, poor speech, and hysteria relative to the non-cannabis using patients. Improvements in symptoms in the cannabis group were more marked through the week when they stopped using the drug relative to those that did not consume cannabis. This is an interesting finding as it indicates that it may have been a specific induced psychosis, with symptoms gradually lessening with cessation from the drug; whereas those with other causes behind their illness showed no changes. Conversely, using a similar research Thornicroft *et al* (1992) found very few demographic or clinical differences using a similar research sample and McGuire *et al*. (1995) also reported that the groups did not differ on their symptoms but the cannabis group had a higher family rate of schizophrenia (7.1%) than controls (0.7%).

The average age for experimenting with cannabis for the first time usually takes place during early adolescence (e.g. from the age of 14) and from a neurodevelopmental view, use of cannabis in itself during this brain maturation stage may lead to mental health problems

(Bossong & Niesink, 2010). It has been suggested that during late maturation roughly from the age of 10-12 years and finishing between the ages of 16-20 years (Spear, 2000), dramatic changes are seen in brain growth during these critical periods for changes in frontocortical regions (Slotkin, 2002; Chambers *et al*, 2003; Nelson, 2004; Cannon *et al*, 2005). During this phase of neuronal plasticity, there is sprouting and pruning of synapses, myelination, changes with neurotransmission and receptor levels which are likely to impact on behavioral and cognitive functions (Katz & Shatz, 1996; Luna, 2009; Rice *et al*, 2002). Endogenous cannabinoid transmitters are central to many aspects of neurodevelopment (see Sundram, 2006; Malone *et al*, 2010) and therefore, exposure to THC during such critical periods in adolescence may disrupt natural maturation, with evidence that this drug has effects on the indirect release of the neurotransmitters dopamine (Beres, 2010), glutamate (Brown *et al*, 2003) and GABA (Schlicker & Kaffman, 2001), as well as on numerous neurodevelopmental processes (see Malone *et al*, 2010). Early exposure to cannabis and higher risk of schizophrenia has been demonstrated in animal (O'Shea *et al*, 2004; Cha *et al*, 2006; Schneider *et al*, 2008) and human epidemiological studies (Andreasson *et al*, 1987; Arsenault *et al*, 2002; Zammit *et al*, 2002; Caspi *et al*, 2005; De Forti *et al*, 2015).

Exploring the link between the use of cannabis and mental health problems is useful in identifying potential risks factors which lead onto more severe psychopathologies, such as schizophrenia (Verdoux, 2004). There are converging findings obtained from prospective based population studies, which suggest that cannabis use might represent an independent risk factor in the onset of psychosis (Andreasson *et al*., 1987; Arsenault *et al*., 2002; van Os *et al*., 2002).

1.4.2 Cross sectional studies

There have been a limited number of cross-sectional studies exploring the link between the use of cannabis and risk for developing psychosis (Verdoux, 2003). Williams *et al*. (1996) was the first to explore cannabis use in relation to positive symptoms (e.g. delusions, magical ideation) using the Schizotypy-A scale (STA; Claridge and Bride, 1995) in two hundred and eleven participants. They found that those people that had 'ever used' cannabis scored higher on this measure relative to those who had never used cannabis, even after controlling for

other drug use. Kwapil (1996) assessed a group of 36 college students at baseline on drug use and schizotypal positive and negative symptoms and then followed up ten years later. Those people with higher positive symptoms had higher rates of substance misuse (including cannabis use); however, substance use at baseline did not predict later psychotic symptoms. Skosnik *et al*. (2001) explored dimensions of psychotic symptoms using the Schizotypal Personality Questionnaire (SPQ; Raine, 1991), which assesses nine subscales (ideas of reference; excessive socials anxiety; odd beliefs and magical thinking; unusual perceptual experiences; odd or eccentric behaviour; no close friends; odd speech; constricted affect) amongst 15 cannabis users (who used at least once per week), 10 ex-cannabis users (with no use in at least forty five days as one criteria) and 15 controls (with no reported lifetime use of cannabis as one criteria). Cannabis users scored significantly higher on all subscales of the SPQ relative to ex users and controls. Nunn *et al*. (2001) also explored dimensions of psychosis using the Oxford-Liverpool Inventory of Feelings and Experiences (O-LIFE, Mason *et al*., 1995) in 196 students; those who used cannabis only, alcohol only, alcohol and cannabis and no alcohol or cannabis. The O-LIFE measures four subscales of psychotic symptoms; positive, negative, cognitive disorganisation and impulsivity non-conformity. An additional measure was used to assess for delusion ideation called the Peters *et al*.'s Delusion Inventory (PDI; by Peters *et al*., 1999). Those participants using cannabis only had higher rates of positive symptoms in the O-LIFE and higher scores on the PDI relative to non-cannabis users, whereas, those using cannabis and alcohol scored lower on the negative measures for introverted anhedonia than any other group. It may be that the cannabis using only group represent a distinct/unusual group where this cohort has a distinct personality type. For example, people may use cannabis (as opposed to any other drugs) specifically for religious or creative purposes; a high level of trait creativity is linked to the positive symptoms in schizophrenia (Shafer *et al*, 2012).

Dumas *et al*. (2002) assessed a group of 232 students who were categorised into past never used, past use, occasional use, or regular use of cannabis (at least twice per week) using the SPQ and four of the Psychosis-Proneness scales by Chapman *et al*. (1994). Those who had never used cannabis reported significantly fewer psychotic-like traits on the psychosis-proneness measures, relative to the ex-cannabis and current cannabis users. Verdoux *et al*. (2003) explored the link between cannabis use and psychosis in a group of 571 females, assessing lifetime and current use of cannabis and other substances. Psychosis proneness was

assessed using Community Assessment of Psychic Experiences (CAPE; Stefanis *et al*., 2002) which has 42 items of positive, negative and depressive symptoms. Cannabis use was categorised into frequency of use, no use in last month, once a month to once a week, more than one time per week. Increased levels of cannabis use were associated with higher frequency of positive and negative psychotic-like symptoms. There were no significant associations found for frequency of cannabis use and depressive scores, nor for alcohol use variables and dimensions of psychosis proneness.

Overall, cross sectional studies converge to indicate that cannabis use (whether current or ex use) is associated with psychotic traits compared to those who report no use. The association does not seem to exist for cannabis and depressive symptoms, nor for alcohol use and dimension of psychosis proneness. Cross-sectional studies are often limited to relatively small sample sizes, and by their nature are a snap shot revealing little about the development of problems, baseline data, and thus causation. Such issues are to some degree addressed by looking at larger populations, or by following samples over longer time periods.

1.4.3 Longitudinal studies

Like cross-sectional, a longitudinal study is observational, but researchers conduct several observations of the same participants over a longer period of time. This gives the benefit of being able to detect developments in the characteristics of the target population (i.e. cannabis users) at both the group and the individual level. The benefit of longitudinal studies is that they extend beyond a single moment in time, and can help to assess some causal effects as opposed to using correlational designs. Please refer to Table 1 as a summary of the most influential longitudinal studies to date assessing the link between cannabis use and risk for schizophrenia.

Table 1: Population-based and birth cohort studies on the association between cannabis misuse and psychosis.

Name	Country	Method	Odds ratio (95% confidence interval)
Swedish Conscripts (Andrèasson *et al*., 1987).	SWE	45,570 participants: follow-up 15 years.	6.0 (4.0-8.9)
Swedish Conscripts (Zammit *et al*., 2002).	SWE	50,087 participants: follow-up 27 years.	3.1 (1.7-5.5)
The Dunedin Multidisciplinary Health and Developmental Team (Arsenault *et al*., 2002).	NZ	1.037 participants: follow-up 15 years.	3.1 (0.7-13.3)
The Netherlands Mental Health Incidence (NEMESIS; van Os *et al*., 2002).	NED	4, 045 participants: follow-up 3 years.	2.8 (1.2-6.5)
Christchurch Health and Developmental Study (CHDS; Fergusson *et al*., 2005).	NZ	1,265 participants: follow-up after 3 years.	1.8 (1.2-2.6
The Early Developmental Stages of Psychopathology (EDSP; Henquet *et al*, 2005).	GER	2,437 participants: follow-up 5 years.	1.7 (1.1-2.5)
The National Psychiatric Morbidity Study (NPMS; Wiles *et al*., 2006).	UK	8,580 participants: follow-up 1.5 years.	1.07 (1.04-1.09)
EDSP (Kueper *et al*., 2011)	GER	1,423: follow-up 8.4 years.	1.9 (1.1-3.1)
Swedish Conscripts (Manrique-Garcia *et al*., 2012).	SWE	50,087 participants: follow-up 35 years.	3.7 (2.3-5.8)

Andrèasson *et al*. (1987) conducted one of the first studies on 45,570 Swedish Conscripts during a fifteen year follow up study assessing the association between those reporting drug use at 18 and later psychotic diagnosis. Those that tried cannabis prior to 18 had a 2.4 risk to develop schizophrenia relative to those that did not use the drug. Heavy cannabis use was categorised as using the drug for more than 50 times and there was a dose-response effect in that heavier use was associated with a greater risk. Van Os *et al*. (2002) followed 4, 045 participants without any baseline psychotic symptoms as assessed by the Brief Psychiatric

Rating Scale (BPRS, Overall & Gorham, 1962) over 3 years, with data re-collection at two time-points, 1 year and 3 years. Baseline cannabis use was a stronger predictor of psychotic symptoms, rather than at the 1-year and 3-year follow-up. More than 50% of the psychosis diagnoses attributed to cannabis use were found if the participants reported using the drug at an earlier age. There were a number of confounding factors in the Swedish Conscript study (e.g. other drug use and personality traits likely to predispose someone to develop a psychotic disorder). Zammit *et al*. (2002; 2004) followed the same cohort up to 27 years and concluded that baseline cannabis use was associated with a greater risk for developing a psychotic disorder and the dose-response relationship remained even after controlling for confounding factors. Similarly, in the NEMESIS study by van Os *et al*. (2001) of 4,045 people without baseline psychotic symptoms, a dose-response effect was found in those using cannabis at baseline had developed a psychotic disorder at the 2-year follow-up period.

In the one birth cohort study, Arsenault *et al* (2002) assessed 1,037 individuals born in Dunedin in 1972-3, with 96% of the sample followed up to age 26. The researchers obtained information on psychotic symptoms at age 11 and drug use was noted at ages 15 and 18. A standard interview was conducted using the DSM-IV. The groups were divided into three based on cannabis consumption at age 15 (just under 30% whom reported three or more times using cannabis and continued up until 18), 18 (just under 32% using three or more times) and controls (65% of the sample whom had reported no use, or once or twice). Psychiatric outcomes by the age of 26 were assessed based on symptoms of schizophrenia and depression and diagnoses of schizophreniform (a form of schizophrenia with a shorter duration of 1-6 months) and depression. Logistic regression revealed that people who reported having used cannabis by age 15 or 18 had higher rates of schizophrenia symptoms (10.3%) than controls (3%). These effects remained even after controlling for baseline psychotic symptoms at 11; the effect was stronger for earlier use of cannabis and developing schizophreniform disorder (e.g. before the age of 15). Cannabis use did not predict any depressive outcomes, but it was the strongest predictor of schizophrenia symptoms from drugs used during adolescence.

Using data from the Early Developmental Stages of Psychopathology (EDSP) 5-year prospective study, of 2,437 participants, Henquet *et al*. (2005) reported that those scoring high on schizotypy reported more psychotic symptoms after cannabis use compared with

individuals with lower schizotypy scores. Kuepper *et al*. (2011) followed up 1,423 from the EDSP studies who were aged 14-24 at baseline. They assessed them at two different time points, which averaged at 3.5 years from the first test, then the last testing session 8.4 years later. The researchers wanted to assess whether continued use of cannabis would affect incidence and persistent psychotic symptoms in the general population. Individuals who reported no cannabis use and psychotic symptoms at baseline, then reported cannabis use 3.5 years later, also experienced an increase in psychotic symptoms over a period of 3.5 to 8.4 years (adjusted odds ratio of 1.9). The incidence rate of psychotic symptoms in individuals exposed to cannabis from baseline to 8.4 years was 31%, compared to 20% in non-exposed individuals; from 3.5 years to 8.4 years, these rates were 14% in users and 8% in non-cannabis users. Interestingly, rates of psychotic symptoms declined upon abstention from cannabis use, but continued to increase with persistent use of the drug.

Fergusson *et al* (2005) conducted a 25-year longitudinal study assessing a birth cohort of 1,265 in a general community sample at age 18, 21, 25 on psychotic symptoms and drug use. A regression model revealed that those reporting daily use of cannabis had between 1.6 and 1.8 higher rates of psychotic symptoms than non-cannabis users. Structural Equation Modelling revealed that the effect of cannabis was stronger for predicting these psychotic symptoms rather than the effect of symptoms on cannabis use. Wiles *et al*. (2006) assessed 8,580 participants aged 16-74 using the revised version of the Clinical Interview Schedule (CIS-R; Lewis *et al*., 1992; Lewis 1994) and Psychosis Briefing Questionnaire (Bebbington & Nayani, 1995); those with psychotic disorder were excluded at baseline and 2,413 completed the follow up interview. Those dependent on cannabis had a higher risk for incidence of psychotic symptoms, and also those engaging in risky drinking behaviours. Other factors involved included life events, such as inhabiting a rural area, and tobacco smoking. Grech *et al*. (1998), in a 4-year follow-up of 119 people diagnosed with schizophrenia, also found that continued use of cannabis was associated with severity of symptoms and longer course of the illness with positive symptoms, than those who did not misuse cannabis. Further to this, Zammit *et al*. (2008) carried out a systematic review on the effects of cannabis on psychotic outcomes, and reviewed 13 studies for rehospitalisation, readmissions, measures for symptoms, measures for treatment, adherence to treatment. Continued cannabis use was associated with poorer treatment outcomes and increased relapse/readmissions. Moore *et al*. (2007) also carried out a systematic review, in this case to

specifically see if cannabis use is associated with psychotic symptoms that persist beyond transient intoxication. This review reported an increased risk of developing psychosis in any individual that reported cannabis use (adjusted odds ratio (Adj OR): 1.41), with a consistent dose response effect (Adj OR 2.09). However, these data from Moore *et al*'s research could be due to alternative explanations (e.g. other drug use or pre-existing personality traits), but Moore *et al* concluded that cannabis use increased risk for developing psychotic disorders and individuals should be advised about this risk. It is somewhat surprising that few studies have controlled for confounds such as alcohol use at baseline, and several other sociodemographic factors. Foti *et al*. (2010) assessed a group of 229 people diagnosed with schizophrenia over a 10-year period, with assessments at five time points (0, 6months, 2, 4 and 10 years), on a range of psychiatric symptoms; psychotic, depressive, negative and disorganised, and controlling for numerous other possible confounds. Lifetime use of cannabis was 66.2% in the sample and a greater total lifetime use was associated with earlier onset of psychosis. Cannabis use in those diagnosed with schizophrenia was associated with worsening of psychotic symptoms and these symptoms were associated with an increase in cannabis use. Importantly, this effect remained even after controlling for gender, age, socioeconomic status, other drug use and medication.

There are several ways to explain the link between cannabis use and psychosis because a causal link has yet to be clearly established. The prominent view is that cannabis use exerts itself as a *component cause*, in that it is neither a necessary (as some people develop schizophrenia without having used cannabis) nor a sufficient cause (as most people do not develop a psychotic disorder from using cannabis). For example, Rothman and Greenland (1998) use a causal model, to explain the schizophrenia risk model divided into key sections representing a constellation of causes that inevitably lead to disease occurrence, and with each element having a causative influence. The causal model contains component causes (e.g. environmental, genetic, cannabis) and a component is necessary for the disorder to occur. A disorder may need a collection of sufficient causes, which are made up of a range of component causes which lead to the disorder along with range of another component cause. Cannabis could therefore be seen as a component in this predictive model for developing schizophrenia; interacting with other components such as genetics (candidate genes), or environmental influences (high stressors). The model put forward for this thesis is an adaptation from the diathesis stress model (Gottesman, 1991) and a new multi-component

model which elaborates more on the known risks of cannabis in this model - please refer to Figure 1. Outcomes for psychological disturbance are inextricably linked to an interaction between early life/neurodevelopmental, vulnerability/genetic, environmental factors and psychosocial stressors; as described in the diathesis stress model. Whereas for the specific link between cannabis use, the component model and the diathesis stress model are missing a number of additional factors which are known to contribute to this risk in developing sub clinical symptoms and behaviours. The prominent view is that early onset of the drug (before the age of 15) is linked to greater risk, as well as the type of cannabis use (primarily skunk and high THC potency cannabis) as well as duration of use. It is clear that not all of those who smoke cannabis will go onto develop subclinical or full blown schizophrenia, but it may that those who smoke cannabis with more of these risk factors in the model would be categorised as having a higher risk, and this wold be demonstrated in the model for the output in that they would be pushed higher up the spectrum in subclinical schizophrenia changes. This therefore would be demonstrated in them showing more symptoms and having greater cognitive disruption as is seen in patients with full blown schizophrenia.

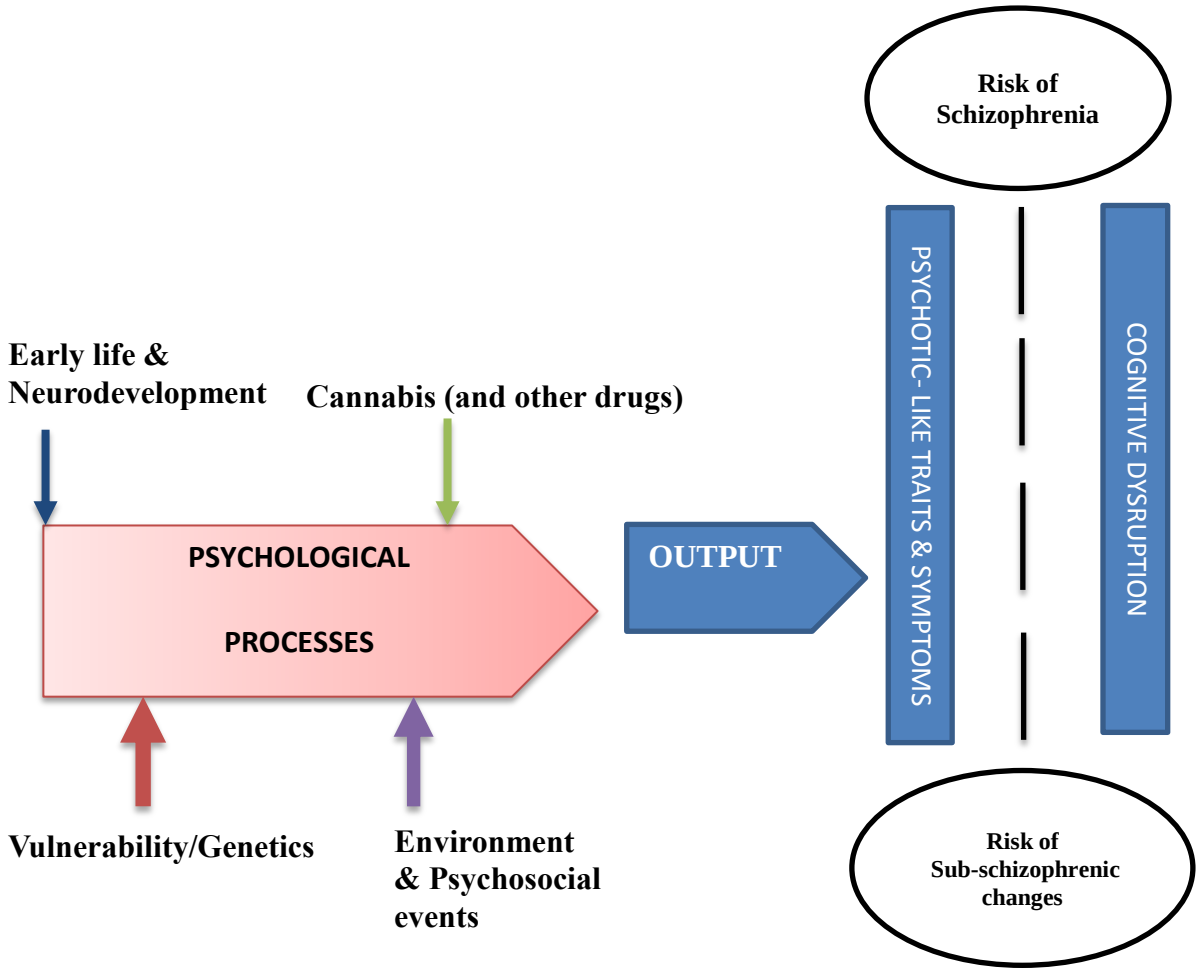

Figure 1: A multi-component model (an adaption of the diathesis stress model) for the link between cannabis use and risk for schizophrenia.

1.5 Rationale

There is strong evidence to support the claim that cannabis use is a risk factor for the development of psychotic symptoms and schizophrenia (see section 1.3 and 1.4). Nonetheless it is also clear that the majority of cannabis users do not go on to develop this disorder. It is argued that the use of cannabis is causing some of the sub-schizophrenia symptoms in some users, and that prolonged use and/or heavy use of stronger versions of cannabis is linked to the onset of induced symptoms in people that may not have developed these symptoms otherwise. The material covered in this chapter does also highlight the extant literature demonstrating some degree of cognitive change, and possibly elevated schizotypy, in recreational cannabis users. Whilst other types of association and other discrete factors may very well be of significance, exploration of cannabis as at least partly causal in these effects is clearly warranted. If cannabis in some individuals may contribute to

or even trigger episodes of schizophrenia, it may very well be the case that this potent psychoactive contributes to changes and/or symptoms which are part of or relate to the constellation of features we see in the full blown disorder. The view of cannabis contributing to psychological change in the direction of schizophrenia (or sub-schizophrenic change) is illustrated in Figure 1, and the evidence presented in earlier sections of this chapter fit with the model, in which cannabis is an added component in the development of pathology. The majority of cannabis users' will have a low risk for developing full blown schizophrenia, but risk of some changes in that direction may be elevated (as demonstrated by having some subclinical symptoms and behaviours. This might be further the case in those with additional underlying genetic or other vulnerability, and those with more of the risk cannabis use variables. This thesis will partially test the model for cannabis use, vulnerability/genetics and explore this in relation to cognitive disruption and psychotic-like symptoms. Whereas, other parts of the model remain untested, such as early life/neurodevelopment, environmental and psychosocial events.

Much of the published research on the cannabis-psychosis link has focussed on schizotypal traits, as opposed to looking at traits combined with cognitive performance and genetic susceptibility. Further to this, research has focussed mainly on clinical samples (e.g. people diagnosed with schizophrenia). These studies are not without their problems as there is heterogeneity in symptoms and behaviours in terms of schizophrenia or psychosis. Findings drawn from clinical studies are difficult to fully explain in terms of causality as a confounding factor is linked to clinical status, which is difficult to control. Therefore, research using non clinical samples may provide a better way of attempting to elucidate the link between cannabis use and psychosis (e.g. Verdoux *et al*., 1998; van Os *et al*., 2000; 2001; Verdoux and Van Os, 2002).

The general aim of the current research thesis is to explore a range of cognitive functions affected by schizophrenia, psychotic-like personality traits, candidate genes for schizophrenia, in a group of recreational cannabis users (free from existing psychopathological disturbance) versus a control sample of non-cannabis users. A key difference in this research thesis is that schizotypy has been measured alongside cognitive performance, in order to see if there is an interaction here and how this then relates to

cannabis use. Study 1 has LI, KB and schizotypy and Study 2 adds greater personality analysis alongside other cognitive tasks known to be sensitive to cannabis use and to schizophrenia.

This research aims to use cognitive tests that appear to especially affected in schizophrenia and link to the neuropsychological underpinning of the disorder (there may be more global deficits in schizophrenics, so tasks that map on to the key theorised changes that take place in schizophrenia are used), and schizotypy, because it exists as a general population personality trait as well as being strongly linked to schizophrenia itself. Under the ideas being explored here is that cannabis use pushes people further along the schizotypy continuum, as part of this sub-schizophrenic change. Furthermore, some risk marker genes will be assessed that have emerged from the extant genetics literature, to see if (as illustrated in Figure 1) cannabis use alongside some degree of genetic vulnerability may be even more likely to produce some level of sub-schizophrenic change.

Study 1 aims to assess selective attention in cannabis users relative to non-cannabis users, through the use of two associative learning tasks. The LI and KB paradigms are utilised to assess the ability to inhibit the processing of irrelevant stimuli which have been previous deemed to be irrelevant. Chapter 2 provides a detailed background to research using the LI and KB tests, as well as the theoretical basis for choosing such tests. Section 1.2 above briefly described some data and evidence to highlight disruption in inhibitory processing and selective attention in cannabis users similar to people diagnosed with schizophrenia. Therefore, due to the mounting evidence, it is expected that the cannabis users should show disruption on these associative learning tasks compared to non-cannabis users.

Study 2 aims to assess attention, executive control and decision-making in cannabis users relative to non-cannabis users. Section 1.2 above highlights some of the key effects cannabis has on these cognitive processes. Chapter 3 provides a detailed theoretical background on these cognitive processes with arguments as to why such tests have been chosen to demonstrate these functions. In brief, executive control will be assessed through the use of the Anti-Saccade Task and an eye-tracking device. Decision-making will be assessed using

the Iowa Gambling Task. Selective/sustained attention and inhibitory control will be assessed through the use of the Continuous Performance Test. Based on earlier research on cognitive dysfunction in cannabis users, it is expected that cannabis users will highlight higher level cognitive processing deficits compared to non-users, thus resembling sub schizophrenia like symptoms (please refer to Chapter 3 for further detail).

Individual differences in psychotic-like personality traits will also be assessed. Study 1 will assess the three factors linked to the structure of schizophrenia symptoms, such as positive, negative and disorganised domains. Study 2 will go further to explore a range of other psychotic-like traits which have been overlooked in the research but are gaining more prominence in the current literature. For example, one would be emotional processing, where there is less clarity in thinking, and also ambivalence as a trait, in that people alternate quite rapidly between emotions (e.g. love and hate for the same thing). Based on earlier research findings it is expected that cannabis users will report experiencing more of these psychotic-like traits and there will be a correlation between earlier onset of cannabis use, frequency and duration of use.

Chapter 4 covers the selection and measurement of a number of genetic markers which have previously been associated with elevated risk for schizophrenia. The aim of this work was to speculatively search for some of these markers amongst the cohorts tested in the behavioural studies, and initially provide a focused genetic picture of the participants across and between groups. This work also provided the possibility to assess the relative contribution that certain genetic risk markers linked to schizophrenia may have on predicting outcomes of performance on the battery of cognitive tasks and personality measures used in Study 1 & 2. It was expected that people with more of these risk marker genes (e.g. variants in the DAOA, COMT, NRG1) and will display more of a personality and behavioural profile similar to that of someone with schizophrenic symptoms.

In particular, it was predicted, in line with the extant literature (explored in more detail in Chapter 4), that people with higher prevalence of the COMT risk haplotype genes, who also use cannabis, may have more sub schizophrenia-like symptoms and behaviours. Due to the lack of evidence looking at cannabis and gene correlates for the neuregulin gene (NRG1) and the cannabinoid genes (CNR1 and FAAH), it is difficult to make clear predictions, so these data were exploratory (please refer Chapter 4 for more detail regarding these genes).

Chapter 5 provides a summary of key results across all of the work. The contributions of the thesis findings to the literature are explored and methodological limitations of this research discussed, alongside suggestions for future work in this area.

Chapter 2: Latent inhibition, Kamin blocking and psychotic-like traits in regular cannabis users

2.1 Introduction

Selective attention is the processing of incoming information, in a quick and rapid way, to selectively filter relevant from irrelevant information (Broadbent, 1958). Selective attention deficits are seen as one of the fundamental dysfunctions in the disorder of schizophrenia; and these are numerously reported in the literature, both from the patient and clinician (MacDonald, 1960; McGhie and Chapman, 1961; Evans *et al*, 2007). As a result of this, there have been many paradigms developed to test selective attention dysfunction, with Latent inhibition (LI) and Kamin Blocking (KB) being two of the most prominent models utilised both in human and animal studies (e.g. Lubow, 2005). Animal studies have shown that LI and KB are disrupted by increased dopaminergic activity, and restored by dopaminergic blockade (Joseph & Jones, 1991). LI and KB tests are measures of associative learning when a certain stimulus comes to be associated with another stimulus or behavior, as through classical or operant conditioning (Pearce & Boulton, 2001). The working memory system plays a key role along with the executive system to form new associations (Tanji & Hoshi, 2001).

2.1.1 Latent inhibition (LI)

LI is defined as occurring when: "A stimulus that is casually familiar enters into new associations more slowly than a novel stimulus" (Lubow & Gerwirtz, 1995, pg 87). It was a term first introduced by Lubow and Moore (1959) to describe the observation that exposure to an irrelevant stimulus impairs the ability to form subsequent conditioned associations with that stimulus. Without LI, ordinary learning would be a cumbersome process. LI promotes the stimulus selectivity required for rapid, efficient learning. LI creates a bias in favour of potentially important stimuli by degrading those stimuli that have been registered as inconsequential in the past (Lubow, 1989).

Lubow (1989) postulates that there would be an overload of information processing, if the brain was required to process all of the incoming input of information, as being similar and of equal importance; but it seems the natural adaptive response evolved for human/animal learning and

attention requires an automatic selection of information that is deemed to be the most important, with inconsequential information being filtered out as unimportant. An everyday application of LI would be the ability to block out irrelevant information on a train journey whilst reading a book; this is generally done in an unconscious manner. People deemed to be high in latent inhibition would easily block out any irrelevant information (i.e. the overload of advertising, high volume of people, other people's distracting behaviours etc.) whereas, those with low latent inhibition would find it difficult to read because they would be easily distracted by the external stimuli within this type of setting.

The majority of LI experiments to date have used the instrumental learning-to-criterion method, with the number of trials to criterion used as dependent variable (Lubow & Gewirtz, 1995). A typical LI experiment for this paradigm would have two separate groups: one pre-exposed (PE) and the other non-pre exposed (NPE). The PE group engage in a masking task which involves listening to a series of nonsense syllables and are asked to select one syllable and count how many times it is repeated. During the PE condition, alongside the nonsense syllables, the participants are presented with a number of trials where the to-be-conditioned stimulus (e.g. a burst of white noise) appears. The NPE group are presented with a similar task for the nonsense syllables, but with the to-be-conditioned stimulus being absent (no white noise). The test phase for all participants involves the to-be-conditioned stimulus (e.g. the white noise) becoming the target for which all participants should respond to (e.g. by pressing a button when a counter incrementally increases); the white-noise always precedes the counter incrementing. The measure is the number of trials-to-criterion (with at least five correct consecutive responses of the paired association between the white noise and counter incrementing). Participants in the PE condition with the white noise take a significantly longer time to learn the association than those who have not been previously exposed to the white noise; this is what is referred to as the latent inhibition effect. The LI effect in healthy individuals has been replicated in numerous studies (e.g. Baruch, Hemesley & Gray, 1988; Gray, Hemsley, & Gray, 1992; Escobar *et al*, 2002; Lubow, 2005; Kaplan & Lubow, 2010).

2.1.2 Latent inhibition and schizophrenia

Support for the model of LI being disrupted in schizophrenia comes from research showing that LI can be attenuated or abolished in rats treated with a dopamine (DA) agonist, such as

amphetamine (Soloman *et al*. 1981; Weiner, Lubow & Felton, 1981, 1984). This effect can be reversed through the administration of a dopamine antagonist (e.g. haloperidol; demonstrated by Christison, Atwater, Dunn, & Kilts, (1988); Solomon *et al*. (1981); Weiner & Feldon, (1987); Weiner and Feldon, & Katz, (1987)). Additionally, Dunn *et al*'s (1993) research indicated that what might be termed the LI 'super' effect (e.g. increased latent inhibition) seems to be specific to antipsychotic drugs. These drug effects on LI fit into the model of schizophrenia being related to deficits in attentional processing (Anscombe, 1987; Mirsky & Duncan, 1986; Nuechterlein & Dawson, 1984) and that the dopamine system has been implicated in playing a role in this attentional dysfunction (Matthysse, 1978; Swerdlow & Koob, 1987).

High levels of dopamine in the ventral tegmental area are associated with a decrease in LI (Swerdlow *et al*, 2003). Other neurotransmitters may also be involved, including glutamate, GABA (gamma aminobutyric acid) and acetylcholine (for reviews refer to Kleinman, Casanova, & Jaskiw, 1988; Owen & Crow, 1987; Reynolds, 1988; Weinberger, 1987). Baruch *et al*'s (1988a) research has demonstrated a loss of LI (i.e. a lack of filtering out of a previously conditioned stimulus that is normally ignored or deemed to be irrelevant) in acute but not chronic schizophrenic patients. However, this effect was not found after the patient was in drug treatment for 6-7 weeks, and there was a positive correlation between lower amount of clinical symptoms and higher LI. Interestingly, patients with acute schizophrenia (at least two weeks into medication treatment) when pre-exposed to the to-be-conditioned stimulus learnt the relationship much faster than healthy controls. This therefore rules out any effects that may have been due to a lack of motivation and/or interference from their clinical symptoms and medication, when considering that schizophrenic patients generally show poorer performance on most cognitive tests (e.g. Heinrichs & Zakzaris, 1998).

2.1.3 Latent inhibition, schizophrenic-like traits and other individual differences

Baruch *et al*. (1988b) used the LI paradigm as a way to assess the general population for individual differences in psychotic-proneness. Prior to this work, psychotic-like traits were assessed utilising measures such as Launey and Slade's (1981) Hallucination scale, Claridge and Brokes (1984) Schizotypal Scale (STA) and Eysenck and Eysenck's (1975) Psychoticism questionnaire. Baruch *et al* found that higher levels of psychotic-like traits were directly

related to lower levels of LI. These psychosis-proneness effects have been replicated by numerous researchers, including Lubow *et al* (1992), De la Casa (1993) and Serra *et al*. (2001). Serra *et al* (2001) investigated learning in chronic schizophrenics and their first-degree relatives versus a healthy control sample. The first-degree relatives were divided into two groups, those who were high/low in schizotypy. The controls showed latent inhibition (i.e. reduction in learning when pre-exposed to the to-be-conditioned stimulus). People diagnosed with schizophrenia and both their schizotypal and non-schizotypal relatives performed much worse overall on basic associative learning, and also showed a loss of LI. Serra *et al*'s findings run counter to previous models in that disruption of LI was due to slower learning in the two conditions, and the controls were faster to learn in the NPE condition. They also found a lack of difference between the schizotypal relatives and non-schizotypal relatives. Taken together, it was argued by Serra *et al* that these differences or lack of findings between the groups may highlight genetic markers of risk for schizophrenia.

2.1.4 Kamin Blocking

Broadbent (1971) describes information processing as a function which limits capacity to avoid overload, and represents parts that are made up of response biases, based on prior experiences and this in turn leads to the inhibition of redundant or irrelevant information. Other models provide support for the inhibition of irrelevant information and this is thought to bias information in an automatic rather than a controlled response (see Schneider and Shiffrin, 1977; Posner, 1982). Kamin Blocking, similar to LI, has been developed to test this model of attentional processing. However LI has been more extensively researched as a paradigm relative to the KB effect.

The Kamin Blocking Effect (KBE) procedure consists of four stages with two experimental conditions: blocking (BL) and non-blocking (NBL); participants are placed in to one of two conditions. During stage one, participants initially complete some keyboard skills by pressing the space bar when they see a yellow cross. In stage two, participants are presented with some coloured shapes on the screen, and in the NBL condition there is no association between any of the stimuli, whereas in the BL condition they see a coloured shape (CS1 – blue square) that is always followed by a second one (the UCS – yellow square). Stage two is entirely observational. During stage three, both groups are asked to predict when the yellow square

should appear and are exposed to the same sequence in which the UCS (yellow square) always follows the same pattern made up of CS1 (blue square) and an additional stimulus (CS2 - a small light 'flanker' stimulus presented both to the left/right of the computer screen). In the final stage, the blue square (CS1) is dropped from the sequence; this time the task is to predict when the yellow square will appear, which is now preceded only by the light flankers when they appear on the screen alongside any coloured square. The KBE is demonstrated as slower learning of this CS2 plus or minus the UCS association, in the BL compared to the NBL condition (e.g. Serra *et al*, 2001). Please refer to section 2.2 below for full details of the method and stimuli.

2.1.5 Kamin Blocking and Schizophrenia

Previous research to test information processing biases in schizophrenia has shown the KBE is affected by the administration of amphetamine (a DA agonist), and like LI this is reversed by the dopamine antagonist haloperidol (Crider *et al*, 1982, 1987; Ohad *et al*, 2003; O'Tuathaigh *et al*, 2003); this finding ties in to the dopaminergic hypothesis of schizophrenia. A Kamin Blocking Effect (KBE) was found between the BL and NBL in healthy participants (e.g. Jones *et al*, 1990). The same KB task was administered to a group of 29 people diagnosed with schizophrenia (14 acute and 15 chronic), and it was found that blocking was not present in acute patients tested within two weeks of admission as opposed to chronic patients stabilised on medication, suggesting that these inhibitory distortions can be found at the earlier stages of the disorders and can thus be treated with anti-psychotic medication. Further research by Serra *et al* (2001) found that KBE is absent in people diagnosed with chronic schizophrenia, schizotypal personality disorder and non-schizotypal relatives.

Moran *et al*. (2003) investigated the KB paradigm in 27 healthy volunteers and 21 people diagnosed with schizophrenia, and also explored schizotypal personality traits. They found a negative relationship between the two subscales of the O-life questionnaire (unusual experiences and cognitive disorganisation) and KB performance. Those people who reported feeling more unusual experiences and cognitive disorganisations were less likely to be affected by the blocking on the KB task which resulted in obtaining a lower score (i.e. they found the association more quickly). They also found that KBE was attenuated in non-paranoid patients -

so those patients not experiencing paranoid symptoms were affected less by the blocking conditions.

2.1.6 Summary and Rationale

Associative learning and the selective attentional processing crucial to this process, appear to be impaired in individuals with schizophrenia and in those scoring high for schizotypy, and can be modulated by drugs affecting dopamine in humans and animals. These findings are consistent with the dimensional view of psychosis, and neurobiological theories relating to aetiology of the disorder. In the LI task, high scorers on schizotypy resemble the performance of people in the acute stages of schizophrenia, in that they are quicker to learn the association in the PE groups. The clearest evidence comes from research looking at differences between high/low schizotypy with high scorers overall on psychosis-proneness measures predicting a reduced LI performance. Findings with the KB task are more variable, but largely support those found for LI. To date no known published research has looked at LI and KB performance in regular cannabis users, apart from my research conducted at undergraduate level (Lynch & Turner, 2006), which found subtle associative learning differences between cannabis users and non-cannabis users. It was found that cannabis users took less time overall on both tasks but mainly for the LI task they were less affected by the pre-exposure conditioning, as is seen in patients in the acute stages of schizophrenia. However, this aforementioned study was limited for a number of reasons.

1. It relied heavily on subjective reporting of abstinence from cannabis and this was not objectively verified through drug screening methods. Subjective reporting is open to bias in terms of the drug group not being honest. Therefore, the differences found arguably could be due to the sub-acute effects of smoking cannabis or other recent drug use
2. There is a lack of a full drug history, to look for use of other substances which might chronically affect cognitive processing, such as use of high doses of cannabis over long periods, dependency on cannabis and use and co-use of other psychoactives – some of this was explored, but only relatively superficially in the earlier work.
3. Participants were not screened for mental health history, both personal and familial to account for a biological predisposition towards schizophrenia.
4. It lacked external validity as most of the participants were undergraduate psychology students who were not offered any remuneration so may not have been that motivated to take part.

The current study aimed to address these issues and further explore the link between smoking cannabis and schizophrenic-like behaviours, by testing a group of cannabis users versus non-cannabis users, on LI and KB performance, to see if there are subtle differences between both groups. Based on the literature reviewed in Chapter 1, it is clear that cannabis use in some people may contribute to schizophrenia. Cannabis use is also frequently associated with acute and sub-acute psychotic-like experiences; in normal, non-pathologised individuals as well as in clinical populations. Such effects may be underpinned by alterations in dopamine produced by THC; acutely, sub-acutely and chronically. If cannabis use is producing such effects at a stable level, elevating schizotypy in many, leading to more serious disturbance in others, then we should also see noticeable differences amongst cannabis users, in other behaviours and cognitive processes affected by psychosis. As such, the filtering out of irrelevant information typically seen in normal healthy adults, but which has been shown to be disrupted in people diagnosed with schizophrenia, those administered with dopamine agonists and high schizotypy scorers may also be significantly affected by regular/frequent cannabis use. The hypothesis therefore is that cannabis users will show moderate impairments on the associative learning tasks for selective attention, namely LI and KB. In addition, both cannabis users and control participants will be classified as either having high or low psychotic-like traits from using their scores on the SPQ-B measure (Raine & Benishay, 1995), and this will be explored to look at differences between these groups on their LI and KB performance. Previous research has demonstrated that individual differences in psychotic-like personality traits results is linked to disrupted associative learning, namely for the LI and KB tests. Therefore it is predicted that those scoring higher for psychotic-like traits will also showed reduced LI and KB performance.

The regular use of cannabis has been associated with experiencing more psychotic-like traits, and this research was presented in the review Chapter one (section 1.4). Therefore it is predicted that cannabis users in this study will report a greater number of SPQ-B traits when compared to non-cannabis users. The review chapter also explored early onset of cannabis use and frequency of use of cannabis as key predictors in schizophrenic-like behaviours. Therefore deficits in LI and KB performance will be mostly marked in those with earlier onset of cannabis use; notably those who first used before age 15. Problems are also more likely to be seen and to be more significant in individuals who use cannabis heavily and/or very regularly (e.g. daily use).

2.2 Method

2.2.1 Participants

Twenty cannabis users and twenty non-cannabis users took part in this study and had an age range of 18-45, with a mean age of 30 (refer to Table 2 for a full list of demographic details). Five additional participants were tested and included in the non-cannabis using group for the secondary analysis, with the age range for all participants from 18-42 and a mean age of 29. Refer to Table 8 for a list of participants' demographic details used in the secondary analysis. Participants contacted the researcher by telephone or via e-mail, in response to adverts (see below), and were screened for eligibility for inclusion. Exclusion criteria were based on those that were currently taking any psychoactive medication, had a diagnosis of epilepsy, had any brain trauma or those cannabis users that did not abstain for two days prior to testing (as verified by a drugs screening kit using an oral salivary assay).

Recruitment

Recruitment was varied to attract a wide range of participants, through advertising in local London newspapers (Camden New Journal), online social networking sites (such as setting up a group on Facebook) and by placing numerous 'wanted' advertisements in Gumtree. Student participants at UEL were contacted via the internal e-mail system and by posters placed on walls throughout the UEL Stratford campus; this also created a snowball effect, with students contacting their friends and family outside of the university. There was a remuneration payment of a £10 love-to-shop gift voucher for participation.

Ethical clearance

Ethical clearance was obtained through the UEL Graduate School; reference = ETH/08/56 (see appendix xvi). All codes and regulations for compliance with the BPS (1978, 2009) Ethical Codes of Conduct for conducting research using human participants were upheld throughout. Example forms can be found in the appendices: information sheet (appendix i), consent form (appendix ii) and de-briefing sheet (appendix iii).

Research setting

Testing took place at 11 am or at 2pm in the recreational drugs lab at UEL or in small teaching rooms when the drugs lab was not available.

2.2.2 Materials

Drugs Test

Salivary drug testing was carried out in all participants using the Multiline Wondfo 6 in 1 Saliva Drug Testing Kit DSW-765 from: http://www.drug-testing-kit.co.uk. These single use tests allowed for the qualitative screening of the following drugs: Amphetamine, Cocaine, Cannabis, Methamphetamine, Opiate, and Phencyclidine.

Cognitive Testing

Latent Inhibition Task

The LI procedure here is a replication of that used in Serra *et al*'s (2001) study. This method consists of two experimental conditions and two stages, with participants from the control and drug groups counterbalanced in a between participants design to allocate them to either the pre-exposed (PE) or the non-pre exposed (NPE) conditions. During the first stage those placed in the PE condition were asked to listen to a tape recording of nonsense syllables (NS; e.g. gid, gad, yik & yak) and, via written and verbal instructions, were asked to count the number of times one of them recurs. The use of NS serves as a distracter, which diverts attention from the true purpose of the task; which has been deemed necessary to demonstrate LI with human participants (Lubow, 1989; Serra *et al*, 2001). Alongside the NS syllables those in the PE condition also hear bursts of low-intensity white noise, the conditioned stimulus (CS), which are randomly superimposed on the recording and are played at different levels of intensity and are played for a total of five minutes along with the nonsense syllables. Those placed in the NPE condition hear the exact same recording of NS without the CS (white noise recording).

In stage two participants are all played the same recording as was in the PE condition above. In other words they are all (in both PE and NPE groups) hear the NS played with the white noise, but this time they are instructed to predict when a counter display will be incremented by pressing the tab located underneath the counter display; increments, which represents the unconditioned stimulus (UCS) are always preceded by the white noise CS. In other words, each time there is a burst of white noise the counter on the screen always increases by 1. The

trials stop after learning has been achieved as a result of getting five correct consecutive trials of the CS (white noise) and counter increasing (UCS). Otherwise testing ends after 25 associations between the CS and UCS. LI is demonstrated as slower learning in the PE than NPE condition, as in the PE condition participants have been previously introduced to the CS without any other consequence and hence should take longer to find this paired association between the CS and UCS. The masking task consisted of a series of 40 nonsense syllables recorded at different intensity levels (70±78 dB) on the same recording for the PE condition and for testing in Stage 2; the recording used a female voice. The LI task involved two stages (one observational and one for the real test phase; please refer to Figure 2 for a visual representation of this tasks design).

Stage One

Pre-exposed condition: A series of NS was presented for about 5 min through headphones (TDK HP100); these were repeated five times in a fixed order, with participants being unaware of when any one series starts and ends. Under both the PE and NPE conditions all participants listened to the masking material with instructions (on-screen) to `choose one syllable and count how many times it is repeated'. In the PE condition 25 bursts of white noise (the to-be-CS) were superimposed on the left-ear of the audiotrack, in conjunction with the nonsense syllables. These white noise burst varied randomly between five intensity levels (range: 64±76 dB) and five durations (range: 1±3 s) throughout the recording and were presented at randomly varied inter-stimulus intervals over the range of 2 ± 22 s.

Non pre-exposed condition: The nonsense syllables were played as in the PE condition minus the superimposed white noise bursts.

Stage Two

Testing session: Participants were told that they had to move on to the next task; and the aim of this task was to predict when they think the counter (on-screen starting at 0 and then increasing to 1, then 2 and so forth) would change, by pressing the left mouse button, on the tab which would be directly underneath the counter. All participants now listened to the recording with superimposed white-noise bursts, as used previously in stage one of the PE condition. Counter increments (the UCS) took place after each burst of white noise (the CS). The learning criterion was based on the completion of five consecutive correct responses (i.e. a button press within the duration of a CS presentation response) of the white noise and counter incrementing.

The test phase was completed either when the learning criterion was achieved or after a total of 25 UCS presentations (i.e. unsuccessful learning). The overall score is the number of trials before they get five consecutive correct presentations of the CS/UCS pairings. Those who did not reach the learning criterion were assigned a final score of 21.

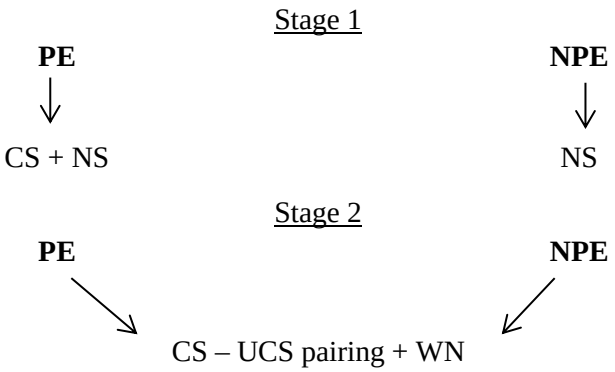

Figure 2: Figurative representation of the LI task

Kamin Blocking Effect

The KBE procedure is based on Serra *et al*'s (2001) study. The KB task involved four stages altogether. The School's programmer designed the task and each stimulus was presented at the centre of the screen for 1.5 seconds on a 9 x 7 inch Dell laptop screen at inter-stimulus intervals of: 2.5 s: 2 x 2 cm crosses and 2x2 squares (seven in total) of different colours (yellow, brown, navy blue, pink, green, purple and red); a pair of light grey 1 x 1 cm triangle vertical flankers were timely placed to display at the centre of the upper and lower margins of the screen, a pair of white 1 x 1 cm square horizontal flankers were timely placed to display at the centre of the left and right side margins of the screen. In addition to this, computer-generated white noises was randomly placed and displayed at different intensity and frequency levels (ranging from 70±80 dB), the white noise were normally presented along with each of the squares and for the same length of time, although there was an exception of this during the pre-exposure phase (see below non blocking condition). The KB task involved four stages altogether.

Stage One: Participants started with some basic keyboard skills to familiarise participants with the materials used, with some of the coloured crosses appearing with the noise. At this stage, participants were asked to press the space bar when they see a cross symbol on the screen.

Stage Two: The participants in the blocking (BL) but not the non-blocking (NBL) condition were introduced to the association between conditioned stimulus 1 (CS1; blue square) and the unconditioned stimulus (UCS; yellow square), as they had to predict when the yellow square would appear (on the computer screen) by pressing the space bar.

Stage Three **(Conditioning):** All participants in both conditions were then introduced to a new association which predicts the occurrence of the yellow square appearing (i.e. the UCS). When combined CS1 (blue square) and conditioned stimulus 2 (CS2; the horizontally placed light grey square flankers) predicted that the UCS will appear. Altogether there were 70 presentations of the coloured squares; included in both the BL and NBL conditions along with 20 trials of the CS1 & 2 preceded the UCS. Participants were instructed to press the space bar when they think the yellow square will appear. This stage ended either after the learning criterion was achieved, which was five consecutive correct responses (i.e. the correct pressing of the space bar preceding five presentations of CS1 and CS2 and the UCS); or the tested ended after 20 presentations of the yellow square, in total.

Stage Four (Test): All participants in the BL and NBL condition received the exact same stimuli. This time the blue square (CS1) was eliminated for predicting the occurrence of the UCS. Altogether there were 160 presentations of the coloured squares which were set to play in a randomised order. The presentation was set-up to include 40 squares (random colours) set to be displayed along with the horizontally placed light grey square flankers (CS2). After each presentation of the CS2 combined with any coloured square would now predict the occurrence of the yellow square appearing next. Participants were instructed to press the space bar when they thought the yellow square will appear. The overall score is the number of trials before they get five consecutive correct presentations of the CS2/UCS pairings. Those who did not reach the learning criterion were assigned a final score of 36; please refer to Figure 3 for a visual representation of this tasks design.

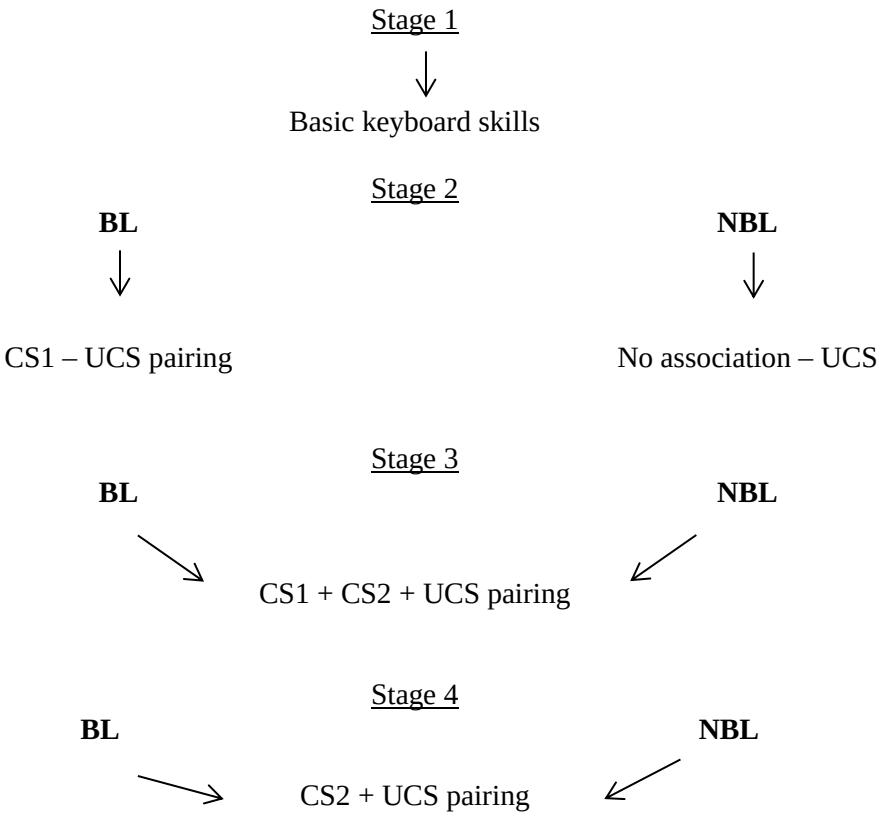

Figure 3: Figurative representation of the KB task

Questionnaires

The Severity of Dependency Scale (SDS; Gossop *et al*, 1995 – see appendix iv)

The SDS was used to assess possible dependency in the cannabis group. It measures compulsive use of a drug along with five questions which relate to the individual's perceived anxieties about their own drug use and feelings of impaired control of the over the use of the drug.

One example question is:

1. *Did the prospect of missing a smoke make you anxious or worried?*

Responses to each question is on a 4 point likert scale (e.g. question 1, 2 & 4 (0 = Never or almost never; 1 sometimes; 2 often; 3 always or nearly always). All items are added to give a total SDS score ranging from 0 – 15. The SDS has been previously used to reliably screen for

dependency and its severity in adults for a number of substances of abuse. Martin *et al*. (2006) verified the validity and reliability of this scale in participants (n = 100) for cannabis use and found good internal consistency (Chronbach's alpha of 0.83) and good test-retest reliability (intraclass correlation coefficient of 0.88).

Schizotypal Personality Questionnaire (SPQ-B; Raine & Benishay, 1995 – see appendix v)
The SPQ-B is an easy to administer questionnaire with 22 items and is based on the most reliable items from the original SPQ-B. The SPQ-B yields a total score along with three main sub-factors: cognitive-perceptual (example question: *Do you often pick up hidden threats or put-downs from what people say or do*), interpersonal (e.g. *People sometimes find me aloof and distant*) and disorganised thinking (e.g. *I find it hard to communicate clearly what I want to say to people*). There is good internal reliability of these sub-scales (ranging from .72 to .80, with a mean of .76 and test-retest reliability (.86 to .95, mean = .90). According to Raine and Benishay (1995) research indicates that the criterion validity was good for correlations between SPQ-B subscales and clinical interview measures of Schizotypal Personality Disorder: the total scale (.66) cognitive-perceptual (.73) and interpersonal (.63), but are lower for disorganised thinking (.36). Participants are required to respond to each question Yes or No; with each Yes response scoring a point. Total scores range from 0-22, cognitive-perceptual 0-8, interpersonal 0-8 and disorganised thinking 0-6.

UEL Drug Use Questionnaire and other participant information (Parrott *et al*., 2000) **– see appendix vi.**

All participants were required to give personal details of both their history and familial history of mental illness and whether they or their immediate family members has a current or had a past diagnosis of anxiety, depression, obsessive compulsive disorder, schizophrenia or paranoia, eating disorders, alcohol or drug dependency. This questionnaire also asked whether they have been hospitalised with brain trauma, or were taking any current medications. They were asked to answer further questions about current and past drug use for Ecstasy, Amphetamine, Cocaine, LSD, Magic Mushrooms, Poppers, Ketamine, GHB, Prozac, Crack, Opiates, Benzodiazepines, Anabolic Steroids, Solvents and other drugs. For each drug a yes/no response was required to indicate whether it had ever been taken; age of first use, how many times they had taken this drug, and how long ago they last consumed the drug. Questions about alcohol, tobacco and cannabis were more extensive to assess for weekly consumption. For

cannabis specifically, questions included which types of cannabis are consumed most often, why they choose to smoke their most consumed type, how many joints smoked per day/week, age of onset, who introduced them to the drug, duration of use, the last time they smoked cannabis and whether they had perceived any acute psychological/health problems from using higher doses of cannabis.

2.2.3 The Experimental Procedure:

Informed consent was obtained and the participants signed the consent form; thereafter the study consisted of three phases:

Phase 1: All participants providing a saliva sample and for drug testing using the Multi-drug One Step: Multi-line 6 Drug Screen Test Device (Oral Fluid) (from drugtesting.co.uk) which includes a test device, collectors (sponge applicator), tubes for collecting sample. Oral fluid specimens were collected using the sponge end of the collector was placed inside the mouth and rubbed around in the inside of their mouth and tongue for a total of three minutes until the sponge becomes saturated. The sponge collector was then removed and fully strained the oral liquid into the collector tube. The oral fluid was transferred by adding three drops of the sample (approximately 100 microlitres) into each well of the test device. Participants are informed not to place anything in their mouth prior to testing (including: gum, food, drink, tobacco products) for at least ten minutes prior to testing. The device was read after ten minutes and a digital picture was taken and stored as a record of the test. All samples were then disposed of through UEL hazardous waste procedures.

Phase 2: Buccal swabs were used in each participant to extract some cheek cells, for subsequent DNA extraction and screening. This procedure and the data obtained are detailed and explored in Chapter 4.

Phase 3: Testing: Administration of the cognitive tests (LI and KB). The participants were counterbalanced for each test: 1) PE condition for LI task and NBL condition of the KB test, or 2) NPE condition for the LI task and BL condition of the KB test. On completion, the participants were asked to complete the SPQ-B, SDS and UEL Drug Use questionnaires.

The study lasted up to 1.5 hours for each participant.

2.3 Results

A primary dataset for the LI task and the KB tasks were analysed and Table 2 highlights the demographics for these groups. However, the primary dataset was changed and revised with five non-cannabis using participants added for a secondary analysis. Three participants were removed based on previous lifetime history of cannabis use in the past 20, 105 and at least 250 times respectively. Further to this, two participants were also excluded who failed to meet the learning requirements for the task (i.e. learn the association between the white noise and counter incrementing and were assigned a final score of 21). Four of the new non-cannabis using participants were allocated to the NPE condition and one to the PE condition. The following results therefore include information from the primary data set for the LI, KB, and drug use variables, in addition to this revised data set, a secondary analysis was performed on the LI and drug use data only; the KB task was not repeated due to complete ceiling effects found in both groups (cannabis users and non-cannabis users).

Data Screening

Normality of variables for the SPQ-B measure and LI, KB data

According to Tabachnick and Fidell (2007) convention alpha levels at 0.01 or 0.001 are used to evaluate the level of skewness and kurtosis with small to moderate samples. All of the SPQ-B scales were positively skewed; the KB and LI were bi-modal. These data were then transformed using logarithms and square roots functions. No substantive difference was found between the transformed and untransformed data, therefore only results using the untransformed data are reported both for the primary and secondary analysis.

2.3.1 Primary analysis for demographic/health details and patterns of drug use

Possible demographic differences between the groups (cannabis and non-cannabis users) were assessed using a t-test analysis for age and Chi^2 analyses for all other measures. From Table 2 it can be seen that no significant differences were found between the cannabis group and non-cannabis using group for demographic descriptors (all $p>0.05$), except nationality; with significantly more British than non-British people in the non-cannabis group ($p=0.028$).

Table 2: Primary results for participants' demographics and personal and familial health information

	Non-cannabis group (20 Participants)	**Cannabis group (20 Participants)**	**Test *T X²***	***p***
Age Range, Mean (SD)	19-45, 28 (7.8)	18-42, 31.85 (7.4)	1.479	Ns
Gender (n=Males/Females)	9/11	14/6	2.55	Ns
Nationality (British/Non British) (n =)	15/5	9/11	3.656	**0.028**
Ethnicities (White European/Black/Asian) (n =)	16/3/1	19/1/0	2.25	Ns
Occupation (Employed/Unemployed/Student) (n =)	11/1/8	9/2/9	0.592	Ns
Health Rating (Poor/Moderate/Fine /Good) (n =)	0/3/7/10	0/3/8/9	0.119	Ns
Personal Mental Health History (diagnosis) (n =)	3	5	0.625	Ns
Familial Mental Health History (diagnosis) (n =)	11	8	0.902	Ns
Brain Injury (yes) (n =)	0	0	-	-
Medication (yes) (n =)	1	0	-	-

Ns = not statistically significant (p>0.05).

Table 3(i) and 3(ii) below shows reported lifetime drug use. Differences between groups for reporting use, amount of use and age of first use/onset were assessed using Mann Whitney U tests (a non-parametric test). Cannabis users reported significantly more lifetime polydrug use (includes having taken two drugs together over a period of time such as MDMA, amphetamine and/ or cocaine: p=0.01). Compared to the non-cannabis users, the cannabis group reported greater use of tobacco, cocaine and MDMA, earlier onset and had higher lifetime use of these aforementioned drugs. There was a trend in the same direction for amphetamine use. Cannabis users were also more likely to have used LSD (p=0.019), Poppers (p= 0.005), ketamine (p=0.09) and Crack cocaine (p=0.04), but no difference for alcohol use, in terms of age of onset, frequency and duration of use (p>0.05).

Table 3(i): Primary results for participants' information about lifetime and current drug use (part A)

Variables	Non-cannabis group (n=20)	Cannabis group (n=20)	U	p (2-tailed)
Cigarettes (n= Yes)	4	13	110.00	**0.004**
Cigarettes/day Mean(SD)	9.50 (7.93)	10.38 (4.77)	105.00	**0.004**
Cigarettes - age of onset Mean(SD)	15.2 (1.70)	16.1 (3.26)	106.00	**0.005**
Cigarettes - * last time used (days)	1(1); 2(1); 10(1)	1(9); 2(2); 7(1); 30 (1)	115.00	**0.01**
Alcohol (n= Yes)	17	19	180.00	**0.01**
Alcohol (units per day) Mean(SD)	9.36 (6.61)	21.5 (39.4)	170.5	Ns
Alcohol – age of onset Mean(SD)	17.31 (3.59)	16.8 (3.72)	175.50	Ns
Alcohol – last time (days)	1(4); 2(1); 5(2); 7(2); 12(2); 14(2); 31(1); 62(1)	1(8); 2(2); 3(3); 4(1); 7(2); 14(2); 120(1)	174.00	Ns
MDMA (n= Yes)	4	14	100.00	**0.002**
MDMA – age of onset Mean(SD)	24 (5.5)	20.1 (4.62)	113.5	**0.01**
MDMA – number of times used Mean(SD)	7.37 (8.51)	144.5 (269.56)	89.0	**0.001**
MDMA – last time used days (SD)	12.7 (3.4)	10.9 (4.58)	105.5	**.005**
Poppers (n= Yes)	5	10	150.0	Ns
Poppers times Mean(SD)	4.6 (4.15)	24.5 (34.4)	138.0	0.054
Ketamine (n= Yes)	0	6	140.0	**0.009**
Ketamine times Mean(SD)	-	22.6 (38.4)	-	-
GHB (n= Yes)	0	2	180.0	Ns
GHB times Mean(SD)	_	1.5(.70)		-
Prozac (n= Yes)	1	0	190	Ns
Prozac times Mean(SD)	0	0	-	-
Poly Drug Use (n= Yes)	3	15	90.0	**0.01**
Current Poly Drug Use (n= Yes)	0	6	-	-

Table 3(ii): Primary results for participants' information about lifetime and current drug use (part B)

Variables	**Non-cannabis group (n=20)**	**Cannabis group (n=20)**	**U**	**P**
Amphetamine (n= Yes)	3	8	150.0	.082
Amphetamine – age of onset Mean(SD)	18.3 (0.57)	18.6 (3.7)	149.5	.082
Amphetamine –times Mean(SD)	13.6 (10.96)	100 (158.49)	146.5	0.066
Amphetamine – last time	-	13.6 (3.54)	-	-
Cocaine (n= Yes)	6	15	110.0	**0.005**
Cocaine– age of onset Mean(SD)	22.2 (4.79)	21.2 (3.8)	115.5	**0.015**
Cocaine – times Mean(SD)	6.1(7.5)	108.4 (172)	130.0	**0.001**
Cocaine – last time used Mean(SD)	1-6 months (2); 3years+(4)	1-2 weeks (2); 1-6months(7); 18-24months(2); 3 years (4)	27	Ns
LSD (n= Yes)	1	7	140.0	**0.019**
LSD- times Mean(SD)	-	8.1 (6.46)	138.8	**0.016**
Benzo (n= Yes)	1	5	160.0	0.08
Benzo- times Mean(SD)	-	5.20 (5.63)	161.0	0.08
Mushrooms (n= Yes)	5	8	170.0	Ns
Mushroom times Mean(SD)	1.6 (0.54)	8.25(6.56)	153.0	**0.001**
Crack (n= Yes)	0	4	160.0	**0.037**
Crack- times Mean(SD)	-	7.25 (6.39)	-	-
Opiates (n= Yes)	1	2	190.0	Ns
Opiate times Mean(SD)	-	4.75 (3.88)	-	-
Steroids (n= Yes)	0	0	-	-
Steroid times Mean(SD)	-	-	-	-
Solvents (n= Yes)	0	2	189.5	Ns
Solvent times Mean(SD)	-	1.5(.70)	-	-

Table 4 reports cannabis use data for both groups. As can be seen the non-cannabis group did report some lifetime use of the drug (n = 6), onset was between the ages of 14-18 years, and last use of the drug was 12 or more years ago. Three of the non- cannabis users were removed in the secondary analysis for long duration and heavy cannabis use in the past (from section 2.3.2).

Table 4: Primary results for participants' information about current and lifetime cannabis use

Variables	**Non-cannabis group**	**Cannabis group**
Cannabis use (current/past/never) (n=)	0/6/14	20
Amount of joints per day Range, Mean (SD)	1-7, 0.45 (1.57)	1-5.50, 2.52 (3.18)
Frequency of use (n=)	2-3 times per month (1); once a month (1) less than once a month (4)	Everyday (3); Almost everyday (7); 3-4 times per week (5); 1-2 per week (2); 2-3 times per month (2); once a month (1)
Cannabis age of onset range & mean (SD)	14-18, 15.16 (1.6)	12-30, 17.4 (4.19)
Cannabis introduction (n=)	Friends = 4; Family = 2	Friends = 16; Family = 4
Cannabis last time (n=)	12 years plus = 6	2 days = (9); 3-7 days = (5); 14 days plus= (3)
Cannabis acute problems (n=)	Yes = 2; No = 4	Yes = 9; No = 11
Types used most often (n=)	Skunk = 4; Grass = 2	Skunk = 9; Grass = 5; Resin = 6
Cannabis duration (n=)	5 years = 1; 7 years = 1	1-3 years (3); 4- 7 years (5); 9-14 years (6); 15 years plus (6)
Cannabis Dependency Score (range & mean (SD)	-	0-12; 3.35 (3.18)

2.3.2 Primary analysis for cognitive outcomes

Latent inhibition

Overall latent inhibition performance, and the number of participants actually learning the association, was compared between *Groups* using a Chi^2 analysis. The cannabis users were far more likely to have learnt the association (i.e. found the association between the white noise and counter incrementing) than the non-cannabis group (p=0.014).

A 2x2 ANOVA was used to look at main effects and possible interactions between *Group* and *Condition* (Pre-exposed versus Non Pre-Exposed). The ANOVA on the primary data set revealed a statistically significant main effect of group (cannabis versus non-cannabis) on LI, (F (1, 36) = 10.3, p = 0.003). In sum, LI was abolished in the cannabis group with no significant difference found between the PE and NPE conditions, whereas this was not the case for the non-cannabis group. This was confirmed by post-hoc follow-up t-tests which revealed a trend towards significance in non-cannabis users with lower scores in the PE condition (t (18) = 1.35, p=0.08), but no differences in the cannabis group in PE and NPE trials (t (18) =.072, p = 0.47). It seems that cannabis users performed better overall on the task and were not affected by the PE condition of the task relative to the NPE condition and relative to the non-cannabis group (see Figure 4 below). No main effect of task was found for condition (PE versus NPE) (F (1, 36) =1.834, p > 0.05) and no interaction was found (F (1, 36) = 1.55, p > 0.05). A follow-up t-test revealed that there was a significant difference between the cannabis users and non-cannabis users in the NPE condition (t (19) = 3.39, p = 0.01), but not in the PE condition (t (17) = 1.32, p > 0.05), with cannabis users performing better under this condition. Please refer to Figure 4 below for a graph illustration of LI performance between cannabis users and non-cannabis users under the PE and NPE conditions.

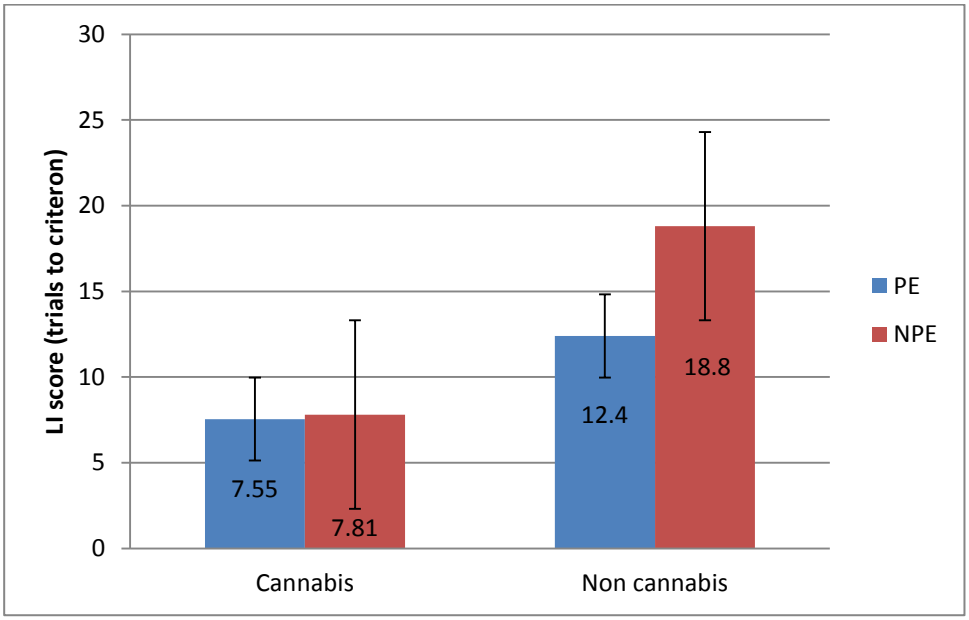

Figure 4: Primary results for Latent inhibition: mean LI scores for cannabis and non-cannabis group in the PE and NPE conditions. LI score represents the mean trials to criterion for finding the association between the white noise and the counter incrementing.

Kamin Blocking

The Kamin Blocking data were analysed using the same methods as for the Latent Inhibition data. There was no difference in learning the association between the groups for the KB task (p>0.05). A 2x2 ANOVA test was carried out to look at the main effects of group (cannabis versus non-cannabis) on condition (BL versus NBL) - refer to Figure 5 below. The preliminary ANOVA revealed no main effect of group (cannabis versus non-cannabis) on KB performance ($p > 0.05$) and no main effect of condition, BL versus NBL ($p > 0.05$). There was a trend towards an interaction (F (1, 36) = 3.53, p = 0.06), with the non-cannabis using group being less affected by the BL condition on their KB performance and this group performed better under the BL condition as opposed to the NBL condition (indicating an abolished KB effect), whereas the regular cannabis users took more time to find the paired association in the BL condition relative to the NBL condition (indicating a normal KB effect). However, on closer inspection it can be seen that the mean scores appeared to be ceiling effects in each of the conditions; the highest possible score is pre-set at 36, with the range of scores for this study falling between 22-36; 45% of the sample obtained the highest score of 36. A Chi^2 analysis revealed that there was no significant difference between cannabis users and non-users for learning the association overall. Therefore, interpretation of these data should consider the high overall means and that there was no significant difference between the groups (cannabis user versus non-cannabis user) for correctly learning the association on the KB task.

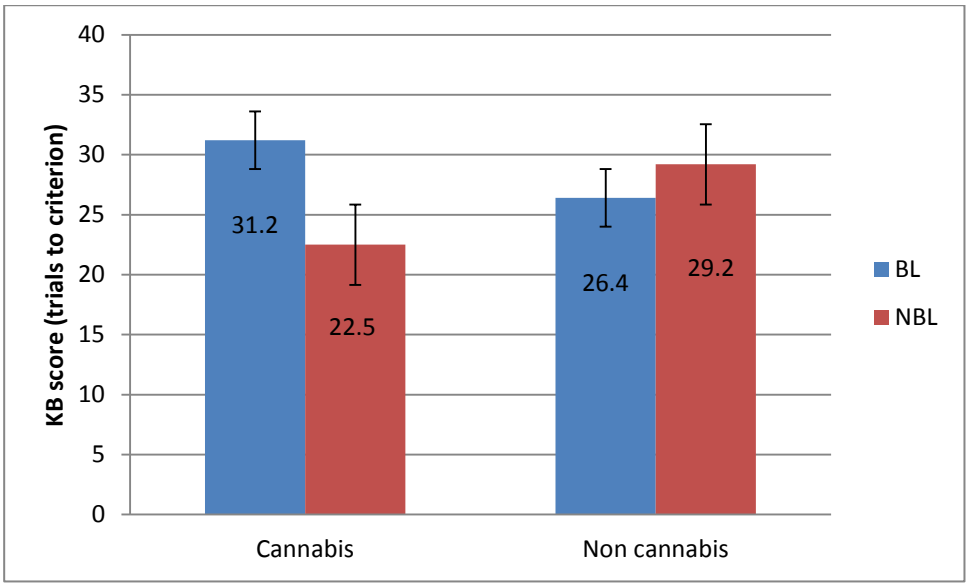

Figure 5: Primary results for Kamin blocking: mean KB scores in cannabis and non-cannabis groups in the BL and NBL conditions.

2.3.3 Primary Analysis for SPQ-B data analyses

The Schizotypal Personality Questionnaire data was assessed using t-tests. There were no significant differences between the cannabis users and non-cannabis users on the SPQ-B total and sub-factor scores (all $p > 0.05$; see Table 5). However, some exploratory analysis revealed differences between the conditions of tasks PE (and NBL) and NPE (and BL) conditions, with those in the PE (and NBL) condition reporting a significantly higher total SPQ-B score (p=0.018).

Table 5: Primary results for a breakdown of participants' SPQ-B variables.

Variables	**Non-cannabis group (n=20)**	**Cannabis group (n=20)**		
	Mean (SD)	Mean (SD)	*T*	*p*
SPQ-IP	1.65 (1.77)	1.8 (0.4)	1.05	Ns
SPQ-CP	1.8 (0.41)	1.75 (0.4)	3.70	Ns
SPQ-DT	1.8 (0.41)	1.85(0.36	4.06	Ns
SPQ-total	6.8 (5.09)	6.3 (0.36)	3.43	Ns
SPQ-total PE (and NBL)/NPE (and BL) conditions	8.31(4.85)/ 4.95(3.69)	8.6(4.15)/ 4.36(2.90)	6.152	**0.018**

2.3.4 Primary analysis for high/low SPQ-B and associative learning task performance

Although there were no clear differences between the cannabis users and non-users for the SPQ-B scores, this variable was subsequently utilised to explore possible effects of schizotypy on associative learning. The group were assessed for high versus low SPQ-B and division of groups was based on the same method used by Laws *et al* (2008). Participants were divided into high/low scorers on the SPQ-B total, for two non-overlapping groups. The SPQ-B mean was 6.5, and the scores ranged from 0-18, thus 5 people were removed who had a score between 6-8; this left 20 people with scores from 0-5 and 14 people with scores from 9-18.

A 2x2 ANOVA assessed possible differences between the *groups* for SPQ-B total scores under both LI *Conditions.* The groups were broken down initially into groups (cannabis versus non-cannabis) for high/low SPQ-total score, but these were too small and imbalanced to make viable statistical comparisons across the conditions (PE versus NPE). Therefore the entire group overall (pooling together cannabis and non-cannabis users) was divided into high/low SPQ-B groups for LI performance (refer to Table 6). A 2 X 2 ANOVA found no main effect LI performance (F (1, 30) =1.511, $p > 0.05$), but did show a main effect for SPQ-total group (high versus low scorers) (F (1, 30) = 4.634, $p = 0.04$) and no significant interaction between LI (PE versus NPE) and SPQ-total group (high versus low scorers; F (1, 30) = 2.577, $p > 0.05$). Follow-up t-tests were conducted and revealed no significant differences between low versus

high SPQ-B scorers in the PE condition (t (15) = 0.692, $p > 0.05$), but a significant difference was found between low and high SPQ-B scorers in the NPE condition, with low SPQ-B scorers taking fewer trials overall to find the paired association t (15) = 4.567, $p < 0.001$). No significant difference was found between conditions (PE versus NPE) for low SPQ-B (t (18) = 0.283, p > 0.05), whereas there was a significant difference between the conditions (PE versus NPE) for high SPQ-B scorers *(t* (10) = 3.198, p = 0.005) who took less time overall to find the paired association under the PE condition.

Table 6: Primary results showing mean LI performance in groups scoring high and low on the SPQ-B

SPQ-total	**High**	**Low**	**High**	**Low**		
	PE		**NPE**		***F***	***P***
LI scores mean (SD)	11.3(8.5)	9.57(8.8)	20.2(1.5)	8.38(8.96)	4.634	**0.04**
(N =)	10	7	4	13		

The same analysis was run for KB outcomes on high/low SPQ-B scores – refer to Table 7. No main effects were found for KB (BL versus NBL) on KB performance (F (1, 30) = 0.348, $p >$ 0.05), no main effect was found for SPQ-total group (high versus low scorers) (F (1, 30) = 0.013, $p > 0.05$); no interaction found between KB (BL versus NBL) and SPQ-total group (high versus low scorers) F (1, 30) 0.054, $p > 0.05$. No follow-up tests were done due to lack of differences found between the group and conditions.

Table 7: Primary results for high versus low SPQ-B scores on KB outcomes.

SPQ-total	**High**	**Low**	**High**	**Low**		
	BL		**NBL**		**F**	***p***
Kamin Blocking Scores	29(8.4)	28.5(10.3)	25.8(11.5)	27.14(9.2)	0.013	**Ns**
(N =)	4	13	10	7		

2.3.5 Secondary analysis for demographic/health details and patterns of drug use

A number of interesting findings were found with the LI data, but there were issues around overlap in cannabis use between the groups and the non-cannabis group appeared to be performing quite poorly compared to the cannabis group. Therefore the primary dataset was revised to remove 5 non-cannabis users. It was 3 of the non-cannabis users who reported previous lifetime use of cannabis, and in addition to this, 2 participants were also removed who had failed to meet the learning requirements for the task (correctly learning the paired association) in the NPE condition. Therefore, 4 new participants were allocated to the NPE condition and one participant to the PE condition. Statistical analyses were the same as those conducted in the primary analyses. Please see Table 8 for demographics of the cannabis and non-cannabis users. The secondary analysis did not include re-examination of the KB task data due to overall poor performance as 50% of the sample reached a ceiling effect of a score of 33, 34, 35 & 36. With the KB data there were no marked differences between groups and so further analyses would be unlikely to reveal any additional findings.

From Table 8, with the removal of the non-cannabis users based on past drug use, this reduced the amount of people in the non-cannabis group reporting a Personal Mental Health History (PMHH) from 3 (in the primary analysis) to 0 (in the new secondary analysis) and this left a significant group difference ($p = 0.04$) between non-cannabis users and the cannabis group. There was also a gender difference between both groups, with significantly more males in the cannabis using group ($p = 0.05$). Compared to the primary analysis there was now no difference between the number of British/non British participants between the cannabis users and non-cannabis users.

Table 8: Represent the secondary results for demographic and drug use details for controls versus the cannabis group

Variable	**Non-cannabis group (20 Participants)**	**Cannabis group (20 Participants)**	***T X²***	***P***
Age Range, Mean (SD)	19-42, 30 (6.8)	18-42, 29 (7.7)	-.801	Ns
Gender (Males/Females) (n=)	7/13	14/6	4.91	**0.05**
Nationality (British/Non British) (n=)	11/9	9/11	0.400	Ns
Ethnicities (White European/Black/Asian/Mixed Race) (n=)	14/3/2/1	19/1/0/0	1.495	Ns
Occupation (Employed/Unemployed/Student) (n=)	11/2/7	(9/2/9)	0.450	Ns
Health Rating (Poor/Moderate/Fine /Good) (n=)	0/1/7/12	(0/3/8/9)	1.495	Ns
Personal Mental Health History (diagnosis) (n=)	0	5	5.174	**0.04**
Familial History (diagnosis) (n=)	9	8	.102	Ns
Brain Injury (yes) (n=)	0	0	-	-
Medication (yes) (n=)	0	0	-	-

Tables 9(i) and 9(ii) below show that the cannabis group frequently reported use of other drugs such as MDMA, ketamine, crack, solvents and LSD; they had significantly more tobacco smokers ($p = 0.001$) with earlier onset of tobacco smoking ($p = 0.002$) and smoked tobacco more often ($p = 0.001$), which is in line with the primary analysis. Whereas the secondary analysis now revealed a difference between alcohol use, with the cannabis group reporting higher use of alcohol per week ($p = 0.02$).

From Table 10, it can be seen that the majority of the cannabis group ($n = 12$) last used cannabis at the specified 2 days abstinence period, with the rest of the sample (n = 8) from 3 - 14 days prior to the testing session. Nearly half of the cannabis sample reported acute problems with higher doses of cannabis and the sample mainly used the skunk variety ($n = 9$). Cannabis dependency scores were on average 3.35 (out of 12); and a score of over 4 is seen as indicative of dependency to the drug.

Table 9(i): Secondary results for participants' information about lifetime and current drug (part A)

Variables	**Non-cannabis group**	**Cannabis group**	**U**	**P**	**Variables**	**Non-cannabis group**	**Cannabis group**	**U**	**P**
Cigarette (Yes) (n=)	3	13	100.0	**0.001**	MDMA (Yes) (n=)	3	14	90.0	**0.01**
Cigarette/day Mean (SD)	6(4.58)	10.38 (4.77)	89.0	**0.001**	MDMA – age of onset Mean (SD)	19.6(5.5)	20.14(4.62)	89.0	**0.001**
Cigarettes - age of onset Mean (SD)	16(1)	16(3.26)	101.0	**0.002**	MDMA – number of times used Mean (SD)	7.66 (10.69)	144.5 (269)	77.5	**<0.001**
Cigarettes - last time used	1 day (1); 2 days (1); 10 days (1)	1 day (9); 2 days (2); 7 days (1); 30 days (1)	107.0	**0.004**	MDMA – last time used	1-6 months(1); 18-24months(1); 3years+(1)	1-2 weeks(1); 1-6months(5); 18-24months(1); 3years+(7)	20.5	Ns
Alcohol (Yes) (n=)	13	19	140	**0.019**	Amphetamine (Yes) (n=)	1	8	130	**0.009**
Alcohol (units per week) Mean (SD)	7.46 (6.89)	20.55 (38.6)	140	**0.021**	Amphetamine – age of onset Mean (SD)	-	18.6(3.7)	130.5	**0.01**
Alcohol – age of onset Mean (SD)	16.5 (15.9)	16.8 (3.72)	170	Ns	Amphetamine – number of times used Mean (SD)	-	100 (158)	126.5	**0.007**
Alcohol – last time Mean (SD)	1(3); 2(1); 5(2); 7(1); 12(1); 14(1); 31(1); 62(1)	1(8); 2(2); 3(3); 4(1); 7(2); 14(2); 120(1)	149.5	Ns	Amphetamine – last time used	_	1-6months(1); 6-18months(1); 3years+(5)	-	-

Table 9(ii): Secondary results for participants' information about lifetime and current drug (part B)

Variables	**Non-cannabis group**	**Cannabis group**	**U**	**P (2-tailed)**	**Variables**	**Non-cannabis group**	**Cannabis group**	**U**	**P (2-tailed)**
Cocaine (Yes) (n=)	3	15	80.0	<**0.001**	Ketamine (Yes) (n=)	0	6	140	**0.009**
Cocaine– age of onset Mean (SD)	23.6 (10.96)	21.2(3.8)	86.5	**0.001**	Ketamine times Mean (SD)	-	22.6(38.4)	140	**0.009**
Cocaine – number of times used Mean (SD)	7.3 (10.96)	108.4(172)	-66.5	<**0.001**	GHB (Yes) (n=)	0	2	180	Ns
Cocaine – last time used	1-6months(1); 3years+(2)	1-2 weeks(1); 1-6months(8); 6-18months (2); 3years+(4)	14	Ns	GHB times Mean (SD)	-	1.5(0.70)	180	Ns
LSD (Yes) (n=)	0	7	130.0	**0.04**	Prozac (Yes) (n=)	1	0	190	Ns
LSD times Mean (SD)	-	8.1(6.46)	130.0	**0.04**	Prozac times Mean (SD)	-	-	190	Ns
Benzo (Yes) (n=)	0	5	150.0	**0.018**	Crack (Yes) (n=)	0	4	160.0	**0.037**
Benzo times Mean (SD)	-	5.20 (5.63)	150.0	**0.019**	Crack times Mean (SD)	-	7.25 (6.39)	160	**0.038**
Mushrooms (Yes) (n=)	3	8	150.0	Ns	Opiates (Yes) (n=)	0	2	180	Ns
Mushroom times Mean (SD)	1.33 (0.55)	8.25 (6.56)	139.0	**0.036**	Opiate times Mean (SD)	-	4.75 (3.88)	180	Ns
Poppers(Yes) (n=)	2	10	120.0	**0.006**	Steroids (Yes) (n=)	0	0	-	-
Poppers times Mean (SD)	4.5(4.94)	24.5(34.4)	114.5	**0.004**	Steroid times Mean (SD)	-	-	-	-
Solvents (Yes) (n=)	0	2	180.0	Ns	Past poly drug use (Yes) (n=)	2	13	90.0	<**0.001**
Solvent times Mean (SD)	-	1.50(0.707)	180.0	Ns	Current poly drug use (Yes) (n=)	0	8	120.0	**0.002**

Table 10: Secondary results for current and lifetime cannabis use

Variables	**Non-cannabis group (n=20)**	**Cannabis group (n=20)**
Cannabis use (current/past/never)(N=)	(0/3/17)	20
Amount of joints per day Mean (SD)	-	2.52 (3.18)
Frequency of use	-	Everyday (3); Almost everyday (7); 3-4 times per week (5); 1-2 per week (2); 2-3 times per month (2); once a month (1)
Cannabis age of onset	15-16	17.4 (4.19)
Cannabis introduction	friends (3)	Friends (16); Family (4)
Cannabis last time (N=)	12 years plus (3)	2 days = (12); 3-7 days = (5); 14 days plus= (3)
Cannabis acute problems (with higher doses of cannabis)(N=)	No (3)	Yes (9); No (11)
Types used most often	-	Skunk (9); Grass (5); Resin(6)
Cannabis duration (N=)	-	1-3 years (3); 4- 7 years (5); 9-14 years (6); 15 years plus (6)
Cannabis Dependency Score Mean(SD)	-	3.35 (3.18)

2.3.6 Secondary analysis for LI performance

In contrast to the primary analysis there was no significant difference found between the cannabis and non-cannabis users for the number of people finding the association between the white-noise and the counter incrementing on the LI task. In contrast to the primary analysis, a normal LI effect was now found in the non-cannabis group, with faster learning in the NPE compared to the PE condition. These data in Figure 6 show that overall the non-cannabis users, in both conditions, took longer to reach the learning criterion compared to the cannabis users. An ANOVA was carried out to see if there was an interaction between group (cannabis versus non-cannabis) and condition (pre-exposed versus pre-exposed), no interaction was found (p = 0.33). There was no main effect of LI for condition (F (1, 36) = 7.81, $p>0.05$), a main effect was found for group (F (1, 36) = 5.83, p = 0.02), with cannabis users taking significantly less time overall to find the association versus those in the non-cannabis group. Follow up t-tests

revealed a significant difference between non-cannabis users in the PE versus NPE conditions (t (18) = 1.36, p = 0.009). Whereas there was no difference between the cannabis group in the PE and NPE conditions (t (18) = 0.72, p > 0.05). A second follow up t-test revealed that there was a significant difference between cannabis users and non-cannabis users on the PE condition (t (17) = 2.58, p = 0.01), with cannabis users taking less time to find the paired association. There was no significant difference found between the Group in the NPE condition (t (19) = 0.970, p > 0.05).

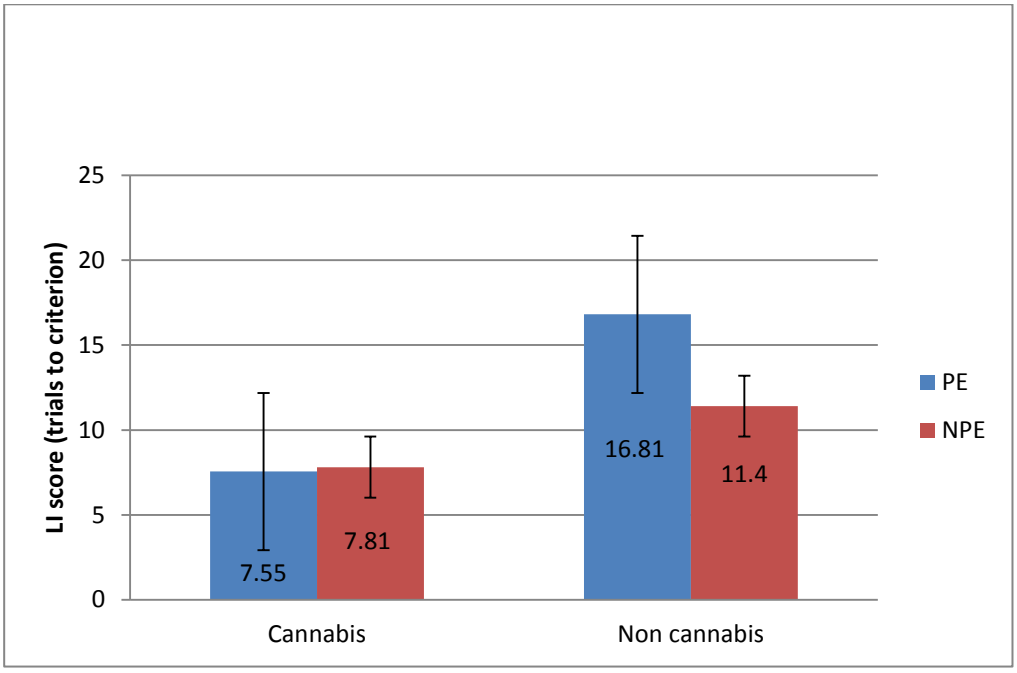

Figure 6: Compared to figure 4, this figure is the secondary result of mean learning scores across group (cannabis versus non-cannabis) and conditions PE (pre-exposed) versus the NPE (non pre-exposed).

2.3.7 Secondary analysis for SPQ-B data analyses

From Table 11, it can be seen that the results were the same for the primary and secondary analysis for SPQ-B outcomes. There were no statistically significant differences between the group (cannabis versus non-cannabis user) for individual differences in psychotic-like traits assessed via the SPQ-B total score and three subscales (all $p > 0.05$). Table 12 shows the 2 *X* 2 ANOVA for main effect of condition for SPQ-B total for cannabis users versus non-cannabis user under the PE condition versus those in the NPE condition. It seems that there was an effect of condition, in that those in the pre-exposed condition (and counterbalanced NBL for the KB task) scored higher on the SPQ-B measure compared to those in the NPE (BL) and this was statistically significant ($p < 0.05$). There was no main effect of cannabis on SPQ-B scores or interaction between both Condition and Group ($p > 0.05$).

Table 11: Represents the secondary data set for SPQ-B scores between cannabis users and non-cannabis user, and for SPQ-B subscales under each condition of the LI task

Variables SPQ-B scores	Non-cannabis group	Cannabis group	F	P
SPQ–IP Mean (SD)	2.5 (1.7)	2.0(1.7)	0.865	Ns
SPQ-CP Mean (SD)	2.5(1.7)	2.5(1.9)	0.007	Ns
SPQ-DT Mean (SD)	2.0(2.2)	1.7(1.7)	0.161	Ns
SPQ-total Mean (SD)	7.05(5.36)	6.3(4.06)	0.248	Ns
SPQ-total PE and NBL/NPE and BL Mean (SD)	9.2(5.94)/ 4.9(3.9)	8.6(4.15)/ 4.36(2.90)	9.79	**0.003**

2.3.8 Secondary analysis for high/low SPQ-B and associative learning task performance

Similar to the primary analysis the group was broken down into high/low SPQ-B scores based on the same method as Laws *et al* (2008). The mean score was 6.67 and the scores ranged from 0-18, 4 people were removed who scored between 6-8, with 22 participants in the low SPQ-B group who scores ranged from 0-5, and 14 in the high SPQ-B group who scores ranged from 9-18. A 2X2 ANOVA was therefore carried out to see if there was any effect of high/low SPQ-B on LI performance. It was found that there was no main effect of LI condition (PE versus NPE) (F (1, 32) = 0.264, $p > 0.05$), a main effect was found for SPQ-total into high/low

for LI performance (F (1, 32) = 6.32, p = 0.017), and an interaction was found between SPQ-total high/low for LI (PE versus NPE) on LI performance (F (1,32) = 4.28, p = 0.05) – refer to Table 12.

A follow-up t-test revealed that there was no significant difference between low and high SPQ-B scorers in the PE condition (t (15) = 0.77, p > 0.05), whereas a significant difference was found between low and high SPQ-B scorers in the NPE condition, with low SPQ-B scorers taking less time to find the paired association relative to the high scorers (t (17) = 3.573, p = 0.001). There was no significant difference found for low SPQ-B scores between the LI conditions (PE versus NPE), (t (20) =1.358, p > 0.05). Whereas the high SPQ-B scorers took less time in the PE condition rather than the NPE condition (t (12) = 2.81, p = 0.008), thus those scoring high on the SPQ-B measure seem to represent an abolishment of LI in this group. However, it should be noted that large means were found for the high SPQ-B scorers in the NPE condition which may represent a ceiling effect. Lack of differences found under the PE condition between high/low scorers might give an indication that the differences found with the cannabis group on LI performance may be a specific effect of the drug as opposed to higher/lower SPQ-B traits.

Table 12: Secondary results for participants' LI performance and SPQ-B (high/Low)

SPQ-total	**High**	**Low**	**High**	**Low**		
	PE		NPE		***F***	***P***
LI scores mean (SD)	12.2(8.6)	10.8(9.02)	20(1.7)	6.12(6.54)	**6.32**	**0.017**
(N =)	11	6	3	16		

2.3.9 Correlations for cognitive, trait and cannabis use variables in the primary and secondary analyses

Finally, a series of correlations were carried out to explore the SPQ-B traits on LI scores and KB scores in primary data analysis (see Table 13) and then for SPQ-B traits and LI scores in the secondary analysis (see Table 14). From Tables 13 and 14, it can be seen that all SPQ-B measures correlated with one another (p < 0.001). In the primary data set it can be seen from

Table 13 that none of the SPQ-B traits was related to KB performance (all $p > 0.05$). None of the SPQ-B variables in the primary analysis was linked to LI performance (all $p > 0.05$), whereas in the secondary analysis the SPQ-B total, and subscales of Interpersonal and Cognitive Perceptual was linked to LI performance (all $p < 0.05$), in that those with higher scores on the SPQ-B measure overall, and for the two subscale IP and CP also was linked to higher scores in the LI task. Higher scores on the LI task represent that it took people longer to find the paired association, so performed less well on the task. Cannabis use variables, in terms of dependency, frequency and duration of use were explored in relation to SPQ-B variables and LI/KB performance (see Table 15). The measure for cannabis dependency was associated with the SPQ-B - IP subscale which is related to interpersonal traits. People who scored more highly on the measure for problems with their cannabis use also reported higher schizotypal traits related to negative symptoms associated with schizophrenia. Higher numbers of joints smoked per week was related to higher SDS and increase in schizotypal traits, which includes the subscales of SPQ-IP, SPQ-CP and SPQ-DT. Age of onset of cannabis use was negatively associated with scores on the SPQ-DT subscale, with earlier onset of the drug related to higher scores on this third subscale of disorganised thinking. Also earlier onset of the drug was associated with lower scores on the KB task. Seven out of twenty of the cannabis cohort reported a Cannabis SDS of four and above and this is seen to be indicative of being dependent on cannabis use, however these regular drug users were able to adhere to the abstinence period for a minimum of two days without using cannabis. The SPQ-B traits were not associated with the LI performance in the cannabis group (all $p>0.05$). Therefore a final correlation analysis (not shown in the tables below) was run for the non-cannabis group only and it was found that LI performance was linked to all of the SPQ-B measures. LI performance was positively associated with SPQ-B total, (r (20) = 0.422, p = 0.03), interpersonal (r (20) = 0.40, p = 0.04), disorganised thinking (r (20) = 0.393, p = 0.04) and a trend for a positive association between cognitive perpetual scores and LI performance (r (20) = 0.349, p=0.006). Interestingly, this was not the same for the cannabis group for traits linked to LI performance.

Table 13: Correlational data in primary analysis for SPQ-B traits, LI and KB outcomes.

Variable	SPQ-total	SPQ-CP	SPQ-IP	SPQ-DT	LI score	KB score
SPQ-total N =	1	**0.795**** 40	**0.764**** 40	**0.817**** 40	0.174 40	0.020 40
SPQ- CP N =		1	**0.378**** 40	**0.528**** 40	0.241 40	0.134 40
SPQ-IP N =			1	**0.418**** 40	0.149 40	0.107 40
SPQ-DT N =				1	0.026 40	-0.195 40

**. Correlation is significant at the 0.01 level (1-tailed).

Table 14: Correlational data exploring the secondary analysis for SPQ-B traits and LI outcomes.

Variable	SPQ-total	SPQ-CP	SPQ-IP	SPQ-DT	LI score
SPQ-total N =	1	**0.828**** 40	**0.814**** 40	**0.863**** 40	**0.336*** 40
SPQ-CP N =		1	**0.491**** 40	**0.595**** 40	**0.322*** 40
SPQ-IP N =			1	**0.552**** 40	**0.312*** 40
SPQ-DT N =				1	0.213 40

**. Correlation is significant at the 0.01 level (1-tailed)
*. Correlation is significant at the 0.05 level (1-tailed).

Table 15: Correlation between drug use characteristics, SPQ-B traits and LI performance in the cannabis group.

Variable	SDS	Age of onset	Joints per week	LI Score	KB score
SPQ-total N =	.288 20	-0.242 20	**.434*** 20	0.217 20	0.045 20
SPQ-CP N =	.095 20	-0.93 20	**0.28*** 20	0.354 20	0.014 20
SPQ-IP N =	0.409 * 20	0.00 20	**0.561**** 20	0.147 20	0.060 20
SPQ-DT N =	0.152 20	**-0.468*** 20	**0.416*** 20	-0.037 20	0.0327 20
SDS N =	1	0.095 20	**0.370*** 20	-0.07 20	0.123 20
Age of onset N =		1	-0.132 20	0.109 20	0**.427*** 20
Joints per week N =			1	-0.234 20	0.189 20

**. Correlation is significant at the 0.01 level (1-tailed)
*. Correlation is significant at the 0.05 level (1-tailed).

2.4 Discussion

The purpose of this first study was to look at the performance of a group of regular cannabis users in associative learning tasks which have previously been shown to be disrupted in schizophrenic populations, first degree relatives of people with schizophrenia and by drugs which affect dopamine. The predictions here, derived from the literature linking cannabis use to psychosis, were that cannabis users would show disruption in the LI and KB tasks because of the chronic effects of this potent psychoactive on dopaminergic and other systems underpinning the attention and associative learning based systems crucial to performance. This study also looked at the personality trait of schizotypy as measured using the SPQ-B.

2.4.1 Latent inhibition and cannabis

Cannabis users seem to be showing a schizophrenic-like profile on the LI task. LI was abolished in the PE condition, with no significant difference found in LI scores between the task conditions (PE versus NPE) for the cannabis users. In the primary analysis, the cannabis users took less time overall to find the paired association between the white noise and the counter incrementing in the PE condition relative to the cannabis group in the NPE condition. Further to this, there was a LI effect in the non-cannabis users but opposite to the expected direction (i.e. faster learning in the PE condition). On closer inspection of the data, 7 out of 20 in the non- cannabis group successfully learnt the paired association overall compared to sixteen out of twenty in the cannabis group. There was no intelligence test administered to see if differences in intelligence was a reason why the non-cannabis group performed badly overall on the LI task. The only clear differences is that more males and non-British participants were in the cannabis using group overall, and there were 5 internal participants (i.e. students/workers at the UEL) compared to the non-cannabis group who used 12 internal candidates.

The study removed 5 people in the non-cannabis group who did not reach the learning criterion (e.g. did not successfully learn the paired association), and those in particular who reported cannabis use in the past. To eliminate the possible influence of these non-users, who did not reach the criterion and those who had previously used cannabis, these data points were excluded and a secondary set of analyses carried out, which also included 5 new participants. Looking at this revised secondary data set, there was now a significant difference between the

PE versus NPE conditions for the non-cannabis group; thus indicating a normal LI effect (i.e. slower learning in the PE condition). Furthermore, in the secondary analysis there was a significant difference between cannabis users and non-cannabis users in the PE condition but not under the NPE condition, indicating that cannabis users overall were less affected by the pre-exposure to the white noise during the masking task (of the PE condition), thus indicating a trend for abolition of normal LI. These LI findings do fit the idea that use of cannabis may be disrupting associative learning in the fashion seen in psychotic populations, first degree relatives and following amphetamine use. Cannabis has been independently associated with deficits in the PFC (e.g. Block *et al*, 2002; Lundquist *et al*, 2001; Solowij *et al*, 2002) and these are linked to attentional dysfunction (e.g. Weinberger *et al*, 2001). Cannabis use is also associated with increased mesolimbic dopamine transmission in the brain (e.g. Tanda *et al*, 1997; Voruganti *et al*, 2001); dopamine is critical for LI performance (Soloman *et al*, 1981; Weiner *et al*, 1981; 1984) and appears to be central in some forms of attentional dysfunction (e.g. Matthysse, 1978; Swerdlow & Koob, 1987; Swerdlow et al, 2003), thus it could be argued that cannabis use is accounting for the disruption of normal LI in this current study.

Further to this, CB_1 receptors have a known role in associative learning in animals (e.g. Gruart *et al*, 2012) and disrupted associative learning has been found in humans using cannabis (e.g. Jager *et al*, 2007; Skosnik *et al*, 2007). Disrupted associative learning (as demonstrated by the Latent inhibition task) is argued to be due to the failure of inhibiting attention to the irrelevant stimuli during the pre-exposure stage, which may be a result of cannabis increasing DA transmission. This DA mechanism may also be involved in producing positive symptoms of schizophrenia when cannabis users are reporting higher rates of paranoia, hallucinations and delusional thoughts (e.g. Crippa *et al*, 2009). Recent research by Granger *et al* (2012) looked at LI performance in healthy controls and assessed them using the O-Life measure and found that the main subscale of 'unusual experiences' linked to paranoia and the positive symptomology was the best predictor of LI disruption. In the current study, the numbers were too small across the groups to make robust statistical comparisons across the SPQ-B subscales for LI performance, but there were differences in LI performance between high and low SPQ-B outcomes (see SPQ-B section 2.4.3 below). What is not clear, if this is a transitory or long term effect of using the drug and/or an underlying vulnerability of executive dysfunction, or an interaction between both cannabis use and executive dysfunction. Realistically longitudinal birth cohort studies are best for providing a clearer picture into the 'cause and effect' specific

drugs may have on the developing brain. There have been many published studies looking at the long-term effects of cannabis on cognition (for a review see Solowij, 2000; 2002). The only published study looking at current cannabis use and former cannabis use (in ex users) on associative learning was conducted by Skosnik *et al* (2012). This study found that ex users performed better than current cannabis users on associative learning, but ex-cannabis users still had problems with the acquisition of conditioned responses and timing on the Eye blink Conditioning (EBC) task compared to healthy controls.

In the primary analysis, low SPQ-B scorers took significantly less time than high SPQ-B scorers under the NPE condition. In the secondary analysis low SPQ-B scorers took significantly longer to find the paired association in the PE condition versus the NPE condition and thus showed a normal LI effect. This was contrasted by high SPQ-B scorer's performing significantly better at finding the paired association in the PE condition relative to the NPE condition, thus indicating abolishment of normal LI in these high SPQ-B scorers. The participant numbers are relatively low when comparing the LI effect between PE and NPE conditions in high and low SPQ-B scores but these findings support previous research in the area (e.g. Baruch *et al*, 1998a; Wuthrich & Bates, 2001; Granger *et al*, 2012). Interestingly, the differences between those with high versus low SPQ-B scores are more apparent under the NPE conditions, with high scorers performing worse which represents poorer basic associative learning skills. The correlation data also highlighted that the non-cannabis group in the secondary analysis scoring higher on the SPQ-B measure and its three subscales was positively associated with higher scores on the LI task, but this was not the case for the cannabis group. Therefore it could be argued that the LI abolition effects in this current study, under the PE condition, are drug specific, as opposed to being linked to the schizotypal personality traits.

2.4.2 Kamin Blocking and cannabis

In the current study cannabis users were slower to learn the association in NBL condition of the KB task relative to the non-cannabis using group. A normal KB effect was found in the cannabis group (e.g. slower learning in the BL relative to the NBL condition), but this was not replicated in the non-cannabis using group. Serra *et al* (2001) found that schizophrenic patients were slower to learn the association in both conditions; and the controls were faster to learn the paired association in both conditions. In Serra *et al*'s study people diagnosed with

schizophrenia who were allocated to the BL condition had a mean score of 19.91 of trials to criterion for KB performance, compared to their schizotypal relatives who had a mean score of 21.70. In the current study the cannabis group had a mean score of 31.2 in the BL condition relative to 26.4 for the non-cannabis using group, which together indicate that both groups had poorer associative learning overall for this task when compared with Serra *et al'*s findings for the KB task. In the non-cannabis group 45% of the sample did not meet the learning criterion versus 60% in the cannabis group, therefore due to lack of differences found in the primary analysis, the KB task was not used in the secondary analysis as performance was poor in both groups. In previous studies, 50% of the clinical sample did not reach the learning criterion of the KB tasks compared to the control sample of 10-20% who do not reach the learning criterion (Oades *et al*, 1996; Moran *et al*, 2003; Moran *et al*, 2008). In some instances participants would be excluded or not put forward for further assessments for behavioural measures (i.e. fMRI assessment: Bott *et al*, 2007). Serra *et al* (2001) excluded those who did not detect the learning association in stage three of the KB task (the same test used in this study), but retained all participants who reached the final testing stage regardless of meeting the learning criterion. This rule of excluding participants who failed at stage three was not applied to the current study.

The lack of effect found in the KB data may also reflect the inconsistencies in findings using this paradigm by other researchers (Jones *et al*, 1990; Jones *et al*, 1991). Jones *et al* (1990) found no difference between schizotypy scorers and the KB task, whereas a later study found significant differences between those with greater positive symptomology of SPQ scores, for those in the acute phase of schizophrenia versus chronic stage of schizophrenia on the KB task (Jones *et al*, 1991). The evidence for both studies was re-examined by Jones *et al* by breaking the participants down into distinct schizotypy groups, on the STA measure and the findings were still less clear (Jones *et al*, 1992). A trend existed for participants scoring high on magical thinking, unusual experiences; these people tended to show less effects from the blocking, which links into the acute positive symptoms (e.g. hallucinations etc.) in the earlier stages of schizophrenic symptoms. However, the authors concluded that the inconsistencies in the evidence for the KB task may be due to the insensitive nature of the between participants design. Jones *et al* (1997) re-looked at the KB using a between and within participants design and found a similar result from before, in that KB was abolished in schizophrenic participants mainly with positive psychotic symptoms; they did not find any difference between relatives of

these patients who were assessed and labelled as a schizotypal and non-schizotypal relative. Further to this, Oades *et al* (1997) found that KBE was attenuated in young non paranoid patients, but this was not the case for those with significant paranoid symptoms. Interestingly, in participants overall (controls and patients) attenuated blocking was linked to higher levels of dopamine activity as measured in 24 hour urine samples Higher levels of dopamine and attenuated blocking ties into the argument of KB performance being linked to genetic differences as certain genes (e.g. COMT) are involved in the breakdown of dopamine (Eisenhofer et al., 2001).

In the current study there were no significant differences found for KB performance between high and low SPQ-B scorers under the conditions BL versus NBL. There have been quite a few inconsistencies using the KB task in Jones' study, (the same task and procedure as used in the present investigation). However, it does seem that some consistent findings have come about using the Oades paradigm, in that KB deficits are found in non-paranoid schizophrenic patients and these results were replicated by Moran et al (2003). Moran et al. also distinguished aspects of schizotypy which reduce blocking and found a negative relationship between KB performance and positive symptoms (e.g. unusual experiences) and disorganisation (i.e. cognitive disorganisation).

2.4.3 SPQ-B, cannabis use and dependency

There were no significant differences found between the cannabis and non-cannabis users on the SPQ-B measure. This does not support previous research (e.g. Skosnik *et al*, 2001; Barkus *et al*. 2008; Friedberg *et al*. 2010). Lack of findings could be due to the low participant numbers with the current study and/or a lack of sensitivity in the SPQ-B as a measure. The SPQ-B requires a simple Yes/No response and many anecdotal responses from the participants related to how they sometimes "felt like that and at other times did not", therefore the participants often marked the response as a straight 'no' and did not allow for any recognition for levels of this trait. A study published by Cohen (2010) adjusted the SPQ measure using a likert-scale and found that it was much more sensitive than the original version for uncovering a psychosis-proneness personality profile.

The amount of cannabis used was explored and heavier use of cannabis was associated with higher number of reported traits on the SPQ-B measure both for positive, negative symptoms and disorganised thinking. This supports previous research by Compton *et al*. (2009) who found that heavier cannabis use in early adulthood is associated with higher rates of schizotypy. Age of onset of cannabis use was negatively associated with scores on SPQ-DT, with earlier onset of use of the drug related to higher scores on this third subscale of disorganised thinking. This differs from Compton *et al*'s (2009) study as early age of onset of cannabis was associated with interpersonal schizotypy. Compton's research, however, used two distinct testing groups (e.g. first-degree relatives of patients versus non-psychiatric controls) and this therefore makes it difficult to generalise these findings against a sample of cannabis users versus non-cannabis users on these SPQ-B traits, especially as the current sample did not report any family histories of psychosis. What is clear is that earlier use of cannabis and higher frequency of use is linked to experiencing more of these psychotic-like traits which arguably may account for the reason why some people at are a higher risk for the development of schizophrenia (e.g. Bossong & Niesink, 2010).

Cannabis dependency was assessed using the Severity of Dependency Scale (Gossop, 1995). It is debatable about the cut-off mark for dependency as there is no single cut-off mark for all drugs of dependence (Trosi *et al*, 1998), however, optimal marks for cut-off points for amphetamine, cannabis and cocaine are reported as 5, 3 and 3 respectively. Martin *et al* (2006) were the first to reliably use the SDS as a screening tool for cannabis dependency in a non-clinical sample of adolescent cannabis users and concluded that the optimal score was four for cannabis dependence. Seven out of twenty of the cannabis group in this current investigation reported a cannabis SDS of four and above, thus indicative of cannabis dependency. A significant association was found between cannabis dependency scores and negative symptomology from the SPQ-IP subscale which relates to interpersonal traits that link to paranoid ideation, social anxiety, no close friends and constricted affect. An important point to make is that SDS scores are not appearing to predict other relationships, as there was nothing clearly linked with this measure to LI scores or other SPQ-B variables. Whereas joints per week (actual use whether dependent or not) correlates to all the SPQ-B variables.

Research on severity of drug dependency (namely cannabis use) and psychotic-like symptoms is very limited but this finding is backed up by one previous study (Hides *et al*, 1997). Hides *et al* used a sample of 153 in-patients diagnosed with schizophrenia spectrum disorder and used the CIDI measure with criteria from the DSM-IV (American Psychiatric Association, 1992) to assess the sample for cannabis dependency, of which 54% met the criteria for cannabis dependence. Individuals with a score greater than or equal to 2 on the Severity of Dependence Scale were nearly 30 times more likely to have a DSM-IV diagnosis of cannabis dependency. In their study, cannabis dependency was linked to having more positive symptoms (odds ratio of 1.09) and negative symptoms (odds ratio of 0.90) associated with schizophrenia (as assessed by the Positive and Negative Syndrome Scale; Kay *et al*, 1987).

Dumas *et al*. (2002) attempts to explain the relationship between cannabis use and schizotypal symptoms in terms of the 'self-medication' hypothesis, with high schizotypal individuals attempting to reduce their negative symptoms using a psychostimulant drug, which in turn may bring about or intensify the positive symptoms. Some researchers now postulate that the endocannabinoid system may underpin vulnerability to schizophrenic-like symptoms and also a vulnerability to cannabis consumption (Schnieder *et al*, 1998; Leweke *et al*, 1999; Bossong & Niesink, 2010). This genetic vulnerability will be assessed by comparing the SPQ-B data across all three studies in Chapter 4 for candidate genes in psychosis-proneness.

2.4.4 Evaluation of research

In the primary analysis the non- cannabis group did not demonstrate a normal LI effect as they achieved fewer trials to criterion in the pre-exposed condition versus the non pre-exposed condition. The cannabis group also demonstrated better associative learning skills under the NPE condition compared to the non-cannabis group. Overall only 35% of the non-cannabis users achieved the learning criterion compared to 80% of the cannabis users. The non-cannabis group overall performed less well, but there was no intelligence test administered to check if there were apparent differences between the group. The non-cannabis group had 3 participants reported heavy lifetime use. Therefore, a new group of 5 non-cannabis users were tested (1 in the PE and 4 in the NPE conditions) and this revealed a true LI effect as they were faster under the NPE condition compared to the PE condition, with 60% of the sample achieving the learning criterion. In the primary analysis most of the participants for non-cannabis users were

internal candidates from the UEL, so it could be argued that there motivation to take part may not have been as high as those willing to travel to the university to take part in the research. However, in the secondary analysis the five new participants were internal candidates and this actually increased performance for the non-cannabis group, as opposed to decrease performance. There were more males in the cannabis using group in both the primary and secondary analysis. This gender difference may have impacted on the findings, as recent research by Kaplan & Lubow (2010) indicated that low schizotypal healthy males, but not females, exhibited LI. Further to this, cannabis users experiment with other drugs and in this current study they reported a greater degree of past and current polydrug use and this must be noted before any firm conclusion can be drawn regarding the impact of cannabis on cognitive functioning; polydrug use may be a key reason for cognitive disruption, as opposed to cannabis use alone (e.g. Croft *et al*, 2001). Cannabis users frequently reported use of other party drugs such as cocaine, amphetamine and high use of MDMA, all which are neurotoxic and affect cognition in their own right (and especially in combination) (e.g. Rogers & Robbins, 2001; Gouzoulis-Mayfrank & Daumann, 2001). Therefore, it is difficult to draw conclusions about the sole impact of cannabis, especially as elevated psychopathology is associated with polydrug use in general (e.g. Parrott et al, 2001). Moreover, having just two-group comparisons (between the cannabis users and non- cannabis users) makes it difficult to assess the true impact of cannabis use, especially as cannabis use is varied within this drug group as well high polydrug use. A way to overcome this for future research would be to use a three-group comparator 1) cannabis use and high polydrug use, 2) cannabis use with low polydrug use, and 3) a non-drug using sample (with little party drug history).

The KB task has not been as extensively researched as a paradigm in human participants as the LI paradigm, and may need much more refining and developing to reduce the inconsistencies found between existing studies. Also, by excluding participants based on not achieving basic associative learning skills in testing stage three would have helped to eliminate ceiling effects. The cannabis group were asked to abstain for at least two days prior to testing and this was assessed objectively though saliva based drug tests, so these LI effects cannot be directly accounted for as acute effects of smoking cannabis. It could be argued that these LI effects are due to withdrawal from the drug (e.g. Pope *et al*, 1995), but these current drug users were able to abstain for two days without any difficulty and those unwilling (or unable) to abstain made this known to the researcher and were not included in the study.

2.4.5 Summary of key findings

Cannabis users seem to have abolished LI and as predicted were showing a more schizophrenic-like impairment on the LI task; they were less affected by the pre-exposure to the white noise under the PE condition. There were no significant differences found in SPQ-B scores between the Groups (cannabis versus non-cannabis) and this measure was limited to a simple Yes/No response, which made it difficult to assess more subtle levels of the traits. Therefore, in studies two and three additional personality measures will be added which assess psychosis-proneness by adopting a likert-scale to explore levels of schizophrenia-like personality traits. The key outcome for the SPQ-B data is that those that reported earlier onset of cannabis use and higher frequency of use (regardless of being dependent or not dependent) reported experiencing more psychotic-like personality traits.

Due to the small numbers in each group (cannabis versus non-cannabis) for the PE/NPE conditions, it was difficult to break these down into low and high SPQ-B scores. The entire group was therefore collapsed into one sample and were allocated to the high or low SPQ-B group based on the criteria set by Laws *et al* (2008). A normal LI effect was found in low SPQ-B scorers, but was abolished in high SPQ-B scorers between the PE and NPE conditions, with high SPQ-B scorers being more distracted by the pre-exposed white noise and learnt to find the association more quickly. There was a lack of differences found under the PE condition between high/low scorers might give an indication that the differences found with the cannabis group on LI performance may be a specific effect of the drug as opposed to higher/lower SPQ-B traits.

Overall the cannabis users do seem to be showing subtle differences in brain inhibitory function akin to previous research with schizophrenic patients, their first degree relatives and high schizotypy scorers. Cannabis users also highlighted individual differences in psychosis-proneness personality traits when they had earlier onset of cannabis, higher frequency of use, and higher levels of dependency to this drug. These findings could be argued to fit with suggestions that regular cannabis use and its association with DA transmission in the PFC is involved in the breakdown of normal information processing and may account for selective

attention dysfunction and the experiencing of more psychotic-like personality traits. These findings also fit in with ideas that cannabis use at earlier ages may disrupt normal neurodevelopment regulated by endocannabinoids; such that the differences observed may be evidence of neuro-difference in users and non-users.

Chapter 3: Decision-making, selective and sustained attention, inhibitory control and psychotic-like traits in regular cannabis users

The growing evidence for a relationship between cannabis use and potential risk for developing schizophrenia was discussed in Chapter one. Chapter two explored the link between cannabis use and schizophrenia-like behaviours, by assessing the performance of a group of cannabis users versus non users on two associative learning tests, namely Latent Inhibition and the Kamin Blocking effect; these tests have been shown to be disrupted in people diagnosed with schizophrenia. The rationale to investigate cognitive disturbances in cannabis users which parallel those found in schizophrenic patients was presented in Chapter 1. In brief, pharmacological evidence suggests that the endocannabinoid (eCB) system has a known role in learning, memory and higher cognitive processing (Herkenham *et al*, 1990). Evidence exists that chronic exposure to cannabinoids can alter the functioning of cognitively relevant neuromodulater systems; e.g. dopaminergic, cholinergic, serotonergic, GABAergic and glutamatergic (Sundram *et al*, 2004). Furthermore, research on administering THC to schizophrenic patients reveal enhanced sensitivity to this psychoactive drug for cognitive dysfunction (e.g. D'Souza *et al*, 2005), and administering THC to normal controls also mimics schizophrenic-like symptoms (e.g. Morrison and Murray, 2009).

Moving on from the findings in the first study, the current chapter aims to further explore whether regular cannabis users show a profile of personality traits and cognitive dysfunctions characteristic of schizophrenia. Secondly, it aims to explore the inter-relationships between personality traits, cognitive performance and cannabis use.

3.1 Decision-making – the Iowa Gambling Task

People diagnosed with schizophrenia have decision making problems (see Alves *et al*, 2006 for a review), which are a result of poor executive functioning (Goldman-Rakic *et al*, 1997, 1999; Rüsch *et al*, 2007). People diagnosed with schizophrenia also demonstrate deficits on cognitive tasks which assess emotion (Kring and Neale, 1996; and see Kring, 1999 for a review). More specifically, those displaying more negative symptoms of schizophrenia (related to the emotional element of affective or motivational symptoms) also show greater deficits on frontal lobe functioning tasks (Wolkin *et al*, 1992). One way that this has been assessed is to look at performance on the Iowa gambling task (IGT), an emotional based neurocognitive learning task specifically designed to tap into 'real-life' decision-making abilities, which requires the individual to simultaneously weigh up the costs and benefits of their decisions. The participants are required to choose cards from one of four available decks of cards, typically labelled as decks A, B, C and D. On selection of these cards a win or lose outcome is displayed on the screen. The difference between the decks of cards are that the first two packs (A and B) provide immediate higher wins (e.g. £100) but can also result in higher losses (e.g. £1125), whereas the other two packs (C and D) provide moderate wins (e.g. £25) or minor losses (e.g. £75). Typically, 'normal', healthy, control participants learn to avoid decks A and B and adopt the more beneficial strategy of opting for packs C and D. Over time there is a net loss of £25 per card from packs A and B, whereas there is a net profit of £25 per card from C and D. The task ends after 100 card selections and the total net score is calculated as a loss or gain from the monetary funds (e.g. £2000) that participants were awarded at the start of the game. Please refer to Table 16 for a summary of the research detailed below on IGT and/or similar tasks to the IGT.

Table 16: A summary of research using the Iowa Gambling Task in cannabis users, schizophrenia, and brain damaged patients.

Study	Participants	Cognitive Task	Main results
Damasio et al (1991)	Clinical – brain damaged patients	IGT	Patients lacked anticipatory response (via SCRs) for riskier decks.
Bechara et al (2000)	Clinical – brain damaged patients	IGT	Poorer decision-making in the patient group.
Verdejo et al (2004)	Substance use disorder	IGT	Poorer decision-making in the patient group.
Bark et al, 2005; Beniger et al 2003; Shurman et al, 2004;	Schizophrenia	IGT	Poorer decision-making in the patient group.
Whitney et al (2004)	Schizophrenia and OCD	IGT	Patients with schizophrenia performed worse than patients with OCD.
Ritter et al (2004)	Schizophrenia and Schizoaffective disorder	IGT	Patient groups performed worse when compared to controls.
Mata et al (2008)	Acute schizophrenia – cannabis use versus no cannabis use	IGT	Patients who used cannabis prior to schizophrenia diagnosis performed worse compared to schizophrenia group who did not use cannabis.
Evans et al, 2005; Premkumar et al, 2008; Turnball et al, 2003	Schizophrenia	IGT	Normal decision making in schizophrenia when compared to a control sample.
Hermann et al, 2009	Cannabis use	IGT	Poorer decision making in cannabis users compared to controls. Dose response effect with higher levels of THC and poorer IGT performance.
Whitlow, 2004	Cannabis use	IGT	Poorer decision making associated with long-term use of cannabis.
Grant et al (2012)	Cannabis use	Cambridge Gambling Task	Poorer decision making in cannabis users compared to controls.
Bolla et al (2005	Cannabis use	IGT & brain imaging	Cannabis users performed worse than controls on the IGT and showed greater activation in cerebellum and less activation in OFC and right DLPFC. Heavy use was associated with greater activation in brain regions with greater CB1 density.
Wesley et al (2011)	Cannabis use	IGT & brain imaging	Cannabis users performed worse than controls on the IGT and showed under-responsiveness in brain areas such as: anterior cingulate, medial prefrontal, and superior parietal cortices, dorsal cerebellum and occipital lobes. Cannabis users had insensitivity to loss from the earlier stages of the IGT.
Yarkomi et al, 2005	Schizophrenia	IGT & brain imaging	Greater attention to reward is associated with greater activation of grey matter volume in PFC in controls.
Shenton et al (2001)	Chronic schizophrenia	IGT & brain imaging	Reduced activation of gray matter volume in PFC for chronic patients.

The IGT was initially developed to assess patients with lesions of the orbito-/ventromedial prefrontal cortex (OFC/VMPFC) which affects learning and decision-making (e.g. Bechara *et al*, 1994). Damage to these brain regions has been linked to impaired emotional expression/feelings, with patients showing impairments in psychophysiological responses to emotional (in relation to neutral stimuli) (Damasio *et al*, 1990). In work exploring his Somatic Marker Hypothesis (SMH) for emotion based decision making, Damasio *et al* (1991) reported that damage to the VMPFC was linked to decision making deficits, particularly for emotion based biasing signals (or somatic markers) when making value led decision. According to Damasio and colleagues, decision making can be viewed as a cost benefit analysis and 'markers' for how punishing or rewarding an action is will be used when a detailed logical analysis cannot be performed in complex situations (Damasio *et al*, 1991; 1994; 2004). Key support for the SMH comes from correlational data on performance on the IGT and Skin Conductance Responses (SCRs) in patients with VMPFC damage and healthy controls. Both groups showed normal SCRs on punishment and rewards at the beginning of the IGT, but soon into the task the controls developed anticipatory SCRs, which were higher for the riskiest card deck. This anticipatory response was absent in the patient group and this correlated with poor performance on the IGT. Bechara *et al* (1996) argued that failure to identify good/bad outcomes in a situation of uncertainty was a result of a failure to activate the somatic marker for learning previous punishments in bad decks, which ultimately results in a lack of sensitivity to the possibility of future punishments on that deck. Thus, based on the SMH, performance on the IGT was seen as people developing 'hunches' for outcomes on the good/bad decks. Opposing theories on IGT performance have been put forward to argue that performance on the test is not due to somatic biases but to cognitive outcome expectancies where people are consciously aware of the outcomes on the task from learning the deck advantage/disadvantage outcomes earlier in the task, and thus they take less risk overall (see Turnball *et al*, 2003). Irrespective of opposing theories on IGT performance it is seen as a robust measure of decision making and findings have been consistent even after changes to its testing parameters (e.g. giving a real financial reward; Bowman and Turnball, 2003) and time delays (Bowman *et al*, 2005).

Relatively consistent findings have been shown in people with damage to frontal brain regions (Bechara *et al* 2000), those diagnosed with substance disorders (Verdejo *et al*, 2004; Bechara *et*

al, 2001) and psychiatric disorders; groups which generally perform much worse compared to healthy controls. For example, Bark *et al* (2005) tested a group of 8 people diagnosed as catatonic schizophrenic and 19 paranoid schizophrenics versus a group of 26 healthy controls and found that the disadvantaged decks were selected more often by the schizophrenia groups. Whitney *et al* (2004) tested a group of 26 people diagnosed as schizophrenic with obsessive symptoms and 26 schizophrenics without obsessive symptoms, and 11 people diagnosed with obsessive compulsive disorder (OCD). There was a trend for both schizophrenia groups to select the disadvantaged decks more often than the OCD group. It could be that the patients with OCD have higher anxiety sensitivities and thus take less risk.

Research which excluded those with a history of substance abuse, indicated that people diagnosed with schizophrenia choose the disadvantaged packs more often (i.e. those with immediate higher reward but greater losses overall) than the advantage packs (i.e. moderate wins but with less loss overall) when compared to a group of healthy controls (Beniger *et al*, 2003; Ritter *et al*, 2004; Shurman *et al*, 2004). Beniger and colleagues compared a group of 36 people with schizophrenia with 18 controls and found that those with schizophrenia using atypical antipsychotic medication performed worse on the IGT. Shurman and colleagues compared a group of 39 people with schizophrenia and 10 controls and found that people diagnosed with schizophrenia had poorer decision making. Similarly, Ritter and colleagues compared 20 people with schizophrenia or schizoaffective disorder with 15 controls and found that controls chose the advantageous packs (e.g. C and D) and this resulted in better decision making overall compared to people with schizophrenia or schizoaffective disorder.

Mata *et al* (2008) assessed 132 people in the acute stages of schizophrenia and tested them on the IGT, and separated the group into those currently using cannabis versus no cannabis use. It was found that patients who had abused cannabis prior to the onset of psychotic symptoms showed poorer performance on the decision-making task. Nevertheless, some studies, which did not account for substance abuse, demonstrated a pattern of normal decision-making on the IGT in schizophrenia (Evans *et al*, 2005; Premkumar *et al*, 2008; Turnball *et al*, 2003).

3.1.1 Cannabis, schizophrenia, neuroimaging and decision-making

Cannabis users have shown to be less risk averse and choose the disadvantaged packs more often relative to non-cannabis users, which resulted in less monetary returns on IGT (e.g. Hermann *et al*, 2009). Hermann *et al* also assessed hair samples of cannabis users, along with their subjective ratings on the trait tridimensional personality questionnaire, in 13 users and 13 controls and this was correlated with performance on the IGT. THC correlated negatively with the last subtrial (cards 80-100 which given an indication of whether the participants learnt the more advantageous strategy) of the IGT (r=-0.67), thus higher levels of THC found in the participant's hair was also indicative of poorer decision-making (e.g. higher loss). Greater use of cannabis was also associated with greater loss overall on the IGT. On the trait measure, the dimension of harm avoidance correlated negatively with IGT (r=-0.43). The last subtrial correlated with adventure seeking, (0.43), negatively with harm avoidance (-0.39), and with reward dependence (r=0.44). More importantly, a regression model indicated that heavy THC consumption rather than personality traits was the strongest predictor of IGT performance. Poorer decision making on the IGT has also been associated with duration of cannabis use, more specifically those with a profile of long-term heavy use chose the riskier card decks with immediate higher gain but higher losses overall (Whitlow, 2004). These studies suggest that cannabis use, particularly heavy use, might lead to poor decision-making. Cannabis use has also been linked to risky decision-making on the Cambridge Gamble Task, an analogue of the IGT (Grant *et al*, 2012).

Brain regions which are rich in CB_1 receptors are linked to decision-making and emotional processing (Herkenham *et al*, 1990; Whitlow *et al*, 2004; Wesley *et al*, 2011). Poor decision-making in cannabis users have also been objectively verified with supporting evidence from brain imaging data. Bolla *et al* (2005) reported differences between cannabis users and non-cannabis users, with cannabis users showing greater activation in the cerebellum and less activation in the right lateral Orbito-prefrontal cortex (OFC) and right Dorso-lateral Pref frontal cortex (DLPFC), areas linked to decision-making (e.g. Bechara *et al*, 1994). Heavy cannabis use showed greater activation in brain regions dense with cannabinoid receptors, such as the cerebellum and hippocampus, and less activation in the medial OFC. Wesley *et al* (2011) reported that cannabis users performed significantly worse than non-cannabis users on the IGT and also demonstrated under-responsiveness in brain areas such as anterior

cingulate, medial prefrontal, and superior parietal cortices; with under-responsiveness in these brain areas as well as portions of the dorsal cerebellum and occipital lobes being associated with monetary losses in the cannabis group. A correlation was also found between early loss evaluations in the non-cannabis group, with early loss evaluation associated with better decision making during the rest of the task, whereas this was not the same for the cannabis group, and the researchers argued that cannabis users had some insensitivity to losses even at the earlier stages of the task. These findings by Wesley *et al* (2011) are supported by growing evidence for abnormalities in affective responding in cannabis users and long terms users (e.g. Degenhardt *et al*, 2003; Skosnik *et al*, 2008). Wesley and colleagues argued that long term users of cannabis and functional insensitivity to monetary losses may be the result of a disrupted cannabinoid system due to long term use of the drug.

Brain activation and functioning in people diagnosed with schizophrenia has also been assessed during the IGT, and it was found that greater attention to reward is associated with greater activation of grey matter volume in PFC in healthy controls (Yarkomi *et al*, 2005), but marked by reduced activation in chronic schizophrenic patients (Shenton *et al*, 2001). These brain scanning data run parallel to the under responsiveness found in the cannabis group by Wesley *et al* (2011), but rather than an under-responsiveness to punishment, people in the chronic stages of schizophrenia differentially respond to rewards when compared to a healthy control sample.

Overall, it seems that emotional decision-making (as measured by the IGT) is disrupted in people with prefrontal brain damage, psychiatric conditions and mainly those diagnosed with schizophrenia. Those people with schizophrenia that use cannabis prior to the onset of symptoms also seem to have poorer decision making abilities (e.g. Mata *et al*, 2008). Further to this, cannabis users seem to perform in a similar way to people diagnosed with schizophrenia, even though performance is correlated with differential responding in brain activation. There are differences observed in drug users' performance depending on the amount of cannabis they consume and the length of period cannabis had been consumed for (e.g. Hermann *et al*, 2009; Whitlow *et al*, 2004).

3.2 Selective attention, sustained attention and inhibitory control

Sustained attention (i.e. maintaining a consistent focus) and selective attention (i.e. focusing on relevant as opposed to irrelevant information) and inhibitory control (i.e. being able to inhibit a response by not selecting irrelevant stimuli) have also been proposed as endophenotypes of schizophrenia. A common measure of attention is the Continuous Performance Test (CPT; Rosvald *et al*., 1956), which exists in a number of versions: for example, the identical pair version where participants are asked to perform a computer task and press the keypad when the same digits are repeated (e.g. 43578 is followed by 43578). Some of these digits are trick digits (e.g. 62897 is followed by 62895) and some are correct (e.g. same digits are repeated) and others are novel digits (e.g. new arrangements of digits follow the preceded trial). Throughout the task distracter digits '12345' are randomly presented (e.g. Cornblatt *et al*, 1989). Earlier studies which used the CPT as a measure of selective and sustained attention analysed measures of perceptual sensitivity (e.g. discriminating targets from non-targets), omission error counts (or sometimes known as motor errors) which represent impulsive responses to filler stimuli (e.g. Dawkins *et al*, 2007) and commission errors (or false alarm) which are incorrect responses to the target. Many recent researchers also focus on reaction times, which reflect the speed for all correct responses as a measure of selective attention (e.g. Chen *et al*., 2004). Please see Table 17 below for a summary of the research using the CPT and/or similar tasks.

Table 17: A summary of research using the Continuous Performance Test in schizophrenia patients, cannabis users and high schizotypy.

Study	Participants	Cognitive Task	Main results
Strauss et al (1993)	Schizophrenia and schizoaffective disorder	CPT	Selective attention deficits associated with higher symptom scores on the BPRS.
Vollema and Postma (2002)	Unaffected first-degree relatives of people diagnosed with schizophrenia.	CPT	Clinical interview SPQ dimensions were associated with deficits in motor and commission errors on CPT, whereas the SPQ binary question was only associated with deficits on the false alarm variable.
Rodríguez-Sánchez et al (2010a)	Schizophrenia and cannabis	CPT	Schizophrenia patients who used cannabis performed worse overall on the CPT.
Rodríguez-Sánchez et al (2010b)	Schizophrenia and cannabis	CPT	No differences were found between people with schizophrenia who used substances and those who did not
Jockers-Scherübl et al (2007)	Schizophrenia and cannabis	CPT	Cannabis use before the age of 17 was associated with better performance on CPT for people diagnosed with schizophrenia, and the opposite was found for controls.
Pope et al (2001)	Cannabis users	CPT	Current cannabis users asked to abstain for 28-days and were assessed on days 0, 1, 7 and 28. There were no differences found between controls and current users on CPT at any of the four testing stages.
Bedwell et al (2009)	Non-clinical	CPT –AX version	Higher scores on the structured clinical interview for Schizotypal Personality Disorder associated with poorer performance on the CPT. In contrast, scores from the psychometric abbreviated SPQ in the same sample did not correlate with accuracy scores on the CPT.
Chen et al (1997)	Non-clinical	CPT AX version.	Adults scoring high on the subscale SPQ-IP for poor interpersonal relationships had lower scores on the CPT.
Bedwell et al (2006)	Non-clinical	CPT	Participants with higher scores on the SPQ-B made more omission errors than controls.
Bergida and Lenzenweger (2006)	Non-clinical	CPT-IP version.	There was a negative correlation between CPT accuracy and the reality distortion subscale scores from the SPQ.

3.2.1 Schizophrenia, cannabis and selective attention

Selective attention deficits have been found in people diagnosed with schizophrenia as well as those in remission (e.g. Amamow and MacCimmon, 1978). Strauss *et al* (1993) assessed CPT performance in a group of schizophrenic patients and also in those with schizoaffective disorder and measured symptoms using the Brief Psychiatric Rating Scale (BPRS; Overall and Gorham, 1980). They found that selective attention was moderately correlated with measures of thought disorder using the BPRS, in that higher symptom scores were associated with greater deficits on the CPT.

Vollema and Postma (2002) used a sample of unaffected first-degree relatives of individuals with schizophrenia to examine schizotypal personality features and CPT. In addition, they used a self-scoring SPQ and also a structured interview for SPQ dimension scores for different factors of schizotypal personality. It was found that the clinical interview dimensions scores for disorganised symptomology was positively associated with false alarms (or motor errors) and commission errors, whereas the SPQ total was only associated with the false alarm variable. As alluded to in the previous chapter, this latter limited finding may in part be because the binary-nature of the SPQ measure may not allow this scale to fully capture all of the schizophrenia-like symptoms which affect cognition.

Rodríguez-Sánchez *et al* (2010a) examined the use of cannabis and its impact on cognition in people in their first episode of schizophrenia symptoms, with 104 people with schizophrenia (with non-affective psychosis) and 37 controls. Patients were classified into cannabis use before the onset of the illness (n=47) and non-cannabis users (n=57). This was a cross-sectional and longitudinal study with assessments at baseline and then at a 1-year follow-up period. It was found that the people with schizophrenia who were using cannabis performed well on tasks for attention. The controls only outperformed the non-cannabis using people with schizophrenia at two different time points on the CPT for selective attention, but there was no difference found between cannabis users and non-cannabis using groups of people with schizophrenia. In a later study, no differences were found between people with schizophrenia who used substances and those who did not (Rodriguez-Jimenez *et al*, 2010b).

Jockers-Scherübl *et al* (2007) investigated cannabis use and cognitive changes in people with schizophrenia and healthy controls. The researchers assessed cognitive effects after a 28-day abstinent period, using two groups of participants: 39 schizophrenics (19 cannabis users and 20 non-cannabis users) and 39 controls (18 cannabis users and 21 non-cannabis users). In contrast to what is expected, cannabis use before the age of 17 was associated with better performance on CPT for people diagnosed with schizophrenia, and the opposite was found for controls.

3.2.2 Cannabis and selective attention

There have been few studies looking at CPT performance and cannabis use in non-clinical samples. Pope *et al* (2001) assessed current and former cannabis users who had smoked at least 5000 joints, versus controls. Current cannabis users were asked to abstain for 28-days and were assessed on days 0, 1, 7 and 28. There were no differences found between controls and current users on CPT at any of the four testing stages (for total correct responses and total errors). The CPT may not have been sensitive enough to assess for selective attention deficits in Pope at al's study compared to Solowij's (1995) study looking at Event Related Potentials (ERPs) in response to processing of irrelevant information (see Chapter 1).

3.2.3 Selective attention and individual differences

Bedwell *et al* (2009) explored the relationship between schizotypal personality dimensions (SPD) and performance on the CPT. The researchers used the AX test where participants had to press the space bar after the letter X was presented on screen, but only if it was preceded by the letter A. Interestingly their research examined CPT performance alongside continuous dimension scores created from a structured clinical interview for schizotypal personality disorder, which looked at the severity and breadth of each symptom. The study found positive associations between interpersonal symptoms and omission errors ($r = .47$) and disorganised symptoms and false alarms ($r = .40$). Higher scores for SPD were associated with poorer performance on the CPT. In contrast, scores from the psychometric abbreviated SPQ in the same sample did not correlate with accuracy scores on the CPT.

Chen *et al* (1997) found that adults scoring high on the subscale SPQ-IP for poor interpersonal relationships had lower scores on the CPT AX. Bedwell *et al* (2006) found that individuals scoring higher on the abbreviated scale for of the SPQ (the SPQ-B) made more omission errors than controls, but this was related to cognitive-perceptual (CP) subscale scores, which is related to more of the positive symptomology. Bergida and Lenzenweger (2006) used the CPT-IP which places more demand on working memory, and found a negative correlation between CPT accuracy and the reality distortion subscale scores from the SPQ. This subscale is similar to the CP subscale in that it represents positive symptomology such as delusions and hallucinations and as such parallels the finding by Bedwell *et al* (2006) for the CPT AX version and CP. Overall, from these studies it seems that selective attention as measured by the CPT can be linked to some schizophrenia-like personality traits.

3.2.4 Brief Summary: Selective attention, CPT and Cannabis

It seems that schizophrenic patients, their first degree relative and people scoring high on positive symptomology and disorganised thinking make more omission errors on the CPT for selective attention. There appears to be a strong genetic loading, as these CPT deficits are also found in first-degree relatives of patients, including their parents, siblings and offspring. The results are contradictory for cannabis use, with one study finding an improvement in CPT performance in patients that used cannabis prior to 17, and in another no difference found between patients that use cannabis versus those that do not. Interestingly, healthy controls seem to perform better than schizophrenics that do not use cannabis, but not people with schizophrenia who use cannabis recreationally. Further to this, no differences were found between cannabis users who underwent a 28-day abstinence period, under four separate testing sessions on the CPT. Inconsistencies within the literature assessing CPT performance may be the result of researchers using different versions of the CPT (see Khan *et al*, 2012).

3.3 Executive control - Anti-saccade task

Executive control is a term used to describe a range of cognitive processes (such as working memory, inhibition, cognitive flexibility) which are involved in the regulation of goal directed behaviour (Rogers and Bennetto, 2000). There are many research paradigms used to assess executive control such as the Stroop Paradigm (Stroop, 1935; Homack, 2004), Trail Making Test paradigm (AITB, 1944; Aruthnott and Frank, 2000), and the Wisconsin Card Sorting Test (Golman-Rakic, 1987; Berman *et al*, 1995) and these demonstrate key functions linked to frontal brain regions (Hallet, 1978). One paradigm which has been widely used is the Anti-Saccade Task (AST; Hallet *et al*, 1978; Everling and Fisher, 1998; Munoz and Everling, 2004). The anti-saccade task is a measure of volitional control of behaviour (eye-movement), whereby participants are asked to focus their gaze centrally and inhibit a normal saccadic response by looking in the opposite direction of a small moving target. The eye movements are measured via eye-tracking equipment. Following the moving object would result in a pro-saccade response, whereas, inhibiting this natural urge to follow the moving target and looking in the opposite direction is referred to as an anti-saccade (Godijin and Kramer, 2007). Anti-saccade responses are seen to be slower than pro-saccades due this condition requiring people to inhibit the prepotent prosaccade response as well generating a correct anti-saccade response (e.g. Masson, 2004). Godijin and Kramer (2007; 2008) argue that outcomes of the task are influenced by inhibition as well as attentional and working memory capacities. Patients with attentional problems and lesions to the DLPFC make more errors on the AST, than on the pro-saccades stage of the task when compared to healthy controls (Guitton *et al*, 1985) and patients with damage to their frontal eye field have slower response times on the AST than healthy controls (e.g. Gaymard *et al*, 1998). A number of variables can be assessed such as error rate, latency and gain. Error rate reflects the number of incorrect anti-saccade or prosaccade responses, latency is recorded as the speed of responding, and gain is a measure of how closely the eye gaze meets the designated target (in the AST the eye gaze should be in the exact opposite location to where the dot has moved to on the screen). Please refer to Table 18 below for a summary of the research using AST and/or similar tasks.

Table 18: A summary of research using the anti-saccade task in schizophrenia patients, brain damaged patients, and cannabis users.

Study	Participants	Cognitive Task	Main results
Masson (2004)	Non-clinical	AST	Anti-saccade responses are slower than pro-saccade responses.
Guitton et al (1985)	Clinical – brain damage	AST	When compared to controls the patients with attentional problems and lesions to the DLPFC make more errors on the AST, than on the pro-saccades.
Gaymard et al (1998)	Clinical – brain damage	AST	When compared to controls the patients with damage to their frontal eye field have slower response times on the AST.
Curtis et al, 2001;Karoumi et al, 2001; Katsanis et al, 1997; Calkins et al, 2001; Reuter and Kathmann, 2004	Schizophrenia	AST	When compared to controls the patients with schizophrenia had poorer executive control.
Bremner et al (2001)	Schizotypal Personality Disorder	AST	Greater deficits were found in patients on the anti-saccade task relative to controls.
Katsanis et al (1997); Ettinger et al (2004)	Schizophrenia and first-degree relatives	AST	Schizophrenic patients performed the worst on the AST.
Browstein et al, 2003; Crawford et al, 1998.	Schizophrenia and first-degree relatives	AST	No difference in AST performance.
Ettinger et al (2006)	Schizophrenia and Non schizophrenia MZ twins	AST	Schizophrenic twins had poorer executive control compared to the non-schizophrenic twins.
Larrison et al (2000)	Non-clinical	AST	Those displaying more psychotic like traits also had poorer executive control.
Graber and Yurgelun-Todd, 2005; Streeter et al, 2008	Cannabis	Stroop Test	Cannabis users had higher commission errors compared to the controls.
Ding et al (2014)	Cannabis	Go/No Go and brain imaging.	Differences were found between cannabis users and controls in brain areas associated with inhibition such as, the inferior parietal lobe, precuneus, right thalamus, premotor cortex, and middle frontal gyrus.
Ploner et al (2002)	Non-clinical samples and acute THC	AST	Healthy volunteers who were administered with 10 mg of oral THC made more anti-saccade errors.
Huestegge et al (2009)	Cannabis	AST	Chronic cannabis users had prolonged latencies for the memory guided AST, in that they could not correctly identify the right location compared to the controls.
Chung et al (2010)	Cannabis use disorder	AST and brain imaging	Increased activation in the PFC and oculomotor control in the frontal eye fields in those with cannabis use disorder compared to controls. Cannabis users' performance was facilitated by punishments rather than rewards.
Abdullaev et al (2010)	Cannabis	AST and brain imaging	Cannabis users had longer reaction times and more errors for processing incongruent stimuli compared to controls. The cannabis group also had greater activation of the PFC during the AST.

3.3.1 Saccadic eye movement and schizophrenia

Abnormalities in saccadic eye movement are now proposed as an endophenotype (e.g. psychobiological marker) associated with schizophrenia; and this is captured using the AST (Curtis *et al*, 2001; Karoumi *et al*, 2001; Katsanis *et al*, 1997; Calkins *et al*, 2001; Reuter and Kathmann, 2004). The existence of abnormalities in eye movement for those diagnosed with a psychiatric condition was first described by Diefendendorf and Dodge (1902). Since then there has been 50 plus peer reviewed articles consistently reporting anti-saccade deficits amongst schizophrenic patients; specifically that such individuals find it more difficult controlling this natural urge to follow the moving target, as opposed to looking to the opposite side (see Turetsky *et al*, 2009).

Similar findings have emerged in high-risk populations (e.g. those diagnosed with schizotypal personality disorder) and greater deficits were found in these groups on the anti-saccade task relative to controls (Bremner *et al*, 2001). Variable results have been found in first-degree relative of those people with schizophrenia (Levy *et al*, 2004; Katsanis *et al*, 1997; Ettinger *et al*, 2004; 2006; Karoumi *et al*, 2001). Katsanis and colleagues assessed a group of 51 people with psychotic symptoms as assessed by the DSM-IV (APA, 1994), 51 of their first degree relatives (e.g. sister, brother, mother) and 38 unaffected healthy controls. It was found that people with schizophrenia made more errors on the AST than their relatives with some psychotic symptoms, whereas those with some psychotic symptoms performed much worse relative to the controls. This reflects some underlying genetic loading for AST performance. However, other studies have found no significant differences between people with schizophrenia and their relatives without schizophrenia (e.g. Browstein *et al*, 2003; Crawford *et al*, 1998).

Ettinger *et al* (2004) examined 24 people with schizophrenia, 24 healthy siblings and 24 controls. People with schizophrenia made more errors on the AST and have reduced gain (ratio of eye over the target velocity) compared to controls. The siblings performed in between the control sample and their relatives with schizophrenia, with deficits mostly marked on the reduced gain of the AST. These data also support the endophenotypic/heritable significance

of AST, further explored by Ettinger *et al* (2006) in a study which used the AST to assess for executive control performance in 10 monozygotic twin pairs discordant for schizophrenia and 10 monozygotic twin pairs without schizophrenia as controls. It was found that the schizophrenic twins made more errors compared to the non-schizophrenic twins healthy control twins. The healthy control twins did not differ from each other, whereas the non-schizophrenic twins performed worse than the comparison healthy control twins on the gain (how close it spatially meets the target) and latency (speed of responding) but did not differ from their own twin on these measures on the AST. AST errors were correlated with negative symptoms in the patients, which contrasted with Ettinger's initial two findings. It can be inferred from these studies that people diagnosed with schizophrenia and those with a great number of schizotypy traits have poorer executive control. Twin studies also highlight a genetic link with executive control problems in people diagnosed or at-risk for schizophrenia.

Deficits on the anti-saccade task seem to be sensitive to the positive symptoms of schizophrenia (O'Driscoll *et al*, 1998; Holahan and O'Driscoll, 2005). The role of schizotypy has also been examined in saccadic eye movements, with higher positive schizotypal traits predicting greater error on the anti-saccade task, rather than negative symptomology (e.g. Ettinger *et al*, 2005). Those displaying more psychotic like traits also had poorer executive control in that they made more errors on the AST (Ettinger *et al*, unpublished research; Larrison *et al*, 2000).

3.3.2 Executive control, cannabis use and brain scan data

Animal models of AST performance indicate that cortical and subcortical structures are involved in the suppression of a saccadic eye-movement, including the DLPFC, the lateral intraparietal area, the frontal eye-field (FEF) and superior colluculus (Munoz and Everling, 2004). Human studies support these animal models and have shown that Structural Eye Field, which controls the FEF and DLPFC and basal ganglia might be involved in the executive control of voluntary eye-movements (Munoz and Everling, 2004). In addition, the DLPFC and the basal ganglia are areas rich in cannabinoid receptors and affected by direct

THC administration Volkow *et al* (1991; 1996). Therefore, it is likely that cannabis use may cause disruption in these areas and affect performance for normal executive control.

There is little published research looking at the effects of cannabis on executive control. Some studies report that cannabis users had higher commission errors using the Stroop task (Graber and Yurgelun-Todd, 2005; Streeter *et al*, 2008). Ding *et al* (2014) reported no differences in the go/no go task in adolescent cannabis users, but in the same study differences were found in fMRI performance between cannabis users and non-cannabis users for differential activation in the brain areas associated with the response inhibition pathways (e.g. inferior parietal lobe, precuneus, right thalamus, premotor cortex, and middle frontal gyrus). Very few studies have been published looking at non clinical samples of cannabis users on the AST. Some studies have looked at the effects of acutely administered THC in healthy controls and chronic users, as well as eye movement control and visual scanning in cannabis users for clinical and non-clinical samples. For example, Baloh *et al* (1979) research was the first to look at the acute effects of THC on controls and observed no effects in saccade control for latencies, peak velocities and visually guided saccades on a memory saccade task (where a target is presented on the sides of the screen and then disappear and participants need to look in the opposite direction but to the exact location of the target; this therefore involves an inhibitory and memory component). Ploner *et al* (2002) replicated these findings but also examined the acute effects of THC on executive control of eye movements and found that from baseline testing 12 healthy volunteers who were administered with 10 mg of oral THC made more anti-saccade errors. Huestegge *et al* (2009) examined the long-term effects of chronic cannabis use using a memory guided AST and they found that chronic cannabis users had prolonged latencies for the memory guided AST, in that they could not correctly identify the right location compared to the control group.

Chung *et al* (2010) assessed responses to rewards presented during the anti-saccade task and brain activation in 12 adolescents (with cannabis use disorder) and 12 controls. The researchers found that brain activation in those diagnosed with cannabis use disorder under the functional magnetic resonance imaging (fMRI) scans found increased activation in the PFC and oculomotor control in the frontal eye fields, which are associated with response inhibition. Monetary incentives facilitated inhibitory control in both groups, however there

was no difference in error rate for neutral and reward conditions, whereas cannabis users performance was facilitated by punishments rather than rewards. Abdullaev *et al* (2010) assessed executive control and fMRI functioning in a group of 14 chronic cannabis users versus 14 non-cannabis users. The chronic cannabis users had longer reaction times and more errors for processing incongruent stimuli compared to the orienting or alerting components of the task, which was reflective of the executive control network. The fMRI data indicated that the cannabis group had greater activation of the PFC during the AST and the researchers argued that the cannabis group had less efficient executive control for attention in tasks which reflect some conflict resolution, and more demands were put on the PFC to deal with this conflict.

3.3.3 Brief summary of AST findings

Neuroimaging data have led to the hypothesis that deficits found with schizophrenic patients on the AST may be a product of pre-frontal cortical dysfunction (Reuter and Kathmann, 2004). The AST assesses for executive control and may provide some indication of those who are liable to be pre-disposed to schizophrenia. The key findings thus far in the research on executive control in people with schizophrenia, their first degree relatives and in those scoring high for schizotypy, is that they make more errors on the anti-saccade task and take longer to respond. Healthy controls that are administered with THC seem to mimic the problems found in the schizophrenia spectrum for AST performance, whereas no problems are found with basic smooth pursuit eye movements. People with cannabis use disorder also demonstrate similar problems on the AST. There has been limited research looking at non-clinical samples of cannabis users versus non users on performance on the AST.

3.4 Individual differences, cannabis use, and cognitive functioning

The review in Chapter 1 indicated that cannabis users score higher on key personality traits which reflect a risk for developing psychosis (Bailey and Swallow, 2004), and furthermore having a greater number of these traits can predict performance on tests with known sensitivity to schizophrenia, such as the IGT (e.g. Hermann *et al*, 2009), AST (e.g. Holahan and O'Driscoll) and CPT (e.g. Bedwell *et al*, 2009). Whilst the first study only included a measure of schizotypy (the SPQ-B, see chapter 2) the current study sought to include several

other measures of potential significance: so in addition to schizotypy, ambivalence, mood and paranoia and impulsivity were included.

3.4.1 Ambivalence and Mood

Ambivalence is seen as a core trait in schizophrenia, where people fluctuate easily between conflicting emotions. For example, people with schizophrenia might have intense feelings of love for someone followed by intense feelings of hatred. Bleuler (1950) believed that ambivalence was one of the four fundamental symptoms that were constant among patients. Meehl (1980; 1999) proposed that ambivalence played a secondary role and that more people are schizotypes, in that they are genetically predisposed to develop schizophrenia, but few do; these people tend to share characteristics similar to schizophrenia patients and also have intense ambivalence. Ambivalence as a psychological construct has been poorly understood (Rawlin, 1986). Rawlin (1986) developed an intense ambivalence 65-item scale and then revised this to a 19-item scale to assess for schizotypal ambivalence. Kerns (2006) used the shortened schizotypal ambivalence scale (SAS) and found that disorganised schizotypy was associated with increased ambivalence, and moreover, ambivalence was strongly associated with decreased emotional clarity (as measured by the subscale 'clarity' on the trait-meta mood scale: TMMS; Salovey *et al*, 1993). Emotional processing is also under investigated in cannabis users. In a recent study, Platt *et al* (2010) identified that heavy cannabis users' ability to understand emotional expressions (e.g. sadness, anger or happiness) was reduced when compared to healthy controls. This possible reduction in ability to identify emotional expressions could also be a sign that cannabis users have difficulty with expressing emotion and thus would be likely to have more ambivalence traits. Therefore in this current study, the cannabis users will also be assessed for emotional processing using the subscale of clarity of thoughts on the TMMS and ambivalence as assessed by the SAS.

3.4.2 Paranoia

Persecutory delusions and paranoia are the second most common symptom of psychosis (Johnson *et al*, 1991), and such experiences/states also appear to be commonplace in cannabis users and following use of cannabis. In non-clinical samples, D'Souza *et al* (2004) conducted a double-blind placebo controlled study and found that participants who were injected with

intravenous THC exhibited a greater display of positive symptoms (including paranoid thinking) compared to those administered placebo. Verdoux *et al* (2003) also found that those who reported experiencing more delusions or hallucinations also showed increases in perceptual experiences after smoking cannabis. Freeman *et al* (2008) assessed levels of delusional thoughts and paranoid thinking and suggested that paranoid symptoms and delusional thoughts are strongly linked with negative affect. Paranoid thinking was assessed for levels of delusional thoughts and persecutory ideas, using the Green *et al* Paranoid Thoughts Scale (GPTS, 1995), which is a 19-item measure with two parts: part A assesses social reference to paranoia (e.g. suspicious of other people), whereas Part B represents ideas of persecution (e.g. people are out to intentionally harm you). Previous research indicates that people who score highly on this measure were three times more likely to infer that neutral stimuli was more threatening in a virtual reality experiment of an underground tube ride, compared to those low on paranoia.

3.4.3 Impulsivity

Impulsivity (or impulsiveness) is defined as multifactorial construct that involves a tendency to act hastily (i.e. acting on a whim) or displaying behaviours characterised by little forward thinking, or consideration of the consequences (Dick *et al*, 2010). Impulsivity has been implicated in numerous psychiatric disorders which includes schizophrenia and substance abuse (Moeller *et al*, 2001; Ouzir, 2013). A person may impulsively use drugs to alleviate self-medicate, or because they are unable to foresee negative consequences associated with this risky behaviour. The schizophrenia-sensitive cognitive assessments discussed above: IGT, CPT and AST are linked together by trait impulsivity, in that performance deficits (in decision-making and inhibitory control) are correlated with higher trait scores of impulsivity (Morgan *et al*, 2006, Jacob *et al*, 2010, Wrege *et al*, 2014). Studies have shown the non-clinical samples of recreational cannabis users showed increased impulsivity personality traits (Verdejo-Garcia *et al*, 2008). It could be argued that cognitive performance may be the result of underlying increased impulsivity in the drug group, as a result of the links between cannabis and impulsive behaviour (e.g. Dalley *et al*, 2011; Robbins *et al*, 2012) and also because the cognitive assessments used in this study are measures of behavioural impulsivity. Therefore, the Barratts Impulsivity Scale (BIS-II, Patton *et al*, 1995) measure will be

included to assess the relative impact of trait impulsivity on cognitive performance in cannabis users.

3.4.4 Rationale

In line with the general hypothesis in this thesis, that cannabis use may shift aspects of behaviour further along a schizophrenic-schizotypic spectrum, and in line with extant data (see above), it is predicted that the sample of cannabis users in this study will show cognitive performance and personality differences from controls, in this direction. In the cognitive assessments, the cannabis users are predicted to show worse performance on the AST, IGT and CPT tests, and on the personality measures to score higher on the traits of schizotypy, paranoia, ambivalence, impulsivity and lower in clarity of thinking.

What is unique about this research work is the combination of cognitive tests and personality measures in one study, and this should allow for some light to be shed on how/if cannabis is affected each variable/measure, and whether it is the cannabis use per se that best explains the differences (especially variables like level of use, age of onset of cannabis), or if a more complex set of interactions best accounts for the data. It is acknowledged that some of these measures may naturally interact, and that, for example, some personality traits may predict cognitive performance, regardless of drug use. Such interactions will be explored using multiple regressions to attempt to statistically account for the relative contribution of cannabis use to cognitive performance outcomes, against that of factors such as schizotypy.

3.5 Method

3.5.1 Participants

Thirty regular cannabis users and thirty non-cannabis users took part in this study, with 16 males and 44 females with an age range of 18-47, and a mean age of 25. The exclusion criteria were use of psychoactive medication, a diagnosis of epilepsy; brain trauma or a positive drug screening result.

Recruitment

The same recruitment methods as previously used in Study 1 (see section 2.2) was applied with the exception of placing an advertisement in the Evening Standard newspaper as opposed to the Camden New Journal. Further, general 'wanted participants to take part in psychological research' posters were placed on the community advertisement boards in the local supermarkets, such as Sainsbury's and Morrsions.

Ethical clearance

Ethical clearance was obtained through the UEL Graduate School (reference = ETH/11/24 – see appendix xvii). All codes and regulations for compliance with the BPS (1996) Ethical Codes of Conduct for conducting research using human participants were upheld throughout.

Research setting

All testing was conducted at the UEL recreational drugs lab, School of Psychology, Stratford campus. A separate lab in the same research suite was used for eye tracking. Testing mainly took place at 11 am or at 2pm.

3.5.2 Materials

Cognitive tests

The anti-saccade task (AST)

The AST was administered using a Tobii eye tracking system (Tobii Studio 1.3; Tobii, 2011). The AST was similar the one used by Ettinger *et al* (2004). A white target of circular shape (approximately 0.3° of visual angle) was presented on a black background using a 17-inch computer monitor. Participants sat in a comfortable chair at a distance of 60 cm from the monitor. A standard (no-gap, non-overlap) anti-saccade task was used. Four practice trials each began with the target in the central location (0°) for a duration of 100ms. Participants were instructed to look in the opposite direction of a moving target on the screen and, secondly, when the target moved to the right the participants were to look in the same distance to the left where the target rests (and vice versa). The experimenter observed the eye tracking in a different screen to validate that participants understood the task.

The real test began by relaying the same instructions to the participants and the trials (video files) were randomised to be presented for a duration of 1000–2000 ms: specifically at 1000, 1250, 1500, 1750 and 2000ms. The target was then stepped to one of two peripheral target locations (±6°) where it remained for 1000 ms, before moving back to the centre for the next trial. Each peripheral location was used 15 times, resulting in a total of 30 trials. Our test had a variation of the AS-T test by adding a new target location of (±12°) repeated the same way 25 times, resulting in an additional 30 trials; so 60 trials overall. Participants were instructed to look at the target while it was positioned in the centre of the screen and to redirect their gaze to the exact opposite location of the target as soon as it moved to its target location. The main emphasis was to assess the inhibition of a reflexive saccade towards the target. Two dependent variables are AST error and latency. IGT error represents an incorrect trial (i.e. following the moving target as opposed to looking in the opposite direction) and latency which is the speed of responding. The preparation and testing took between 20-30 minutes.

The Iowa Gambling Task (IGT)

The IGT is the same version as the original used by Bechara, Damásio, Tranel and Anderson (1994). The computerised task was run using a laptop computer and mouse. Participants had to choose from a deck of four cards which appeared on the screen named A, B, C or D. The cards are set in a pre-determined order for payoffs and losses. Decks A and B are considered *disadvantageous*, with high immediate gains but also expensive losses, producing a net loss of 250€ every 10 cards. Decks C and D are considered *advantageous* ones, with smaller immediate gain and smaller losses, causing a net gain of 250€ every 10 cards. The participants are instructed to 'win as much money as they can' by picking one card at a time from each of the four decks (A-D). The participants could do this in any order until the computer instructs them to stop at the 100th choice. Participants are initially given a budget of €2000 and can see their wins/losses at the top of the screen, which is updated after each card choice. IGT dependent variables are IGT total score and IGT learning score. The total score is calculated by subtracting the total number of cards selected from the disadvantageous pack (A and B) from the total number of cards selected from the advantageous pack (C and D). The learning score is calculated over the 5 blocks of 20 cards assessing the difference between the number of cards picked from *advantageous* decks (C and D) minus those picked from *disadvantageous* ones (A and B). No financial incentive was given for the IGT and the task took up to 15 minutes to complete.

Continuous Performance Test (CPT)

The CPT was similar to that used by Connors (2000) and it was administrated to all participants on a Laptop computer. The practice test involved the presentation of 20 number sequence with digits presented at 1000 millisecond intervals, with participants asked to press the mouse button when they have seen the presentation of a specific pattern of numbers. The test involved the presentation of 75 separate 5-digit numbers (e.g. 43578) (at 500 millisecond intervals) played in a random order. Participants are instructed to press the keypad when the same digits are repeated (e.g. 43578 is followed by 43578). Some of these digits are trick digits (e.g. 62897 is followed by 62895) and some are correct (e.g. same digits are repeated) and others are novel digits (e.g. new arrangements of digits). Throughout the task the digits '12345' are presented 3 times in between each of the 5-digit number presentations and these are known to the participants as 'distracters' - the participants are initially told to ignore these

stimuli. CPT dependent variables are: Hit rate (reacting correctly to the target stimuli), Hit reaction time (index of the speed for the correct response) and commission error (or sometimes known as false alarm) equals an incorrect response to the target, omission errors represents the number of incorrect motor responses (index of the amount of incorrect responses to trick, novel or distracter stimuli). The time taken to complete the task was up to 20 minutes.

All of the tests were counterbalanced: 1) IGT, CPT and AST; 2) CPT, IGT, and AST, 3) AST, IGT and CPT, 4) IGT, AST, and CPT; 5) CPT, AST and IGT; 6) AST, CPT and IGT.

Questionnaires

Severity of Dependence Scale (SDS) (Gossop *et al*., 1995 - see appendix iv) and Schizotypal Personality Questionnaire (SPQ–B) (Raine and Benishay, 1995 - see appendix v) – both questionnaires are described under section 2.2.2

UEL drug use questionnaire (Parrott *et al,* 2001) (see appendix vi)

Please refer to Chapter two (section 2.3) for a full description of the Personal History Questionnaire and Lifetime drug use questionnaire, the SPQ-B and the SDS.

The Paranoid Thoughts Scale (GPTS; Green *et al*, 2008 - see appendix vii)

The GPTS is a trait measure of paranoia with two 16-item scales assessing ideas of social reference and ideas of persecution. Participants were asked to rate their agreement with statements referring to experiences, thoughts or feelings over the last month, scoring from 1 to 5, where 1 = "Not at all" and 5 = "Totally". They were asked to complete both Part A and Part B and instructed not to "rate items according to any experiences you may have had under the influence of drugs". A total score is for each scale is obtained by summing Part A or Part B, respectively and higher scores indicate greater levels of paranoid thinking. The internal consistency of the scale and test–retest reliability are good and convergent validity has been demonstrated with the Paranoia Scale (Fenigstein & Vanable, 1992).

Part A is a self-report measure of social reference of paranoid thinking in the past month and consists of a list of sixteen items. Two example questions were:

9. I was convinced that people were singling me out

16. It was hard to stop thinking about people talking about me behind my back

Part B is a self-report measure of the occurrence of persecutory ideation in the past month. It contains a list of sixteen items and two example questions were:

3. People have intended me harm

13. The thought that people were persecuting me played on my mind

Trait meta-mood scale (TMMS; Salovey *et al*., 1995 - see appendix viii)

The TMMS is a measure of emotional intelligence with 30-items and has three subscales: attention to feeling, clarity and repair. Similar to Kern *et al's* (2005) study *o*nly the Clarity subscale of the TMMS was used in this study to assess how much someone understands their own emotional states. The Clarity subscale, consists of 11-items (5 reversed scored); an example question would be "sometime I can't tell my feeling are". Participants were asked to read each statement and indicate the degree to which they agreed or disagreed with each using a tick box likert-scale ranging from Strongly Disagree (1) to Strongly Agree (5). The subscale of Clarity has adequate internal consistency (0.87) and good convergent and discriminant validity (Solovey *et al*, 1995)

Schizotypal Ambivalence Scale (Rauling, 1986 - see appendix ix)

The schizotypal ambivalence scale (SAS) is a 19-item true/false questionnaire which is designed to assess the trait of ambivalence, which is seen as one of the core characteristics of schizotypy and schizophrenia. Participants were asked to answer each item by circling T (True) or F (False) and were required to respond to all items even if they were unsure of the answer. A total SAS score is obtained by summing all of the (True) responses (i.e. score range 0-19). The SAS has good internal consistency reliability (.84) and correlates moderately with other psychometric measures of schizotypy. Example questions were:

1)	T	F	Often I feel like I hate even my favorite activities.
8)	T	F	I always seem to have difficulty deciding what I would like to do.
10)	T	F	Love and hate tend to go together.

Barratt Impulsiveness Scale version 11 (BIS-11: Patton *et al*., 1995 - see appendix x)

The BIS-11 is a 30-item questionnaire designed to assess general impulsiveness which is broken down into six BIS-1st order factors (attention, motor, self-control, cognitive complexity, perseverance, cognitive instability) and three BIS-2nd order factors (attentional impulsiveness (attention and cognitive instability), motor impulsiveness (motor and perseverance), non-planning impulsiveness (self-control and cognitive complexity). The items are scored on a four point scale from 1 (Rarely/Never), 2 (Occasionally), 3 (Often) to 4 (Almost Always/Always). Example questions are: "I do things without thinking"; "I act on impulse"; "I often have extraneous thoughts when thinking". A total BIS score is obtained by summing the first and second-order factors. The reported internal consistency coefficients for the BIS-11 total score range from 0.79 to 0.83 for different populations groups: under-graduates, substance-abuse patients, general psychiatric patients, and prison inmates (Barratt *et al*, 1995). In a review by Stanford *et al,* 2009 internal consistency for the First order subscales were: Attention (0.72), Cognitive Instability (0.55), Motor (0.64); Perseverance (0.27), Cognitive Complexity (0.48), SC = Self-Control (0.72) and in the Second order: Attention (0.74), Motor (0.59), Non-Planning (0.72).

3.5.3 Procedure

The procedure was similar to that of study one for informed consent (see appendix xii), drugs screening and DNA screening (see section 2.2.3 for further details). The participants then completed the cognitive assessments and questionnaires. All participants' were de-briefed accordingly (see appendix xiii) and if any questions arose about the true nature of the study, these were effectively addressed at this particular time. Participants were provided with an information sheet (see appendix xi) that contains the relevant contact details of professional bodies, who directly deal with questions relating to mental health issues and drug use advice. Participants were awarded between £15-£20 remuneration for participation.

3.6 Results

Data Screening

Normality of variables for the SPQ measure

Data were explored using boxplots to look for outliers defined as one and a half times the length of the box from either end of the box. The SPQ-B measure and its subscale of 'interpersonal' had 2 outliers removed: 1 from the cannabis group and 1 from the non-cannabis group. Part A of the GPTS had 6 outliers removed: two from the cannabis group and 4 outliers for the non-cannabis group. Part B of the GPTS had five outliers removed, three from the cannabis group and two from the non-cannabis group. The BIS-2nd order had one outlier removed from the cannabis group data. Data from all the cognitive tests were generally normally distributed, although some minor changes were required as follows:

- 2 anti-saccade error scores were removed from the cannabis user group data
- 2 outlier scores were removed from the IGT dataset in the non-cannabis group
- Several outliers were removed from the CPT data: 1 for hit rate in the cannabis group; 2 for commission errors in the cannabis group; 3 for motor errors (1 in the non-cannabis group and 2 from the cannabis group) were removed which resulted in better distribution.

Skewness and kurtosis of each data set after exclusion of outliers were all below 0.2 or 0.1 respectively. In the cannabis group, there was missing data for profit/loss made after every 10 card selections in one participant due to technical issues and having to use a different version of the IGT, however, the overall total IGT score was obtained for all participants. Due to some technical problems with the Tobii eye tracking equipment, 4 participants' in the cannabis group and 8 participants' data in the non-cannabis group was not obtained on the AST. There was 1 missing data point in the non-cannabis group for the CPT.

3.6.1 Demographic/health details and patterns of drug use

Possible differences in the mean ages of the participant groups (cannabis and non-cannabis) were analysed using the t-test statistic, whilst gender and other demographics were explored using Chi² tests. It can be seen from Table 19 that there were significantly more males and white Europeans in the cannabis group, as well as lower health ratings (measured on a 4-point likert scale), and more personal and familial mental health diagnosis histories in this group (such as depression and anxiety). The cannabis sample in the Study 2 had slightly older participants in this cohort compared to Study 1.

Table 19: Demographic and health details for cannabis users and non-cannabis users

	Non-cannabis group (30 Participants)	**Cannabis group (30 Participants)**	**Test** X^2	***p***
Age Range, mean (SD)	18-37, 24 (5.5)	18-47, 26 (8.6)	0.938	ns
Gender (Males/Females)	4/26	12/18	5.45	**0.02**
Nationality (British/Non British)	20/10	22/8	0.31	Ns
Ethnicities (White European/Black/Asian/Mixed Race)	15/4/7/7	21/7/1/1	8.11	**0.04**
Occupation (Employed/Unemployed/Student)	10/2/18	11/3/16	0.365	Ns
Highest Qualification (GCSE/ A level or further/ Degree/ Post grad/ PhD)	0/22/3/4/1	2/25/2/1/0	3.191	Ns
Health Rating (Poor/Moderate/Fine /Good)	0/7/6/17	1/11/12/6	9.13	**0.02**
Personal Mental Health History (diagnosis)	2	10	6.66	**0.02**
Familial History (diagnosis)	8	17	5.55	**0.03**
Brain Injury (yes)	0	0	-	-
Medication (yes)	1	0	-	-

Table 20 below summarises other drug use in the cannabis and non-cannabis users. Group comparisons, just including the data for each drug from those participants in each group admitting to have used that drug, was assessed as follows: polydrug use was assessed using a Chi^2 analysis amount of use, age of onset was assessed using Mann Whitney U tests. Cannabis users reported more drug use than non-cannabis users for alcohol, MDMA, poppers, ketamine, GHB, Prozac, amphetamine, cocaine, LSD, Benzodiazepine, Mushrooms, and Opiates as well as other drugs not listed (e.g. legal highs) and reported more unusual experiences from these drugs. Only 2 participants reported current polydrug use (2 or more drugs as well as current cannabis use). Higher drug use was reported in the cannabis group in Study 2 (see Table 20) with 15 out of 20 cannabis users reporting polydrug use. Further, the sample in this study was also different to the previous study sample (in Study 1) due to the history of opiates and crack cocaine.

Table 20: Other drug use in cannabis and non-cannabis user groups.

Variable	*Cannabis group*	*Non-cannabis group*	*U x²*	*p or ns*	*Variable*	*Cannabis group*	*Non-cannabis group*	*U x²*	*p or ns*
Cigarettes (n= Yes)	(26)	(4)	32.269	**<0.001**	Amphetamine –times Mean(SD)	121(243)	1-2, 1.5(0.7)	-1.537	ns
Cigarette/day Range, Mean(SD)	1-30, 7.5(6.5)	2-30, 14.25(11.7)	-1.133	Ns	Amphetamine – last time	1-2 weeks (2); 6-12 months 2); 3+years (3)	3+ years (1)	1.5	ns
Cigarettes - age of onset Mean(SD)	16.4(3.64)	14.75(2.9)	-0.676	Ns	Cocaine (n= Yes)	(20)	(2)	23.25	**<0.001**
Cigarettes - last time used (n=)	That day (21) one day (5)	That day (2) one year (1) one year plus (1)	-1.735	Ns	Cocaine– age of onset Mean(SD)	14-30, 20(4.4)	19-23, 21(2.82)	-0.288	ns
Alcohol (n= Yes)	(28)	(22)	4.320	**0.04**	Cocaine – times Mean(SD)	91(249)	27 (32.5)	-0.344	ns
Alcohol (units per week) Mean(SD)	12.7(6.5)	4.43(6.98)	-3.081	**0.002**	Cocaine – last time used Mean(SD)	1-2 weeks (6); 1-6 months (4); 6+-18 months (4); 18+-24 months (2); 3+years (4)	6+-18 months (1); 3+years (1)	10.5	ns
Alcohol – age of onset Mean(SD)	16.5(3.17)	17.37(2.51)	-1.058	Ns	LSD (n= Yes)	(6)	(0)	-	-
Alcohol – last time (n=)	1 day (7); 1 week (19); 1 month (3); 1 year+ (1)	1 day (1); 1 week (12); 1 month (6); 1 year (2)	-2.257	**0.024**	LSD- times Mean(SD)	87(140)	-	-	-
MDMA (n= Yes)	(16)	(2)	15.56	**<0.001**	Benzo (n= Yes)	(4)	(0)	-	-
MDMA – age of onset Mean(SD)	20(4.68)	16.5(2.1)	-1.273	Ns	Benzo- times Mean(SD)	127(162.8)	-	-	-
MDMA – number of times used Mean(SD)	121(243)	1.5(0.7)	-1.993	**0.03**	Mushrooms (n= Yes)	(13)	(0)	-	-
MDMA – last time used (n=)	1-2 weeks (4); 1-6 months (5); 6+-18 months (2); 18+-24 months 2); 3+years (3)	3+ years (2)	3.0	**0.03**	Mushroom times Mean(SD)	1-365, 35(99)	-	-	-
Poppers (n= Yes)	(12)	(1)	11.88	**0.001**	Crack (n= Yes)	(6)	(1)	4.05	**0.05**
Poppers times Mean(SD)	105(246)	-	-1.617	Ns	Crack- times Mean(SD)	45.5(78)	50	-	-
Ketamine (n= Yes)	(9)	(0)		-	Opiates (n= Yes)	(4)	(0)	-	-
Ketamine times Mean(SD)	27.5(25.5)	-	-	-	Opiate times Mean(SD)	95(179)	-	-	-
GHB (n= Yes)	(4)	(0)	-	-	Steroids (n= Yes)	(0)	(0)	-	-
GHB times Mean(SD)	135(265)	-	-	-	Steroid times Mean(SD)	-	-	-	-
Prozac (n= Yes)	(2)	(0)	-	-	Other Drugs 1	(2)	(0)	-	-
Prozac times Mean(SD)	7(4.24)	-	-	-	Other Drugs 2	(2)	(0)	-	-
Solvents (n= Yes)	(2)	(0)	-	-	Poly Drug Use (n= Yes)	(2)	(1)	20.26	-
Solvent times Mean(SD)	1.5(.70)	-	-	-	Current Poly Drug Use (n= Yes)	(2)	(0)	-	**0.006**
Amphetamine (n= Yes)	(7)	(1)	5.192	**0.026**	Unusual Experiences (n= Yes)	(5)	(0)	-	-
Amphetamine – age of onset Mean(SD)	19 (4.26)	16.5(2.1)	-1.098	Ns					

Table 21 below indicates that the amount of use per day was quite varied between one joint to twenty joints per week. Most of the participants were introduced to cannabis via friends, the mean duration since last use was 3.6 days (note a 2 day abstinence period was requested); the main type used was of the skunk variety, the participants varied in terms of how long they used cannabis for ranging between 1-35 years, with mean years at 10.53. The cannabis dependency scores ranged from 0-12, with a mean score of 4.9. Twenty people scored a dependency score of 4 and above which indicates dependency of cannabis within this sample.

Table 21: Patterns of cannabis use in the cannabis group.

Variables	**Range or n**	**Mean (SD)**
Number of joints per day (range and mean (SD)	1-5.50	2.52 (3.18)
Frequency of use -Joints per week (range, mean (SD)	1-20	3.6(3.9)
Cannabis age of onset (range and mean (SD)	12-23,	15.56 (2.84)
Cannabis introduction (n=)	Friends = 26; Family = 4	
Cannabis last time (days) range and mean (SD)	2-14	3.6(2.4)
Cannabis acute problems (n=)	Yes = 12; No = 18	-
Types used most often (n=)	Skunk = 16; Grass = 11; Resin = 3	-
Cannabis duration range and mean (SD)	1-35	10.53 (9.29)
Cannabis Dependency Score range and mean (SD)	0-12	4.9(3.37)

3.6.2 Trait measures

Table 22 shows the means and SDs for the trait measures. One-way ANOVAs were carried out to see if there were any significant differences between cannabis users and non-cannabis users on the trait measures. It was found that cannabis users scored significantly higher on the Schizotypal Personality Questionnaire (SPQ) for total scores (F (1, 56) = 11.88, p = 0.0004) and the two subscales of cognitive perceptual (F (1, 56) = 8.945, p = 0.004) and disorganised thinking (F (1, 58) = 13.8, $p < 0.001$); but not on the subscale for interpersonal of SPQ-IP. Cannabis users also reported experiencing more paranoid thinking, with significant differences found between scores on part A and part B of the Paranoid Thoughts Scale in relation to social reference to paranoia (F (1, 52) = 4.994, p = 0.03) and ideas of persecution (F (1, 53) = 7.36, p = 0.009). Cannabis users scored significantly lower than non-cannabis users on emotional processing as measured by the clarity subscale on the trait meta-mood scale (F (1,58) = 7.666, p = 0.008) and statistically higher on the Ambivalence measure as assessed by the Schizotypal Ambivalence Scale SAS (F (1, 58) = 4.339, p = 0.04). Cannabis users also scored significantly higher than non-cannabis users on impulsivity for the Barratt Impulsivity Scale-first order (F (1, 58) = 11.266, p = 0.001) and second order factors (F (1, 57) = 10.095, p = 0.002).

Table 22: Schizotypy, paranoia, emotional clarity, ambivalence and impulsivity scores in cannabis and non-cannabis user groups.

Trait measures	**Cannabis user**		**Non-cannabis user**			
	Mean	**SD**	**Mean**	**SD**	***F***	***P***
SPQ-CP	2.8	1.9	1.73	1.74	8.945	**0.004**
SPQ-IP	2.7	1.87	2.03	1.77	1.91	ns
SPQ-DT	1.96	1.30	0.97	0.97	13.8	**<0.001**
SPQ-total	7.30	3.57	4.28	3.04	11.88	**0.004**
GPTS Part A	25.7	8.2	20.88	4.98	4.994	**0.03**
GPTS Part B	20.3	4.50	17.64	2.65	7.36	**0.009**
TMMS Clarity	35.2	6.04	39.2	5.21	7.666	**0.004**
SAS	7.83	4.79	5.23	4.87	4.339	**0.04**
BIS-first	68.37	9.11	60.6	8.8	11.266	**0.001**
BIS-Second	67.34	7.01	60.6	9.11	10.095	**0.002**

3.6.3 Cognitive outcomes

Table 23 presents the data from the AST, IGT and CPT in cannabis and non-cannabis users. In the gambling task, there was a trend for cannabis users to select more for the risk decks A&B more often (t (56) = 1.55, p = 0.06) which resulted in the cannabis users winning more money on the IGT (F (1, 56) = 3.791, p = 0.05) than non-users (see Figure 7). Further to this, higher use of cannabis (number of joints smoked per week) was correlated with an increase in riskier decision making (r = -0.326, p = 0.03). There were no significant differences found in the other neurocognitive tests

Table 23: Anti-saccade (AST), IOWA gambling (IGT) and Continuous performance task (CPT) scores in cannabis and non-cannabis user groups

Cognitive measure	**Cannabis user**		**Non-cannabis user**			
	Mean	**SD**	**Mean**	**SD**	***F***	***p***
AST-error	26.4	20.63	21.7	26.8	0.682	ns
AST-latency (ms)	306.27	89	316.78	56.97	0.194	ns
IGT-total	1258	933	1687	723	3.791	**0.05**
IGT_net score	-16	4.428	-6.85	20.30	1.55	0.06
CPT-Accuracy	20.31	4.26	19.90	3.93	0.07	ns
CPT-Commission errors (CE)	6.35	7.06	8.57	5.69	1.292	ns
CPT-Response Time (RT)	579.79	139.8	577.50	90.6	1.465	ns
CPT-Motor errors (ME)	1.22	1.25	1.62	1.67	0.02	ns

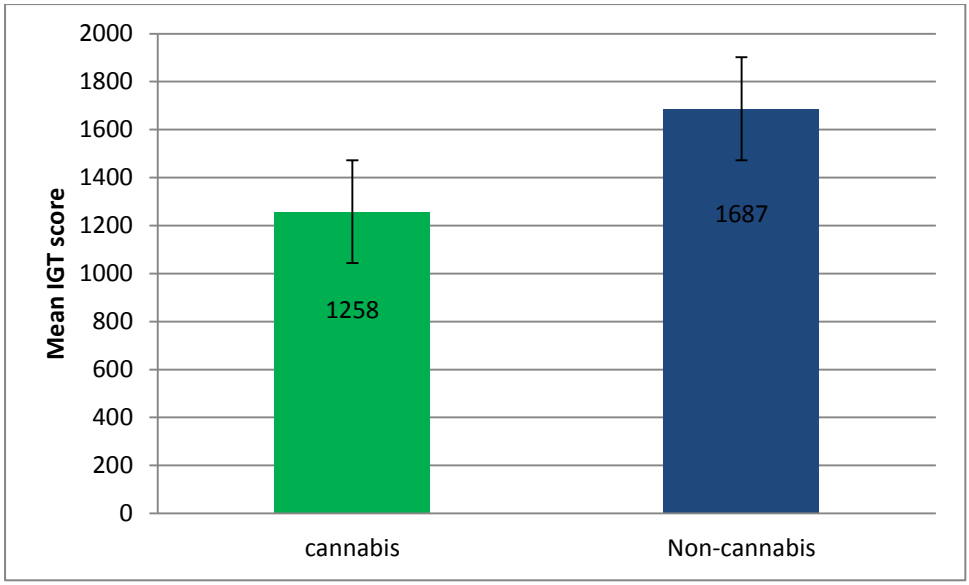

Figure 7: Represents the difference between cannabis users and non users on risky decision-making as assessed via the IGT.

3.6.4 High/Low SPQ-B and Cognitive Variables

The participants were divided into two non-overlapping groups based on the mean SPQ-B scores (M = 6.2, SD = 4.06) and the data from 5 participants at the mid-point were removed (Laws *et al*, 2008). 33 participants were in the low SPQ-B group and scores ranged from 0-6 and 22 participants in the high SPQ-B group with scores ranging from 8-17. The mean scores on the cognitive tests in these high and low SPQ-B scorers are summarised below in Table 24. A third analysis was run to check for performance between these groups for high/low SPQ-B in terms of their cognitive performances. A one-way ANOVA revealed there was a significant difference between high and low SPQ-B groups on the AST error measure (F (1, 41) = 9.143, p = 0.004), with high SPQ scorers making more errors on the AST, but there was no difference found in latency. No significant differences were found between high and low SPQ groups on IGT performance.

No significant differences were found between high and low SPQ-B scorers for CPT accuracy, motor errors or commission errors, but high SPQ scorers had faster reaction times on this task (CPT-RT; (F (1, 56) = 4.689, p = 0.035).

Table 24: Cognitive test results between high and low SPQ-B scores.

Cognitive Tests	**High SPQ-B**		**Low SPQ-B**			
	Mean	**SD**	**Mean**	**SD**	***F***	***p***
AST-error	35.07	27.22	14.29	14.03	9.143	**0.004**
AST-latency (ms)	305.55	92.40	351.80	53.8	0.552	ns
IGT-total	1379.5	822	1547	930	1.019	ns
CPT-accuracy	20.24	3.47	20	4.52	0.049	ns
CPT-RT	558	125.71	603	103.15	0.049	ns
CPT-ME	1.27	1.49	1.57	1.70	1.965	**0.035**
CPT –CE	6.73	4.68	7.969	7.47	0.488	ns

3.6.5 Correlations for Cognitive and Trait Variables

A series of Pearson correlations were conducted to look at associations between the personality trait measures and the cognitive task outcomes (see Table 25). A second correlation analysis was performed on the cannabis group only, to explore the relationship between cannabis use variables (e.g. age of onset, dependency, duration and joints per week) on cognitive outcomes (see table 3h). There was a medium negative relationship between CPT accuracy and CPT motor errors, with greater accuracy linked to fewer motor errors. There was a negative medium relationship between CPT mean RT with SPQ-IP, with those reporting more traits linked to the interpersonal dimension of schizotypy being slower to respond on the CPT. There was a medium positive relationship between AST error and SPQ total, with higher error linked to experiencing a higher amount of psychotic like traits. SPQIP was also linked with AST latency, with a negative medium relationship between greater levels of interpersonal schizotypy and taking longer to respond on the AST task. A similar finding was found for SPQIP and AST error as well as BIS-2nd and AST error, with greater levels of interpersonal schizotypy and impulsivity linked to greater AST error.

Table 25: Correlations between personality trait measures and the cognitive test outcomes

Variable	CPT-RT	CPT CE	CPT ME	AST-Err	AST-latency (ms)	IGT-total
CPT-accuracy	*0.356		**-0.552			
CPT_CE	+-0.259					
AST-latency (ms)				*-0.34		
IGT-total					*-0.31	
SPQ-total				**0.44		
SPQ-IP	**-0.39	*0.211		**0.48	*-0.37	
SPQ-CP						*-0.26
GPTS-part A		*0.211				
BIS-2nd				**0.46		

**. Correlation is significant at the <0.001 level (1-tailed)
*. Correlation is significant at the 0.01 – 0.05 level (1-tailed).

Table 26 below highlights a statistically significant medium positive association between the first order impulsivity scale and joints per week (JPW) with higher use linked to experiencing more impulsive thinking. A medium negative correlation was found between JPW and IGT, with an increase in joints smoked per week being linked to less monetary return on the gambling task. There was a strong positive correlation between JPW and impulsivity for the first order factor, with an increase in joints per week linked to cannabis users having higher trait impulsivity. There was a negative medium association between clarity of thoughts and duration of cannabis use, with longer use linked to feeling less clear in thinking. Duration of use was also negatively associated with GPTS partA and the SPQ-B subscale of interpersonal thinking, with shorter duration of cannabis use associated with higher schizotypal interpersonal deficits and higher social reference to paranoia.

Table 26: Correlation data for cannabis use variables and trait/cognitive outcomes

Variable	JPW	Duration
AST- latency		*0.363
IGT- total	*-0.326	
CPT- RT		
TMMS- clarity		*-0.389
GPTS-partA		*-0.334
GPTS-partB		
SPQ- IP		*-0.342
BIS-Ist	*0.435	
BIS-2nd		

*. Correlation is significant at the 0.01-0.05 level (1-tailed).

3.6.6 Regression analysis for cognitive and trait outcomes

A number of regression analyses were performed to look at individual differences in personality and cannabis use, to see which best predicts outcomes on each of the cognitive measures. The main predictor variables (PVs) were cannabis use, all of the personality trait measures (i.e. SPQ-B total, SAS, GPTS A and B, BIS-1st/2nd, TMMS-clarity) and the model was adjusted for sex due to a significantly greater number of males in the cannabis group. Sex was not a significant predictor/covariate.

1. For the CPT data, none of the regression models were significant.
2. The IGT was entered into the regression model as the criterion variable and the model was not significant and none of the PVs significantly predicted IGT outcomes.
3. AST latency was entered as the CV but the model was not statistically and no PVs significantly predicted outcomes on this measure. Anti-saccade task error as a CV was statistically significant (F (9, 36) = 2.390, p=0.031; R^2 43%, Adj R^2 28%). Performance on the AST was predicted by psychotic-like personality traits and impulsivity as shown below in Table 27. In that higher impulsivity and increased psychotic-like personality traits was linked to poorer performance, resulting in more error on the AST.

A separate regression analysis removed the non-cannabis users from the model to assess the individual differences in the cannabis users on the SPQ-B total, SAS, GPTS A and B, BIS-1st/2nd; TMMS-clarity; and the model was adjusted for sex. There were no significant predictors of these criterion variables on cognitive performance on IGT, AST and CPT, and none of the models were significant (*p>0.05).* There was a trend for the SPQ-B total to

predict outcomes on the AST for error (F (5, 25) = 2.003, p = 0.056, in that higher psychotic-like traits were associated with more error on the AST.

Table 27: Trait predictors of anti-saccade task performance

Predictor variable	***Beta***	***p***
SPQ total	0.584	0.002
BIS-1st	0.745	0.008
BIS-2nd	0.726	0.007

(sex, GTPS parts A and B, SAS, TMMS-Clarity were not significant PVs)

3.6.7 Regression analysis for cognitive and trait outcomes and cannabis

To further explore outcomes on these tasks, the cannabis use variables were entered into separate regression models as PVs to determine whether level of dependence, length of use and/or age of onset impacted on cognitive task performance. The SPQ-B total was added to these regression analyses to check if individual differences in this personality measure are predictive of cognitive performance more so than cannabis use per se.

1. The IGT model was not statistically significant but the main predictor variable for IGT performance was joints per week (t (23) = 2.127, p = 0.04) indicating that outcomes on the IGT (i.e. risky decision making) were linked to heavy cannabis use.
2. CPT accuracy as the CV was not significantly predicted by the PVs and there were no significant findings for CPT RT, CPT motor errors and CPT commission errors.
3. AST error as a CV was significantly predicted by SPQ-total (t (19) = 2.728, p = 0.01. Adj R^2 0.127) but the model was not significant. AST latency as a CV was not significant.

3.7 Discussion

The main aim of this study was to compare cannabis users and non-cannabis users on cognitive tests with known sensitivity to schizophrenia and also to assess individual differences in schizotypal traits. The cognitive assessments measured decision-making using the IGT, attention using the CPT and executive control using the AST. Additionally, this study looked at personality variables as possible predictors of cognitive task performance in both the whole group and in cannabis users. In line with the model that cannabis use may act to modulate cognitive function, in a direction more akin to that seen in schizophrenic and high schizotypy individuals, it was predicted that we would see riskier decision making, disruption attention and impaired executive control in the cannabis group.

3.7.1 Decision-making

In line with the general model/theme of the thesis it was predicted that cannabis users would show worse performance on the IGT. The current study revealed impairments in decision-making for IGT performance in cannabis users who selected the decks which had immediate higher gains but also higher losses overall (see Figure 7), and therefore made a significant loss in comparison to the non-cannabis users. This is in line with previous research (e.g. Hermann *et al*, 2009; Bolla *et al*, 2003; Wesley *et al*, 2011).

Heavy cannabis use was also correlated with poorer decision-making on the IGT, which suggests riskier decision making, and may help to explain why people continue to use the drug despite its potentially negative effects. Previous research by Whitlow *et al*, (2004) and Hermann *et al* (2009) support this finding as they found poorer outcomes on the IGT in those that had used cannabis heavily relative to those with only partial use. Further studies have found that cannabis intoxication was associated with poorer IGT performance compared to controls (Lamers *et al*, 2006). Lamers *et al* (2006) also showed that cannabis users performed significantly worse on the IGT even after 15 days of abstinence. Similarly, Verdejo- Garcia *et al* (2007) found deficits in IGT performance in cannabis users even after 25 days of abstinence.

Other factors may have been involved in these decision-making strategies, for example, emotional and motivational processes may have played a part. It may be that the cannabis users have an increased sensitivity to rewards and insensitivity to losses or risk aversion, in that they have specifically chosen to use an illegal substance for personal gratification and discount the risks associated with this psychoactive drug. Previous research has found evidence to suggest that drug users reduce the value of a reward when the there is a delay in receiving this (e.g. Coffey *et al*, 2003). Further to this, Bolla *et al* (2002) showed that cocaine users show hyperactivation in the OFC and ventral striatum, areas known to be involved in the evaluation of a reward (e.g. O'Doherty, 2004). Whereas, in contrast to this, cannabis users in Bolla *et al*'s study showed decreased activation in the OFC and heavier cannabis users showed greater activation in the posterior cingulate and parahippocampal regions, which are known to be involved in working memory. The most robust finding in the acute effects of cannabis use is that it is known to disrupt short-term memory, whereas it is argued that long-term use may result in disrupting higher level cognitive processing (e.g. Solowij *et al*, 1995; Pope at el, 1995; 2003). Therefore, cannabis users may perform less well on the gambling task due to the impaired ability to track task contingencies (e.g. learning that packs A and B yield higher rewards but overall higher losses) and this may be related to impaired short-term memory updating rather than an enhanced sensitivity to the rewarding effects. However, a standard measure of memory performance was not applied in the current investigation so the results of the current study cannot discriminate between these alternative explanations.

Wesley *et al* (2011) used Magnetic Resonance Imaging (MRI) and reported that cannabis users performed similarly to the controls at the beginning of the task, but performed less well towards the end of the IGT, a pattern of findings that is echoed in the present study. Wesley and colleagues reported that controls displayed greater activity to losses in the anterior cingulate cortex, medial frontal cortex, precuneus superior parietal lobe, occipital lobe and cerebellum relative to cannabis users. Further to this, the activation in controls was positively correlated with losses over time, which suggests that cannabis users may be less responsive to the punishments of the task. Being less responsive to emotional component of the task also links into the arguments of the density of CB_1 receptors in the amygdala, which is linked to emotional responding and fear responding (Lin, Mao and Gean, 2006). Therefore, heavy and prolonged cannabis use may disrupt normal emotional processing, with participants showing

less responsiveness to the negative effects, which could be a reason of why they continue to misuse cannabis as they respond less to its negative impact.

Sevy *et al* (2007) compared a group of people with schizophrenia with concurrent cannabis use disorder with schizophrenics without concurrent cannabis use, and to a healthy control group. It was found that both schizophrenia groups were impaired and did worse of the IGT. However, there was no difference found on the IGT for patients with and without concurrent cannabis use. Problems on the IGT may be more likely related to factors predisposing the person to schizophrenia and/or linked traits. In this study, participants were divided into high and low SPQ-B total scores but that this did not impact on the IGT total score. Those with high scores on the personality measure made less money than those with low SPQ scores, but this finding was not statically significant. Therefore, IGT performance in this study was not directly affected by personality profile and was more closely linked with cannabis use *per se*.

3.7.2 Executive control

In line with the general model/theme of the thesis it was predicted that cannabis users would show worse performance on the AST. Although the raw data showed a possible trend with cannabis users making more errors on the anti-saccade task and being faster to respond relative to the non-cannabis group, these differences were not statistically significant. This is similar to the finding by Chung *et al* (2010) who looked at adolescents with and without cannabis use disorder and found no difference in performance on the AST. In the current study people that use cannabis recreationally and abstained for two days did not show any disruption in AST performance, although some trends existed for deficits to be more pronounced in the heavier users of the drug. For example, there was an association between increased amount of cannabis used per week and length of use was positively correlated with reaction time on the AST, thus the more cannabis smoked and a longer duration of use were both associated with faster responding on the AST. Cannabis use varied across the group, so it may be that if heavier cannabis users were assessed as a single group, then deficits would be more prominent for this drug group.

In order to explore the possible influence of schizotypal personality traits on task performance the group was then divided into high and low SPQ scorers. Those scoring high on this measure made twice as many errors on the AST. This finding is supported by previous research (Holahan and O'Driscoll, 2005; Ettinger *et al*, 2005; Ettinger *et al*, 2006). Ettinger *et al* (in press) assessed for low and medium schizotypy and found that those people displaying more psychotic like traits had greater deficits on the AST. Larrison *et al* (2000) found more errors on the AST in a group of people scoring high on the full version of the SPQ. This aforementioned research is similar to that of the current study, thus it seems that the shorter version of the SPQ-B was sensitive in finding differences in AST performance.

3.7.3 Selective attention

No clear differences were found between cannabis users and non-cannabis users on the CPT for accuracy, speed and number of motor and commission errors. Research in the area of performance on the CPT in schizophrenic patients tend to show that schizophrenic patients perform less well for selective and sustained attention, whereas the research on cannabis use in clinical samples and in non-clinical samples has been less clear. This may help to explain why no difference was found between the cannabis users and non-cannabis users. For example, Rodríguez-Sánchez *et al* (2010) found no difference between schizophrenics who used cannabis versus those schizophrenics that did not use cannabis. Jockers-Scherübl *et al* (2007) found differential effects on CPT performance in schizophrenics who use cannabis versus controls who use cannabis after a 28-day abstinent period. Performance was impaired in healthy controls if they used cannabis before the age of 17, but performance was improved in patients that started cannabis before the age of 17. In the current study, the mean age of onset/first use of cannabis was 15.5, but this variable was not correlated with CPT outcomes. The current sample was compared to non-cannabis users, as opposed to a patient group, and further to this the abstinence level was set at 2-days, which may have yielded different findings on CPT outcomes. Even after the abstinence period of 28-days it seems that Pope *et al* (2001) could not detect any differences on the CPT in cannabis users versus controls. The researchers argued that the test battery for selective attention was not as sensitive as ERP study by Solowij *et al* (2002), nor was the CPT as sensitive at the selective attention LI task used in Study 1 (see Chapter 2).

CPT performance was not correlated with the SPQ-B subscales for positive symptomology and disorganised thinking. Interestingly, Bedwell, Kamath and Compton (2009) found an association between CPT performance and disorganised and positive schizotypy when participants were assessed using a structured interview for severity of symptoms, as opposed to the psychometric assessment schizotypal personality dimensions. When the group were divided into high and low SPQ-B scorers, no differences were found between the groups for accuracy, motor or commission errors. However, there was a difference found for reaction times, with high SPQ-B scorers responding more quickly on the CPT compared to the low SPQ-B scorers.

3.7.4 Individual differences, cannabis use and cognitive performance

Individual differences in personality were explored between the cannabis users and non-cannabis users for those traits commonly found to be elevated in those diagnosed with schizophrenia (namely schizotypy, paranoia etc.). Cannabis users scored significantly higher on all of the trait measures of schizotypal personality (from the SPQ-B), paranoia (part A and B of the GPTS), ambivalence (SAS) and impulsivity (first and second order factors of the BIS). Findings were less clear for emotions as assessed by the TMMS subscale for clarity. These data are therefore broadly in line with previous research that has demonstrated that cannabis users experience more psychotic-like traits (Arsenault *et al*, 2004; Henquet *et al*, 2003; van Os *et al*, 2002; Stefanis *et al*, 2004; Skosnik *et al*, 2008; Dumas *et al*, 2004 Kuepper *et al*, 2011) and higher and impulsivity (Schmid *et al*, 2004; Barkus *et al*, 2008).

Clarity of thoughts on the TMMS was negatively correlated with duration of cannabis use, and the first order factor of the BIS was positively correlated with joints per week (JPW). Thus longer use of cannabis was linked to more emotional confusion and increased use of cannabis was linked to multiple aspects of impulsivity (across the spectrum of motor, attention, cognitive complexity, perseverance, and cognitive instability impulsivity constructs). In addition, there were also weaker trends (less than 0.05 but greater than 0.01) linking level of cannabis use (JPW) to riskier decisions on the IGT and increased impulsive traits. Thus heavy use of cannabis was more closely linked with greater deficits in decision making, as well as being associated with higher impulsivity.

3.7.5 Regression analysis

Regression analyses were performed to assess the relative contribution of possible predictor variables (e.g. cannabis use and SPQ-B, paranoia measures - social reference part A and ideas of persecution part B; ambivalence, clarity of thoughts and impulsiveness) on cognitive performance. Some of the predictor variables did predict cognitive performance. The IGT in the cannabis group alone was linked to the amount of cannabis used per week. Thus risky decision making seems to be affected by heavier use of cannabis. In the first regression analysis, anti-saccade task error was significantly predicted by SPQ total and the first and second order of the BIS and was the only significant regression model with adjusted R^2 at 28% shared variance. Thus it seems that psychotic personality traits and impulsivity were the best predictors of outcomes on the AST, as opposed to cannabis use. Therefore, it could be argued that schizotypal traits and impulsivity are seen as a deficiency of inhibitory control, as having higher levels of these traits was associated with poor performance on the AST.

3.7.6 General Limitations

There are several limitations to this study. There may be residual effects of cannabis may have impacted on the study, as neurobehavioural effects linked to heavy use of cannabis include deficits in memory and executive functioning (Bolla *et al*, 2002; Pope *et al*, 2006; Solowij *et al*, 2006). Most studies have an abstinence period of 12-72h and therefore it is difficult to elucidate these findings in terms of it being residuals or withdrawal effects. The cannabis users in this current study abstained for two days, and 20 out of 30 participants scored in the range for cannabis dependency, but interestingly dependency was not linked to any of cognitive outcomes. Participants in this current study were asked to abstain for two days (at least 48h), so any effects could be residual rather than acute effects of the drug. Residual means the lingering effect the drug could still have and produce two different mechanisms which are difficult to independently assess (Whitlow *et al*, 2004). For example, Pope *et al* (1995) argue that the residual effects may be due to the presence of the drug residue, either a dopamine agonist, or metabolites, which continue to have an intoxication effect after the peak acute effects of the drug has gone, for several hours (Grotenhermen, 2003) and up to 6 hours (e.g. Crean *et al*, 2012). Further to this, a residual effect can also indicate that even after complete elimination of the dopamine agonist there is still changes that can persist, which indicate that there may be neuroadaptions (e.g. persistent changes that

remain and are caused by the continued drug use). Or alternatively differences found could be due to withdrawal effects, such as irritability, negative affect, aggressiveness (Kouri *et al*, 1999). Withdrawal symptoms are seen to first appear after 24h upon abstinence of the cannabis (Budney *et al*, 2003). Further, the drugs testing kit used in this study were for screening purposes only and did not assess for drugs within the system. Given that cannabis has a half-life it can last up to one month in the body (Agurell *et al*, 1986). There was an imbalance of some demographic and personal health information in both groups, for example, there were a significantly greater number of males in the cannabis group. However the regression models were adjusted for sex and this factor did not predict performance on any of the cognitive assessments. The participants were selected using a range of recruitment methods, so as to avoid using a convenience sample (i.e. the student cohort only). A wider range of external participants were also recruited via social networking methods, newspapers, and general 'research participants wanted' posters were displayed on large supermarkets which attract a wide range of members from the general public. However, 50% of the sample was made up from the UEL student cohort which limits the generalizability of the findings. In this current study the cannabis users reported a greater degree of past and current polydrug use. Therefore, it is possible that other drug use or polydrug use *per se* may be a key reason for cognitive disruption, as opposed to cannabis use alone (e.g. Croft *et al*, 2001).

3.7.7 Final summary

Overall, this chapter has provided experimental evidence to suggest that cannabis users made riskier decisions on the gambling task when compared to a group of non-cannabis users. An increase in joints per week was associated with performance on the task, in that those who smoked more joints also took more risks overall. Both of these findings fit in with the main theme in that the cannabis use and heavier use of the drug show disrupted cognition akin to those with schizophrenia and high schizotypy. Cannabis users make riskier decisions which may be the result of responding less to punishments (e.g. losses) on the Iowa Gambling Task, as has been previously shown in brain scans of cannabis users when completing this task (e.g. Bolla *et al*, 2002) combined with the pharmacological effects cannabis has on the brain associated with fear in the amygdala (e.g. Phan *et al*, 2008).

There was a lack of findings for the anti-saccade task and CPT between the cannabis users and non-cannabis users. As previously discussed, conflicting CPT outcomes in clinical and non-clinical samples may be due to the number of variations of the task used. However, faster reaction times on the CPT were linked to negative symptomology as assessed using the SPQ-B and it may be that outcomes are more pronounced in people with greater negative symptoms, as these are more closely linked to the genetic component of schizophrenia (e.g. Bassett *et al*, 1993). Also, those with more negative symptoms are harder to treat with anti-psychotic medications so it may be that CPT performance deficits are more closely associated with those in the chronic stages of schizophrenia; rather than the milder changes, predicted in this work, that may be associated with regular cannabis use in non-pathologised individuals.

The AST has been less widely assessed in cannabis users and it seems that cannabis users display more psychotic-like traits, particularly those who use cannabis more heavily. The anti-saccade task performance was also moderately accounted for by personality factors which could be argued to constitute a profile resembling schizophrenic-like symptoms mainly in the cannabis using group. The data could be taken to suggest that these traits resemble a liability for schizophrenia, a finding backed up by previous research (e.g. Ettinger *et al*, 2000; Larrison *et al*, 2002).

Taken together, these data do somewhat suggest that the cannabis users in this sample present with a personality and behavioural profile similar to people diagnosed with schizophrenia. These data, along with that from Chapter 2, will be further explored in Chapter 4 to look at cannabis use, candidate genes for schizophrenia, psychotic-like personality traits and cognitive performance.

Chapter 4: Exploration of schizophrenia-linked candidate gene markers in the cannabis and non-cannabis using study cohorts and possible links to cognitive and trait data

Chapters 2 and 3 have summarised the findings from a range of cognitive assessments and personality measures administered to regular cannabis users and non-user controls. The core rationale of this work is that cannabis use, which appears to contribute to schizophrenia in a minority of users, may produce trends towards schizophrenia-like traits in a much larger number of regular users; and that this may then be observable in schizophrenia-sensitive assessments. In the first study, for example, LI was abolished in cannabis users, paralleling the effects seen in schizophrenics and first degree relatives. The second study revealed impairments in decision-making for IGT performance in cannabis users; and an increase in joints per week was associated with performance on the task, in that those who smoked more joints also took more risks overall. Faster reaction times on the CPT were linked to negative symptomology as assessed using the SPQ-B. The anti-saccade task performance was moderately accounted for by personality factors which could be argued to constitute a profile resembling schizophrenic-like symptoms mainly in the cannabis using group. Taken together, these data do somewhat suggest that the cannabis users in this sample present with a personality and behavioural profile similar to people diagnosed with schizophrenia.

An important consideration however is the role of genetic risk factors, linked to schizophrenia and psychosis, which may underlie a possible exacerbation of certain traits when the factor of cannabis use is added (see Chapter 1). This chapter therefore looks at the DNA data from participants (section 2.2.1 (primary analysis) and 3.51) and then explores possible interactions between a subset of these schizophrenia-linked markers and the data from the previous chapters (sections: 2.3.2; 2.3.3; 3.6).

4.1 Genetics of schizophrenia

Schizophrenia is associated with multiple genes, each suggested to have a relatively small risk effect (for a review see Saha *et al*, 2005; Plomin *et al*, 1994). Schizophrenia is likely to result from interactions between such risk genes (epistasis) as well as between genes and environmental factors (van Os *et al*, 2008). There is growing evidence that novel

chromosomal mutations may be involved in the aetiology of schizophrenia (Bassett *et al*, 2010). Multiple family studies have shown that rates of schizophrenia are higher in relatives of people diagnosed with schizophrenia, than in the general population (van Os *et al*, 2008). First degree relatives are at a 3-15% risk of developing schizophrenia and healthy controls are at 0.5-1% risk (Shih *et al*, 2004). The relative contribution of genetic and environmental factors has been investigated in twin and adoption studies and the concordance rate for schizophrenia is 45%-75% among monozygotic twin pairs but only 4%-15% among dizygotic pairs (Lictermann *et al*, 2000; Cannon *et al*, 1998; Sullivan *et al*, 2003). These studies show the impact of genes as well as non-genetic effects. A meta-analysis of 12 schizophrenia twin studies found that estimates of heritability in liability to schizophrenia to be 81% (e.g. Sullivan *et al*, 2003). Studies have shown that schizophrenia is 10 times higher among individuals who are adopted away from a parent diagnosed with schizophrenia, than those who are adopted away from an unaffected mother (Kety *et al*, 1994; Gottesman and Shields, 1982). One major study by Tiernari *et al* (2004) followed children up from birth longitudinally to 21 years to assess for rearing patterns and risk for developing schizophrenia spectrum disorder (SSD). They found that only those children already at risk for developing schizophrenia were affected by disordered rearing patterns, but the discordance rate for identical twins developing schizophrenia was less than 50% so this does not discount the effects of the environment. Most researchers now look at the interplay between genetics and environments, to further understand the risk of developing schizophrenia (Harrison and Weinberger, 2005). It would not be one single gene that accounts for schizophrenia; it is likely to be multiple genes that have an additive effect of acting in a combined fashion.

4.1.1 Molecular genetics

Molecular genetics started in the 1980's with the introduction of modern technologies such as Polymerase Chain Reaction (PCR) methods, which allowed researchers to amplify the regions of DNA of interest for further investigation (e.g. Strachan and Read, 1999). There are two main approaches for identify candidate genes: linkage analysis and association studies (e.g. Badner *et al*, 2002; Hamshere *et al*, 2004). Linkage studies are conducted on two or more affected individuals and are studies to assess for genomic regions which could indicate the transmission of the disease. Statistical analyses are performed to see is two segments of DNA are transmitted to the offspring higher than what would be expected by

chance. Researchers scan the genome for several markers for possible disease genes and this approach has been successful for some disorders such as neurofibromatosis and hereditary non-polyposis colon cancer (e.g. Saha *et al* 2005; Frogatt *et al*, 1995). However, some linkage analysis can be limited as it can only identify a region on the genome, which may contain a large number of genes, so it does not link the disease with a specific gene(s). Early linkage studies of schizophrenia had a few genomal regions that reached statistical significance but failed to be positively replicated (e.g. Jurewicz *et al*, 2001). Thus there are issues with lack of statistical power, small effects of individual genes and the use of a large number of markers in each of the studies (e.g. Alaerts *et al*, 2009). There has been over 20 genome-wide linkage studies of schizophrenia and several have shown the same linkage in two or more samples. For example Badner *et al* (2002); Lewis *et al*, (2003); Ng *et al*, (2009) conducted meta-analyses of schizophrenia linkage and identified chromosomal regions, 1q, 2q, 3p, 4q, 6p, 8p, 11q, 13q, 14p, 20q, and 22q, but 8p was confirmed by all three meta-analyses. Thus, the risk for schizophrenia might be explained by multiple genetic markers, but Haraldsson *et al* (2011) argue that this does not rule out the existence of single marker which confers the highest risk. Association studies compare the frequency of alleles in previously identified candidate genes and then compare these to an unrelated sample of affect individuals with a healthy control sample from the same population. An allele is one part of a pair of genetic markers that are positioned on a specific part of the chromosome. In contrast to linkage studies, association studies can identify specific genes, which came about due to the technological advancement of DNA methods, such as PCR. However, the lack of knowledge of how certain genes functions, lack of replication and how certain genes are involved in the aetiology of schizophrenia from linkage studies proved to be limiting for association studies. One key problem was false positive findings due to a large number of markers being analysed which resulted in multiple testing (Haraldsson *et al*, 2011).

Researchers can now identify candidate genes within the linkage region and apply linkage disequilibrium (LD), which assesses for the non-random association of alleles at two or more loci or on the same or different chromosomes (Saha *et al* 2005; Frogatt *et al*, 1995). LD allows for the testing of specific variant within the gene such as single nucleotide polymorphisms (SNPs). Each individual has many SNPs but each SNP occurs in about 1% of the population and the tiny changes in the DNA sequence may reflect a liability for the development of schizophrenia, where there is the transformation of one DNA molecule to

another (e.g. a C (cytosine) to a T (thymine) and these are linked across generations. Those variants within alleles that are said to be in LD do not travel randomly but are transferred together across generations. Therefore when researchers find a disease variant more frequently in patients compared to healthy controls, then this finding is not caused by multiple testing, and can constitute as a disease marker. Several sets of these SNPs are referred to as a haplotype and analysis of a haplotype is useful in detecting true effects from a SNP from it LD with another SNP. Haplotypes are elaborated below under the discussion of each candidate gene for schizophrenia, but in short the statistical power of haplotype analysis is increased as fewer SNPs are needed to identify genetic traits involving multiple SNPs by detecting a few SNPs from a haplotype. Further advancements in the 21st century has allowed research to conduct genetic wide association studies on thousands of participants, where researchers use microarrays or chips to rapidly scan 300,00-1,000,000 SNPs to find markers associated with that disease; analyses can be done to control for multiple testing (e.g. Harrison and Owen, 2003; Haraldsson *et al*, 2011).

4.1.2 Genetics of sub clinical schizophrenia

Family (e.g. Pogue-Geile, 2003; Kendler *et al*, 1985), twin and adoption studies (e.g. Torgersen *et al*, 2000) agree that schizotypal personality traits are genetically continuous with schizophrenia, in that domains of personality pathology aggregate in relatives of people diagnosed with schizophrenia. As with family studies there are issues of resolving the relative contribution of genetics from the environmental influences in risk for schizophrenia. Since 2002, positional cloning methods have been successful in identifying candidate gene for schizophrenia which has been replicated in many studies (e.g. Fanous *et al*, 2007; Lien *et al*, 2009; Picchioni *et al*, 2010). A meta-analysis by Sullivan *et al* (2003) suggests that the presence of allelic or locus heterogeneity in schizophrenia may compromise the statistical power to detect, identify or replicate candidate genes for schizophrenia. Fanous *et al* (1997) suggest that studying subclinical traits (i.e. schizotypy) in the unaffected relatives of people diagnosed with schizophrenia should help to increase the statistical power and resolve some inconsistencies. Fanous and colleagues were the first researchers to do this and performance a genome scan correlation, which used genetic linkage data to test for a genetic correlation between a disease and an aetiological relevant trait, in unaffected relative of PDS in the Irish study of high density schizophrenia families (ISHDSF). Using 3 independent samples of 90

Irish Multiplex families the results were compared to those from a genome scan of narrowly defined schizophrenia in the same sample. The researchers observed a significant relationship between schizotypy scores for these two phenotypes genome wide and chromosome 5q, 6p, 6q, 8p, 9q and 10p detected links for schizophrenia but were not as powerful for schizotypy.

Several scans of the genomes have been conducted and produced significant scores for chromosomal locations, but the discovery of predisposition genes has remained elusive (e.g. Lewis *et al*, 2003). There are a number of issues which hinder genetic research. One would be the diagnostic and symptomatic complexity of schizophrenia, and a diagnosis of the disorder is made at a clinical level, rather than from a clear aetiological viewpoint. Further to this, schizophrenia is a common disorder which may represent a common genetic liability with high frequency in the general population. There is a general overlap of psychiatric disorders, so highlighting specific genes for schizophrenia is difficult due to the co-morbidity with other disorders. Lastly, the genetic and environmental variables may only provide a small effect, which act together in an additive fashion, and results in someone having vulnerability for developing schizophrenia (e.g. Gottesman and Erlerimeyer-Kimling, 2001). As a result of these issues the research into candidate genes for schizophrenia has not been ground-breaking, but progress has been made due to the invention of new efficient tools for analysing DNA (e.g. O'Donovan *et al*, 2003; Harrison and Weinberger, 2005).

A number of candidate genes have been implicated in the disorder which has been successfully replicated: DAOA or known as DAOA/G72, COMT, and neuregulin 1 (NRG1), (e.g. O'Donovan *et al*, 2003; Owen *et al*, 2004; Plomin *et al*, 2001). Research on these candidate genes, as well as the cannabinoid receptor gene 1 (CNR1) and the FAAH gene will be addressed below. Further to this, research presented here focuses on how these genes are linked to cognition disruption and subclinical personality traits in relation to schizophrenia. Lastly, a rationale is provided to account for why these are candidate genes are being explored in relation to cannabis use and risk for schizophrenia.

4.2 D-amino acid oxidase activator (DAOA)

D-amino acid oxidase activator (DAOA), located on chromosome region 13q33.2, codes for a mitochondrial protein which plays a role in cellular energy, cell death, and cell growth (Kvajo *et al*, 2008). Post-mortem studies of schizophrenic brains show an overexpression of the subtype DAOA/G72 (Korostishevsky *et al*, 2004). DAOA is understood to interact with the enzyme D-amino acid oxidase (DAO) which then activates DAO (Chumakov *et al*, 2002). DAO is important as it oxidises D-serine, which can powerfully activate the NMDA-type glutamate receptor (e.g. Harrison and Owen, 2003); a receptor highly implicated in the aetiology of schizophrenia as well as other psychiatric illnesses such as bipolar disorder (Chumakov *et al*, 2002; Jamra *et al*, 2006; Jansen *et al*, 2009; Ma *et al*, 2006). D-serine was found to be higher in serum of people diagnosed with schizophrenia (e.g. Hashimoto *et al*, 2003) and it was higher in the cerebral spine fluid (CSF) of drug naïve schizophrenic patients (e.g. Hashimoto *et al*, 2005) and therapeutic benefits have been reported in treatments with D-serine (e.g. Tuominen *et al*, 2006). Schizophrenia susceptibility has been mapped to chromosome 13q by many research teams using genetic wide studies (e.g. Blouin *et al*, 1998; Bruzstowicz *et al*, 1998; Shaw *et al*, 1998; Camp *et al*, 2001; Lin *et al*, 1997). Jamra *et al* (2006) argued that even though inconsistencies exist in data from genome-wide studies, the linkage data for chromosome 13 is the most convincing across the genome.

DAOA has been studied for interactions with other candidate genes for schizophrenia, for example, Nicodermus *et al* (2006) assessed for 6 SNPs in the DAOA gene and found some interactions with the G/G version of the COMT gene (see section 4.3). Variation in the DAOA gene has also been linked to differential treatment outcomes in relation to anti-psychotic medication (Pae *et al*, 2010). DAOA has also been linked to neurocognitive functioning in schizophrenic groups, particularly in tasks which place demands on the medial temporal lobe (MTL); an area thought to play a significant role in the aetiology of schizophrenia (Harrison, 2004; Heckers, 2001). Evidence exists for DAOA playing a modulatory role on brain activity in the MTL mainly for hippocampal and parahippocampal function. For example, Goldberg *et al* (2006) investigated the relationship between cognitive performance and variants of the DAOA gene and found that variant M24 T/T was associated with impairment in 1-back task performance (which assesses working memory), less activation in the hippocampal and parahippocampal regions and weaker BOLD activity

during the working memory task. Hall *et al*. (2008) demonstrated similar effects in participants at high risk for schizophrenia, with weaker scores on the Hayling Sentence completion task, differential activation in the left hippocampus and parahippocampus and in the PFC area in relation to increased task difficulty, in the T/T genotypes. Therefore this research additionally provides some support that variation of the DAOA gene impacts on PFC functioning, an area that is vitally important for cognitive dysfunction in schizophrenia. Effectively the role of DAOA gene variants has also been explored in animal research, and the evidence of the distribution of DAOA, the effects of variants on behaviour, knock-out manipulations and pharmacological challenge all provide some support for the role of DAOA with regards to schizophrenia (see review Drews *et al*, 2012).

4.3 Catechol-*O*-methyl transferase (COMT)

The Catechol-*O*-methyl transferase (COMT) gene is located on chromosome 22q11.2 and is a major catalysing enzyme for dopamine (DA) and noradrenalin (NA). A single nucleotide substitution (G→A) at 472 bp of exon 4 produces the Val/Met variant also referred to as SNP rs4680 Val158Met, which causes a functional difference in the breakdown of DA and NA, as the homozygotic Valine allele (i.e. Val/Val) has a three-to-four fold higher activity than the homozygotic Methionine allele (i.e. Met/Met); the heterozygotes (i.e. Val/Met) have an intermediate level of activity (Lotta *et al*, 1995; Lachman *et al*, 1996). The COMT has links to the disorder of velocardiofacial syndrome (VCFS; Thomas and Graham, 1997; Bassett and Chow, 1998) and people with this disorder have significantly higher rates of schizophrenia (10-31%) than the general population (1%) (Shprintzen *et al*, 1992; Murphy *et al*, 1999; Murphy and Owen, 2001). Around 1-3Mb of DNA deletion including the COMT gene in this chromosomal region is linked to the clinical features of VCFS (e.g. Murphy *et al*, 1997).

The COMT gene has been widely reported to be implicated in the aetiology of schizophrenia, but its role remains controversial and results are ambiguous (see Hosak *et al*, 2007 for a full review). SNP rs4680 G/A also known as the Val/Met variant has been widely researched with positive (e.g. Egan *et al*, 2001; Li *et al*, 1997; Kunugi *et al*, 1997a; 1997b) and negative findings (e.g. Chen *et al*, 1999; Daniels *et al*, 1996; Fan *et al*, 2005) reported in relation to risk for developing schizophrenia. Other studies link those carrying the A/A or Met/M*et*

*al*lele with schizophrenia (e.g Ohmori *et al*, 1998) and increasingly more links have been made with the G/G or Val/Val allele and schizophrenia (e.g. Wanodi *et al*, 2003). Handoko *et al* (2005) reported a strong association with COMT haplotype rs737865/rs4680/rs165599 (G/G/G) and schizophrenia. COMT plays a significant role in the metabolism of dopamine with the A/A allele associated with low activity of dopamine metabolism; and vice versa for the G/G alleles (Bilder *et al*, 2002). The A/A SNP genotypes has been linked to better cognitive performance (Gallinats *et al*, 2003; Bilder *et al*, 2002; Bruder *et al*, 2005) and G/G carriers were linked to worse cognitive performance (Goldberg *et al*, 2003; Bruder *et al*, 2005). Those with A/A SNP genotypes have been linked to superior cognitive performance (Sheldrick *et al*, 2008). Those homogenous for the G allele were linked to higher schizotypy scores and G/A carriers appear to show a weak association with disorganised thinking for schizotypy in males but not females (Avramoupoulos *et al*, 2002). However, other researchers (e.g. Strous *et al*, 2006) have found no differences between rs4680 genotypes for clinical symptomology. There has been a significant association found between rs4680 G/A carriers and higher scores on blunted affect in schizotypy (Wang *et al*, 2010). Carriers of the G/G genotype had higher schizotypy state psychopathology scores and higher negative and disorganised thinking scores (Smyrnis *et al*, 2007). Whereas Sheldrick *et al* (2008) reported that A/A carriers reported experiencing higher SPQ scores for disorganised thinking.

The COMT SNP rs4680 has been widely studied in relation to cannabis use. Caspi *et al*'s (2005) longitudinal reearch was one of the first studies to report that males (who used cannabis before the age of 15) with the G allele had higher risk for psychotic symptoms. Whereas Zammit *et al* (2011) failed to replicate these findings. Henquet *et al* (2006) found a 3-way association between THC administered to people diagnosed with schizophrenia, carriers of the G/G allele and higher rates of psychotic symptoms. Animal studies support the links between cannabis and the COMT gene, as COMT knock-out mice demonstrate sensitivity to chronic THC exposure on behaviours related to psychosis and memory (O'Tuathaigh *et al*, 2010). Pelayo-Teran *et al* (2010) reported that age of first use of cannabis was later in carriers of the A/A genotype. Van Wickel *et al* (2008) reported that a haplotype (rs46333; rs4680) of the COMT gene and earlier onset of cannabis (less than 16 years) was associated with greater scores on the BPRS. Costas *et al* (2011) reported that rs4680 G/A doubled the probability of lifetime cannabis use compared to G/G carriers.

In sum rs4680 has been extensively studied, and despite some inconsistencies in the data is widely considered to be a possible risk marker for schizophrenia; and will be hypothesised as such in this current study. In addition to this, SNP rs165599 and SNP rs737865 will be added as together with rs4680 these three SNPs have been consistently reported as a risk COMT haplotype, with C-G-G positively associated with schizophrenia (De Rosse *et al*, 2006; Schifman *et al*, 2002; Handoko *et al*, 2004). In addition, the T-A-A haplotype has also been linked to schizophrenia and the T-G-G has been characterised as a protective haplotype (Kotrotsou *et al*, 2012).

4.4 Neuregulin1 (NRG1)

The NRG1 gene is located at chromosome 8p 13 and it plays an important function in many organs including the heart, breast and nervous system. In the nervous system the NRG1 is involved in many important functions such as neuronal specification, synapse formation, myelination, regulation of NMDA, GABA and nicotinic receptors to name a few. NRG1 expression has been found in the nervous system in the prefrontal cortex, hippocampus, cerebellum, and substantia nigra, in animals (Kerber *et al*, 2003) and humans (Harrison and Law, 2006). NRG1 plays a central role in cognition (Harrison and Law, 2006) and it has been widely linked to genetic susceptibility to schizophrenia (Steffansson *et al*, 2003; Zhao *et al*, 2004; Zou *et al*, 2005). Steffansson and colleagues found that SNP rs221553 (C allele) was over-represented in people diagnosed with schizophrenia and a 5 marker haplotype (known widely as the Icelandic haplotype - including SNPs rs221553, rs241930, rs243177 and microsatellites 433E1006) doubled the risk for schizophrenia. These findings have been replicated with positive (Williams *et al*, 2003; Zhau *et al*, 2004; Bakker *et al*, 2004) and negative findings (Kampman *et al*, 2004; Duan *et al*, 2005; Thistleton *et al*, 2004). Whereas, Georgieva *et al* (2008) reported that it was the T allele that was over-transmitted in people diagnosed with schizophrenia. One meta-analysis of 13 population studies confirmed the association between the NRG1 gene and schizophrenia with positive findings for rs241930, rs23177, rs221132 and rs221553, and two microsatellites 478B14-848 and 420 M9-1395 (Li *et al*, 2006). The most robust SNP to be consistently associated with schizophrenia was rs221553 and all of the published research up to 2006 was included in a meta-analysis but this SNP was not significant; and the Icelandic haplotype was the only marker to reach

significance levels. Few brain scan studies have been conducted in relation to SNP rs221533 to explore its links with schizophrenia. For example, Winterer *et al* (2008) found that white matter was reduced in medial frontal cortex for those carrying the risk 'C' allele. Further, Suarez-Pinilla *et al.* (2015) in a 3-year follow-up study of people in their first episode psychosis and healthy controls found that the risk C allele was significantly associated with increased lateral ventricle volume across time. After the follow-up period it was found that the C allele carriers had significantly less white matter compared with participants who were homozygous for the T allele.

In animal research, NRG1 knockout gene mice display more attentional problems (Rimmer *et al*, 2005) and social behaviour problems but their memory was intact (O'Tuathaigh *et al*, 2007). Interestingly, these mice had less cognitive impairment when exposed to THC, but had higher levels of anxiety (Karl *et al*, 2010) and male rats with this manipulation were more sensitive to the psychotropic effects of THC (Long *et al*, 2010). Since SNP rs221533 has been widely studied and has produced the most consistent findings, it was included as a risk marker in this current study.

4.5 Cannabinoid receptor gene and the Fatty Amide Hydrolase (CNR1 and FAAH)

The endogenous cannabinoid system and its links to schizophrenia were outlined in Chapter 1. The cannabinoid receptor (CNR1) and the fatty acid amide hydrolase (FAAH) genes are located on chromosomes 6 and 1 in the 6q15 and 1p33, respectively (Mastuda *et al*, 1990); this region is considered to be a susceptibility locus for risk of schizophrenia (Cao *et al*, 1997). The CNR1 and FAAH genes have been implicated in the risk for schizophrenia, with the CNR1 gene receiving more support (Dawson *et al*, 1995) compared to the FAAH gene (e.g. Martinez-Gras *et al*, 2007; Costas *et al*, 2012). One SNP in particular on the CNR1 gene (the rs1049353 G/G genotype) has been associated with schizophrenia (e.g. Leroy *et al*, 2001), although some researchers have not replicated this finding (Ujike *et al*, 2002). An AAT triplet repeat on the CNR1 gene has also been associated with schizophrenia (Zhang *et al*, 2004), though again this is not an unequivocal finding (Tsai *et al*, 2000; 2001). There have been differences found in sensitivity to THC and P300 responses with this AAT triplet under the THC condition (Stadelmann *et al*, 2011).

The FAAH gene is strongly linked to cannabis dependence (Sipe *et al*, 2002; Morita *et al*, 2005). In SNP rs324420, the A/A variant has been more commonly found in those diagnosed with schizophrenia with co-morbid substance dependence. Schact *et al* (2009) reported that A/A or C/A genotypes were linked to having more cannabis withdrawal and Sipe *et al* (2002) reported that individuals with the A/A genotype reported more dependence on cannabis. Cannabis users with the A allele also displayed more negative psychotic like symptoms (as assessed with the disorganised thinking measure of the SPQ (Arias *et al*, 2010). MRI scan data in people with the A allele showed lower threat related activity in the amygdala and higher reactivity to reward, and this cohort reported lower anxiety and impulsivity scores; whereas the C allele was associated with higher anxiety and higher threat related brain activity (Hariri *et al*, 2009). Given that SNP rs1049353 on the CNR1 gene and SNP rs324420 on the FAAH gene has been widely studied, both SNPs were included as risk markers in this current study.

4.6. Summary and Rationale

Overall, this is a relatively new area examining schizophrenia-linked genotypes in relation to performance on neuropsychological/cognitive tests which are sensitive to schizophrenia. Other genes have been implicated in the aetiology of schizophrenia (e.g. DISC1 (Disrupted in Schizophrenia), and DTNBP1 (Dysbindin; Stefansson, 2008,2009; Harrison and Owen 2003; O'Donovan *et al* 2003, 2008; Owen *et al* 2004), but the DAOA, COMT and NRG1 markers were chosen as these genes have been most widely researched with relatively more consistent findings in relation to schizophrenia. Whilst it is acknowledged that this work is speculative, the following predictions were made with regards to each marker:

DAOA

The T/A genotype for SNP rs142129 has been linked with schizophrenia and the T/T genotype within the literature which is linked to poorer cognitive performance, particularly in those diagnosed with schizophrenia (e.g. Goldberg *et al*, 2006).

- Given the consistent findings with SNP M24 rs1421292, this will be put forward as a risk marker in this current study.

COMT

There has been conflicting findings for the COMT gene in relation to SNPrs4680 (G/G, G/A and A/A) genotypes and schizophrenia. However, most of the current research converges to report that the G/G allele is the strongest predictor of being associated with schizophrenia.

- Therefore, it is likely that those carrying the G/G allele will be more like to have higher SPQ scores.
- Having the A/A allele has been linked with increased cognitive performance and the G/G allele has been linked to worse cognitive performance, so it is predicted that the A/A carriers will have better cognitive performance on each of the cognitive tests.
- Further to this, it is predicted that those people who use cannabis with the risk G allele will be more likely to have higher subclinical schizophrenia symptoms, as well as higher cognitive disruption.
- A secondary analysis will explore variation in the at-risk COMT haplotype. This at-risk haplotype has not been investigated in cannabis users, but it is likely that cannabis users with this at-risk haplotype will highlight subtle differences in performance in cognitive functioning and individual differences in psychotic-like trait compared to non-cannabis users.

NRG1

- For the NRG1 gene, given the consistent findings with SNPrs221533, it is likely that those carrying the C allele will have more schizophrenia-like traits and behaviours. This combined with cannabis use may be predictive with the highest trait scores and performance patterns more closely aligned to schizophrenic type behaviour in the tasks.

CNR1 and FAAH

These two genes are less researched in schizophrenia, but given the nature of this study looking at the possible links between the cannabinoid system and schizophrenia, these markers may prove highly pertinent.

- For CNR1, it is likely that those individuals with the rs1049353 G/G genotype will show responding biases in the direction of schizophrenic traits and behaviours, which may be even more likely, or increased, in the presence of concurrent cannabis use.

- The FAAH SNP rs324420 A/A genotype may be linked to higher psychotic-like traits and cognitive dysfunction, most notably when combined with cannabis use.

Combined effects

- It is predicted that those with a profile of multiple risk alleles from the candidate schizophrenia genes (i.e. the presence of two or more from the DAOA, COMT, CNR1, FAAH and, NRG1 markers) may show greater biases on cognitive tests and schizophrenia-like personality symptoms, in the direction of schizophrenia-like deficits and differences.

- In line with the general hypothesis that cannabis use and gene markers serve as distinct and possible component causes of schizophrenia, it is predicted that cannabis users with multiple risk alleles will show the strongest bias towards a schizophrenia like behavioural profile in the measures employed.

4.7 Method

4.7.1 Participants

The samples from 50 cannabis users and 50 non-cannabis users were made up of a combination of the 40 participants from Study 1 and 60 participants from Study 2. In total, there were 67 females and 33 males, with an age range from 18-47 (mean of 27; SD 7.7). The participants were from a variety of different ethnicities and nationalities.

4.7.2 Materials

Please refer to Chapter 2 (section 2.2.2) and Chapter 3 (section 3.5.2) for information on ethics documentation and drugs screening. The SPQ-B, latent Inhibition task is outlined in section 2.5.2. The Iowa Gambling, Continuous Performance and Anti-Saccade tasks are outlined in section 3.5.2.

4.7.3 Genotyping

DNA was collected using buccal swabs (*Copan Diagnostics)* to extract some cheek cells, by rubbing the swab vigorously on the inside cheek at least 6-10 times. Intensive and extensive preparatory work and validation testing was completed from 2009-2012 (see appendix xiv). Initially the samples were found using the Primer Blast website: http://www.ncbi.nlm.nih.gov/tools/primer-blast/ Each primer was 20bp in length, and one forward and one reverse primer for each SNP was ordered from and synthesised by MWG-Biotech, UK. Table A (in Appendix xiv) highlights 20 SNPs that were used in the initial investigation and these were then used for PCR primer testing. Testing was conducted on three SNPs with different amounts of DNA, primer, magnesium chloride, PCR master mix and water to achieve the optimal outcomes. The washed and prepared PCR products for each of the SNPs were confirmed that DNA is present in each of the samples from electrophoresis (via the gel capture by appearance of the florescent rings); the product was stored in the 96 well plates, at -20 degrees. The SNP extension primer was carried out using the Beckman Coulter Primer Extension Kit for the Genome Lab ™ and AB gene ready mix. The SNP primers were created using the Primer 3 blast function and successful primers should be in the range of 60 degrees – 75 degrees and a Poly (T) tail was added to the 5' (please refer to in

Appendix xiv Table C3, for a list of SNP primers and Table D4 for the primers with their Poly (T) tails. The SNP analysis was run according the Beckmann and Coulter manual for the CEO 8000 software. 10 separate multiplexing analyses were conducted and some of these worked and others did not (see Figure D4 and E5, in Appendix xiv). The in-house method used resulted in relatively weak and inconsistent genotyping despite repeated attempts and alterations in method:

1. changes in DNA concentration during each of the PCR runs
2. changes in concentrations of the SNP PCR primers
3. rewashing the DNA for more purified DNA concentrations

All of the intensive lab work was conducted over a period of 36 months (at a minimum of 10 hours of lab work per week). At this point, to facilitate the process and spend no further time on this aspect of the work, samples were sent to K-biosciences (LGC Genomics, Hertfordshire). Therefore all of the PCR samples (n=100) were washed using the Qiagen kit and then run through the PCR, using the same protocol as Table B2 (in Appendix xiv), but for only 7 out of the 20 SNPs were selected to be sent externally to K-Biosciences (see table F6, in Appendix xiv). The first batch of analysis (which included the SNP primer PCR products) had a poor success rate at K-Biosciences (under 60%). Therefore a secondary batch was sent which including all of the original DNA samples and were cleaned to increase the % of purified DNA. All DNA samples (n=100) were eluted in 10mM Tris buffer and re-sent to K-Biosciences for a final attempt to genotype the DNA without any PCR or SNP primers, and it was much more successful with a hit rate of (89%-99%). The SNP markers were genotyped using the KASP™ method, a competitive allele-specific polymerase chain reaction incorporating a fluorescent resonance energy transfer quencher cassette, for further details see: http://www.kbioscience.co.uk/reagents/KASP.html

4.8 Results

4.8.1 Data screening

Data were screened and cleaned in the second and third chapters, but owing to the small number of participants outliers were not removed in the combined psychological measures and DNA data analyses. However, a check for the standard deviations (SDs) was run to make sure that outliers did not affect the results. Due to the high SDs one participant (from the non-cannabis group) was removed from the CPT motor error data. Additionally, two participants were removed from the final analysis assessing cannabis use variables as co-variates, as both reported excessive amounts of cannabis per week (e.g. 120 joints per week). There were missing genetic data for some participants for the following SNPs: SNP rs142129 (n=2); SNP rs737865 (n=3); SNP rs4680 (n=10); rs165599 (n =11); rs1049353 (n =1); rs324420 (n =5); SNPrs221533 (n=3). For the COMT haplotype those with missing data in any of the three SNPs were excluded, so there were 18 missing data for the COMT haplotype. The findings are reported as either significant (p<0.05) or leaning towards significance (p≤ 0.15) are discussed below.

4.8.2 Hardy Weinberger Equilibrium (HWE)

The Hardy Weinberger Equilibrium (HWE) theory states that the percentage of each genotype should remain constant in a population (Nature, 2013). To test that HWE was met the HWE Calculator was used from Court (2005-2008). This calculation is based on a similar principle to Chi ^{2} analyses, and were run for the entire group to look for differences (in observed and expected frequencies) in genotype for each SNP (see Table 28). In addition, Chi^2 analyses were run between groups (e.g. cannabis user and non-cannabis user) for frequencies of genotype and alleles (see Table 29). If the p-value was < 0.05 then the result is not consistent with the HWE. Also for those data with cells < 5 then an F statistic was run using SPSS version 18.

4.8.3 HWE Result of Genotyping data

Table 28 below summarises, for each gene SNP, the frequency distribution of corresponding genotypes across all participants (cannabis and non-cannabis users, from studies 1 and 2). Chi² analyses were run to look at differences and it was found that there were significant differences for the frequency of occurring alleles under each genotype, with the homogenous recessive allele (referred to a q in Table 28) occurring less than the homogenous dominant alleles (referred to as p in table 28). SNP 9 p (or allele T) occurs more frequently in the sample at 57%, as opposed to q (or the A allele) at 43%. Two SNPs are not consistent with the HWE due to $p<0.05$ for COMT rs737865 and CNR1 SNP rs1049353 having zero participants carrying the A/A allele. Having larger sample sizes would be ideal for making the data becoming more consistent for frequency of alleles.

Table 28: Distribution of SNP dominant and recessive genotypes in the whole group and chi-square analyses.

Gene	SNP	Genotype frequencies			X^2	*p*	[1]Allele	
DAOA	rs142129	T/T	T/A	A/A			p	q
		30	51	17	0.35	0.56	0.57	0.43
COMT	[2]rs737865	T/T	T/C	C/C				
		57	27	13	8.68	**0.003**	0.73	0.27
COMT	rs4680	G/G	G/A	A/A				
		39	36	15	1.73	0.188	0.63	0.37
COMT	rs165599	G/G	G/A	A/A				
		15	43	31	0.001	0.99	0.46	0.54
CNRI	rs1049353	G/G	G/A	A/A				
		93	6	0	0.10	0.75	0.97	0.03
FAAH	rs324420	C/C	C/A	A/A				
		57	29	9	3.08	0.08	0.75	0.25
NRG1	rs221533	T/T	T/C	C/C				
		75	19	3	1.58	0.21	0.87	0.13

[1] In each table the allele is listed a 'p or q'; a standard procedure in the genetics literature to denote the dominant and recessive alleles.

[2] In the literature the rs737865 is also referred to as G/G, G/A or A/A genotypes. The C allele of the rs737865 corresponds to the G allele in the literature.

Table 29 below highlights the occurrence of each SNP and their corresponding genotype in the cannabis users and non users. Distributions of the CNR1 SNP rs1049353 A/A, and NRG1 SNP rs221533 (C/C) were not consistent with the HWE; each having less than five in one cell for both cannabis and non-cannabis users. The FAAH gene is not consistent with the HWE in the cannabis users as there were less than five participants having the A/A genotype. The SNPs that remained consistent with the HWE in both groups was COMT rs4680 and rs165599 and COMT rs737865 for the non-cannabis users; the FAAH gene for the non-cannabis users all *p*-values are greater than 0.05. When the cannabis group was compared to the non-cannabis group for frequency of alleles there was a trend for significance for variation in the DAOA gene X^2 (2) = 7.231, $p = 0.055$. The T/A genotype occurred much more frequently in the cannabis users.

Table 29: Distribution of SNP dominant and recessive genotypes in cannabis and non-cannabis users, and chi square analyses.

SNP		**Genotype**			**X^2 (HWE) within each group**	***P***	**Allele** **p**	**q**	**X^2 - between the cannabis users and non users**	***P (2-tailed)***
		T/T	T/A	A/A						
DAOA rs142129	Cannabis user	9	31	9	3.44	0.06	0.86	0.14	7.231	0.055
	Non-cannabis user	21	20	8	0.727	0.39	0.89	0.11		
		T/T	T/C	C/C						
COMT rs737865	Cannabis user	29	12	7	6.46	**0.01**	0.73	0.17	0.418	0.949
	Non-cannabis user	28	15	6	2.66	0.10	0.73	0.17		
		G/G	G/A	A/A						
COMT rs4680	Cannabis user	19	14	9	3.61	0.06	0.60	0.40	2.012	0.729
	Non-cannabis user	20	22	6	0.00	0.989	0.65	0.35		
		G/G	G/A	A/A						
COMT rs165599	Cannabis user	7	20	15	0.006	0.94	0.40	0.60	0.016	0.910
	Non-cannabis user	8	23	17	0.002	0.96	0.40	0.60		
		G/G	G/A	A/A						
CNR1 rs1049353	Cannabis user	48	2	0	0.02	0.88	0.98	0.02	0.753	0.329
	Non-cannabis user	45	4	0	0.089	0.76	0.95	0.05		
		C/C	C/A	A/A						
FAAH rs324420	Cannabis user	27	15	4	0.78	0.37	0.75	0.25	0.209	0.941
	Non-cannabis user	30	14	5	2.53	0.11	0.75	0.25		
		T/T	T/C	C/C						
NRG1 rs221533	Cannabis user	37	11	1	0.03	0.86	0.87	0.13	0.810	0.882
	Non-cannabis user	38	8	2	2.72	0.10	0.88	0.12		

4.9 Genotyping data by measure and groups

4.9.1 SPQ data: studies 1 and 2 (whole sample)

Table 30 presents the SPQ data for all participants from studies 1 and 2, broken down by SNP genotypes across the 5 key gene markers. An ANOVA revealed a significant difference between participants with variation in the NRG1 rs221533 on scores in the SPQ-CP subscale ($F(2, 94) = 3.183$, $p = 0.046$), with the post-hoc Tukey test revealing that this finding was attributable to the significance between the T/C and T/T genotypes ($p = 0.05$). There were also trends represented by low marginally non significant differences for the SPQ-DT subscale for the COMT rs165599 G/G genotype (F (2, 24) = 2.623, p = 0.078) in that these carriers reporting experiencing more disorganised thinking (e.g. problems with communication). Whereas, the opposite was found for the NRG1 rs2215533 T/T (F (2, 94) = 2.468, p = 0.09), in that they reported less disorganised traits relative to the T/C and C/C genotypes.

4.9.2 SPQ data: studies 1 and 2 (cannabis users and non-cannabis users)

Data was taken from studies one and two from the SPQ-B personality measure. Table 31 provides the mean scores along with the respective schizophrenia candidate genes in both the cannabis users and non-cannabis users for the SPQ-B total scores along with three of its subscales for disorganised thinking, interpersonal and cognitive perceptual. A one-way ANOVA revealed that there were no significant differences found between variation in each of the genes and their respective genotypes. A trend was found for variation in the NRG1 gene for SNPrs221533 for cognitive perceptual with the T/C genotype scoring higher on SPQCP than T/T and C/C (F (2, 91) = 2.43, p = 0.09). A trend found for variation in the COMT gene for SNP rs165599 and outcomes on the SPQDT (F (2, 83) = 2.509, p = 0.08).

Table 30: SPQ total, cognitive perceptual (CP), interpersonal (IP) and disorganised thinking (DT) scores explored across SNP genotypes in all participants.

			SPQ total			**SPQ CP**			**SPQIP**			**SPQDT**		
Gene	**SNP**		**Mean (SD)**	***F***	***p***	**Mean (SD)**	***F***	***p***	**Mean (SD)**	***F***	***p***	**Mean (SD)**	***F***	***p***
DAOA	rs142129	T/T (22)	5.53 (5.0)	1.05	0.355	2.18 (2.3)	0.405	0.668	2.22 (2.34)	1.99	0.141	1.64 (1.73)	0.368	0.693
		T/A (37)	6.92 (3.99)			2.4 (1.62)			3.00 (2.00)			1.57 (1.625)		
		A/A (14)	6.11 (3.37)			2.0 (2.0)			1.92 (1.59)			1.357 (1.736)		
COMT	rs737865	T/T (42)	6.23 (5.59)	0.84	0.436	2.59 (2.03)	0.520	0.596	2.33 (2.21)	1.913	0.153	1.66 (1.88)	1.283	0.282
		T/C (23)	6.0 (3.84)			1.896 (1.65)			2.739 (1.86)			1.08 (1.08)		
		C/C (8)	7.77 (3.37)			1.625 (1.59)			3.25 (1.83)			2.25 (1.58)		
COMT	rs4680	G/G (31)	6.64 (4.66)	1.66	0.196	2.45 (2.01)	0.631	0.534	2.806 (2.22)	1.643	0.199	1.677 (1.93)	0.984	0.378
		G/A (28)	5.14 (4.02)			2.035 (1.83)			1.96 (1.77)			1.250 (1.32)		
		A/A (14)	7.13 (5.74)			2.28 (1.85)			3.214 (2.08)			1.857 (1.61)		
COMT	rs165599	G/G (13)	8.06 (4.68)	1.45	0.241	2.384 (2.18)	1.995	0.142	2.84 (2.07)	0.655	0.522	2.23 (1.48)	2.623	0.078
		G/A (36)	6.09 (4.41)			2.53 (2.05)			2.50 (1.99)			1.22 (1.64)		
		A/A (24)	5.87 (4.02)			1.79 (1.44)			2.50 (2.24)			1.66 (1.71)		
CNR1	rs1049353	G/G (68)	6.4 (4.28)	0.76	0.385	2.26 (1.87)	0.188	0.666	2.58 (2.08)	0.02	0.889	1.602 (1.685)	2.137	0.147
		G/A (5)	4.8 (3.76)			2.20 (2.49)			2.2 (1.92)			0.80 (1.095)		
		A/A	-			-			-			-		
FAAH	rs324420	C/C (45)	6.47 (4.19)	0.09	0.918	2.33 (1.78)	1.101	0.337	2.48 (2.12)	0.421	0.658	1.511 (1.604)	0.194	0.824
		C/A (21)	6.55 (4.68)			2.33 (2.24)			2.57 (2.11)			1.76 (1.81)		
		A/A (7)	5.88 (3.48)			1.57 (1.61)			3.00 (1.82)			1.142 (1.67)		
NRG1	rs221533	T/T (56)	5.83 (4.31)	2.25	0.111	2.08 (1.95)	3.183	**0.046**	2.571 (2.147)	0.353	0.702	1.375 (1.59)	2.468	0.09
		T/C (15)	8.05 (2.91)			3.066 (1.53)			2.6(1.8)			2.133 (1.88)		
		C/C (2)	6.00 (4.26)			1.00 (1.41)			2.00 (2.82)			2.0 (1.41)		

Table 31: SPQ total, cognitive perceptual (CP), interpersonal (IP) and disorganised thinking (DT) scores explored across SNP genotypes in cannabis users and non-cannabis users

		(n =)	*Cannabis user*	*Non-cannabis user*	*F or*	*p*	*Cannabis user*	*Non-cannabis user*	*F or*	*p*	*Cannabis user*	*Non-cannabis user*	*F or*	*p*	*Cannabis user*	*Non-cannabis user*	*F or*	*p*
Gene	**SNP**	**genotype (cu: ncu**	**SPQ total Mean (SD)**	**SPQ total Mean (SD)**			**SPQ IP Mean (SD)**	**SPQ IP Mean (SD)**			**SPQ CP Mean (SD)**	**SPQ CP Mean (SD)**			**SPQ DT Mean (SD)**	**SPQ DT Mean (SD)**		
DAOA	rs142129	T/T (9; 21)	8 (4.21)	4.47 (5.0)	0.361	0.698	8.0 (2.0)	1.61 (2.18)	1.44	0.24	2.88 (2.36)	1.76 (1.94)	0.05	0.95	2.11 (1.45)	1.095 (1.60)	0.02	0.98
		T/A (31; 20)	6.9 (3.6)	6.95 (4.81)			2.61 (1.83)	3.20 (2.19)			2.64 (1.64)	2.25 (1.55)			1.83 (1.44)	1.50(1.79)		
		A/A (8; 8)	6.4 (3.7)	5.75 (3.0)			1.4 (1.50)	2.75 (1.28)			3.0 (2.29)	1.62 (2.06)			2.0 (1.66)	1.37 (1.99)		
COMT	rs737865	T/T (29; 28)	6.41 (4.07)	6.03 (5.15)	0.705	0.497	2.0 (1.85)	2.39 (2.26)	1.99	0.14	2.75 (2.01)	2.25 (1.99)	0.40	0.672	1.72 (1.6)	1.39 (1.93)	1.07	0.35
		T/C (12; 15)	6.91 (3.26)	5.27 (4.21)			3.08 (1.78)	2.40 (1.91)			2.75 (1.66)	1.53 (1.55)			1.41 (1.08)	1.33 (1.49)		
		C/C (7; 6)	8.71 (3.15)	3.87 (4.63)			3.00 (1.53)	3.66 (2.25)			2.57 (1.98)	1.83 (1.32)			3.14 (0.89)	1.16 (1.33)		
COMT	rs4680	G/G (19; 20)	6.21 (3.95)	7.05 (5.32)	1.203	0.305	2.16 (1.38)	3.05 (2.58)	1.37	0.26	2.47 (1.86)	2.55 (2.09)	0.42	0.614	1.74 (1.63)	1.45 (1.95)	0.79	0.456
		G/A (14; 22)	5.57 (8.13)	4.86 (4.54)			2.07 (1.9)	1.90 (1.97)			2.64 (1.94)	1.64 (1.62)			1.07 (0.92)	1.32 (1.67)		
		A/A (9; 6)	8.66 (3.94)	4.83 (1.94)			3.22 (2.68)	2.66 (1.21)			3.00 (1.94)	1.17 (0.75)			2.44 (1.51)	1.0 (1.2)		
COMT	rs165599	G/G (7; 8)	7.85 (3.71)	8.25 (5.65)	1.339	0.268	2.57 (1.62)	3.37 (2.32)	0.579	0.56	3.0 (2.3)	2.37 (2.13)	2.00	0.141	2.43 (1.40)	2.5 (1.77)	2.50	0.09
		G/A (20; 23)	6.95 (3.99)	5.34 (4.69)			2.40 (1.57)	2.22 (2.2)			3.0 (2.13)	2.13 (1.94)			1.75 (1.58)	1.0 (1.68)		
		A/A (14; 17)	6.35 (3.85)	5.47 (4.19)			2.29 (2.4)	2.59 (2.09)			2.0 (1.36)	1.53 (1.46)			2.07 (1.59)	1.35 (1.62)		
CNR1	rs1049353	G/G (48; 45)	6.91 (3.78)	5.84 (4.73)	0.356	0.552	2.39 (1.84)	2.51 (2.21)	0.037	0.85	2.7 (1.93)	1.95 (1.72)	0.06	0.804	1.92 (1.47)	1.38 (1.76)	1.59	0.211
		G/A (2; 4)	6.50 (4.94)	4.00 (3.5)			3.5 (2.12)	1.75 (1.5)			2.5 (0.70)	1.75 (2.87)			1.0 (1.41)	0.50 (1.0)		
		A/A (0; 0)	-	-			-	-			-	-			-	-		
FAAH	rs324420	C/C (27; 30)	6.37 (3.95)	6.56 (4.46)	0.019	0.981	2.11 (1.78)	2.76 (2.14)	0.569	0.57	2.7 (2.2)	2.16 (1.58)	0.98	0.378	1.63 (1.44)	1.63 (1.73)	0.062	0.940
		C/A (15; 14)	7.73 (3.55)	5.28 (5.49)			2.86 (1.73)	2.14 (2.44)			2.93 (1.53)	1.93 (2.4)			2.20 (1.52)	1.21 (1.80)		
		A/A (4; 5)	9.0 (2.16)	3.4 (1.81)			4.0 (1.41)	2.40 (1.52)			2.0 (2.0)	1.0 (0.70)			3.0 (1.41)	0		
NRG1	rs221533	T/T (37; 38)	6.18 (3.95)	5.47 (4.67)	1.899	0.156	2.24 (1.86)	2.47 (2.22)	0.329	0.72	2.33 (1.99)	1.89 (1.82)	2.43	0.09	1.67 (1.35)	1.11 (1.66)	2.319	0.104
		T/C (11; 8)	8.81 (2.35)	7.0 (3.42)			2.81 (1.78)	2.75 (1.91)			3.82 (1.17)	2.37 (1.40)			2.45 (1.80)	1.87 (1.64)		
		C/C (1; 2)	8.0 (-)	5.0 (5.65)			3.0 (-)	2.0 (2.83)			2.0 (-)	1.0 (1.41)			3.0 (-	2.00 (1.41)		

4.9.3 LI data: study one (whole sample)

ANOVAs were performed for the entire group to assess for possible differences between SNP types and cognition: with associative learning assessed by LI test (see Table 32). For the LI data, There was a non-significant difference between carriers of SNP rs737865 on the COMT gene and LI outcomes (F (1, 33) = 2.526, p = 0.09).

Table 32: [3]LI performance data explored across SNP genotypes in all participants

Gene	**SNP**	**genotype (n=)**	**Cognitive data mean (SD)**		
			LI Mean (SD)	***F***	***P***
DAOA	rs142129	T/T (11)	16.27 (7.68)	2.414	0.103
		T/A (22)	9.40 (8.67)		
		A/A (7)	11.42 (8.92)		
COMT	rs737865	T/T (23)	13.69 (8.82)	2.526	0.09
		T/C (11)	12.0 (8.57)		
		C/C (3)	2.0 (-)		
COMT	rs4680	G/G (13)	8.38 (8.8)	1.846	0.174
		G/A (19)	13.57 (8.31)		
		A/A (4)	7.25 (9.18)		
COMT	rs165599	G/G (2)	10 (1.41)	0.182	0.834
		G/A (18)	11.5 (9.67)		
		A/A (16)	13.06 (8.70)		
CNR1	rs1049353	G/G (39)	11.89 (8.73)	1.125	0.270
		G/A (1)	2 (-)		
		A/A			
FAAH	rs324420	C/C (22)	12.64 (8.81)	0.028	0.868
		C/A (13)	13.15 (8.93)		
		A/A (0)	0		
NRG1	rs221533	T/T (29)	11.96 (9.22)	0.160	0.853
		T/C (6)	9.66 (8.75)		
		C/C (2)	11.0 (-)		

[3] Due to relatively small numbers, in the context of genotyping work, the LI data was grouped together for the PE and the NPE conditions and put together as one score for LI associative learning, rather than as a LI score which looks specifically at the conditioning phase.

4.9.4 LI data: study (cannabis users and non-cannabis users)

The cannabis and non-cannabis user groups SNP profiles were assessed on cognitive outcomes including LI. The cognitive performance data are presented by SNPs and group in Table 33 below. ANOVAs conducted on the dataset revealed a significant main effect for COMT gene SNP rs737865 and LI outcomes (F (2, 31) = 3.89, p = 0.03), cannabis users with T/T and T/C genotypes took significantly less time to find the paired association compared to carriers of these alleles in the non-cannabis group. There were no other clearly significant effects in the cognitive data.

4.9.5 IGT, CPT and AST: study 2 (whole group)

Selective/sustained attention/executive control assessed by the CPT (see Table 34), executive control assessed by the AST (see Table 35), and decision-making assessed by the IGT (see Table 36). After controlling for multiple testing, SNP rs1049353 on the CNR1 gene was significantly associated with higher commission error scores in the G/A genotype participants compared to those with the G/G version (F (1, 57) = 8.96, p = 0.004). There was a weak and non-significant trend for those with SNP rs1049353 G/G to be significantly faster on the CPT than the G/A genotype (p = 0.08). There was also a possible trend for COMT rs165599 G/A to be associated with higher motor errors than G/G and A/A genotypes (p = 0.085). Variation in the DAOA, NRG1 genes was not linked to CPT performance.

Table 33: LI performance data explored across SNP genotypes in cannabis users and non-cannabis users

		Cannabis user	***Non cannabis user***			
Gene	**SNP**	**LI Score Mean (SD)**	**LI Score Mean (SD)**	***F***	***P***	**Geno-type (n =)**
DAOA	rs142129	15 (8.48)	16.5 (8.05)	0.31	0.59	T/T (2; 9)
		5.87(7.18)	18.8 (4.02)			T/A (16; 6)
		15.0 (8.48)	10 (9.62)			A/A (2; 5)
COMT	rs737865	9.84 (8.9)	18.7 (5.9)	3.89	**0.03**	T/T (13; 10)
		4 (4.38)	15.0 (7.87)			T/C (3; 8)
		2 (-)	2 (-)			C/C (1; 1)
COMT	rs4680	3.0 (2.5)	17 (8.39)	1.25	0.30	G/G (8; 5)
		10.85 (8.93)	15.16 (7.8)			G/A (7; 12)
		2.5 (0.70)	12 (12.72)			A/A (2; 2)
COMT	rs165599	9.0 (-)	11.0 (-)	0.039	0.96	G/G (1;1)
		8.3 (9.5)	14.6 (9.26)			G/A (9; 9)
		6.6 (7.5)	16.9 (7.17)			A/A (6; 10)
CNR1	rs1049353	8.0 (7.9)	15.6 (7.92)	-	-	G/G (19; 20)
		2.0 (-)	-			G/A (1; 0)
						A/A (0; 0)
FAAH	rs324420	5.77 (6.46)	17.38 (6.95)	0.351	0.59	C/C (9; 13)
		12.57 (9.64)	13.83 (8.86)			C/A (7; 6)
						A/A (0; 0)
NRG1	rs221533	8.66 (8.80)	15.5 (8.57)	0.39	0.56	T/T (15;14)
		4.23 (3.20)	20.5 (0.70)			T/C (4; 2)
		-	11.0 (9.89)			C/C (0; 2)

Table 34: The Continuous Performance Test (CPT) for Accuracy (Acc), Response Time (RT), Motor Errors (ME) and Commission Errors (CE) data explored across SNP genotypes in all participants

Gene	SNP	Geno-type (n=)	Cognitive data											
			CPT Acc Mean (SD)	***F***	***p***	**CPT RT Mean (SD)**	***F***	***p***	**CPT ME Mean (SD)**	***F***	***p***	**CPT CE Mean (SD)**	***F***	***P***
DAOA	rs142129	T/T (18)	19.63 (5.72)	0.82	0.44	563.36 (90.0)	0.10	0.90	1.16 (1.15)	1.254	0.294	10.94 (9.28)	0.97	0.39
		T/A (28)	18.96 (5.63)			542.41 (194.04)			1.53 (1.6)			8.37 (9.83)		
		A/A (10)	21.4 (2.36)			563.87 (239.49)			2.2 (2.25)			6.2 (5.63)		
COMT	rs737865	T/T (34)	20.29 (4.87)	0.98	0.38	574.38 (153.38)	0.31	0.74	1.84 (2.18)	0.408	0.667	9.29 (8.30)	0.18	0.83
		T/C (16)	18.18 (5.92)			539.20 (193.31)			1.46 (1.47)			8.37 (10.86)		
		C/C (10)	19.1 (4.3)			533.05 (227.00)			1.3 (1.15)			7.4 (8.85)		
COMT	rs4680	G/G (26)	20.34 (4.98)	1.02	0.37	552.19 (197.05)	0.12	0.89	1.48 (1.47)	0.423	0.657	9.96 (9.45)	0.29	0.75
		G/A (17)	19.03 (5.66)			554.14 (206.98)			1.87 (1.87)			8.11 (11.06)		
		A/A (11)	18.00 (5.17)			583.59 (119.60)			2.09 (2.2)			7.81 (6.67)		
COMT	rs165599	G/G (13)	19.69 (4.03)	1.38	0.26	544.78 (212.99)	0.23	0.80	1.00 (0.91)	2.59	0.08	9.46 (10.24)	0.24	0.79
		G/A (25)	19.28 (3.49)			575.97 (178.81)			2.4 (2.25)			9.08 (9.94)		
		A/A (13)	17.0 (6.63)			538.40 (181.10)			1.5 (1.82)			7.40 (4.05)		
CNR1	rs1049353	G/G (54)	19.43 (5.33)	0.43	0.52	570.34 (161.71)	0.32	0.08	1.69 (1.98)	0.104	0.75	7.68 (7.7)	8.96	**0.004**
		G/A (5)	21 (1.58)			424.49 (291.3)			1.4 (1.14)			19.6 (15.56)		
		A/A (0)												
FAAH	rs324420	C/C (35)	19.29 (6.09)	0.15	0.86	523.45 (178.41)	1.59	0.21	1.36 (1.94)	0.958	0.390	8.6 (10.02)	0.83	0.44
		C/A (16)	20.12 (3.36)			606.43 (184.97)			1.94 (1.77)			7.19 (5.23)		
		A/A (9)	19.44 (3.36)			603.59 (130.66)			2.22 (1.98)			12.0 (10.17)		
NRG1	rs221533	T/T (46)	20.09 (4.61)	1.67	0.20	554.22 (170.60)	0.23	0.80	1.69 (1.94)	0.376	0.688	9.22 (9.93)	0.50	0.61
		T/C (13)	18.08 (6.37)			578.84 (204.53)			1.66 (1.87)			6.69 (4.73)		
		C/C (1)	13 –			467.69 -			-			13.0 -		

There was no evidence of any significant influence of the SNP genotypes for DAOA, COMT, CNR1, FAAH and NRG1 in relation to anti-saccade task (AST) outcomes (See Table 35; all comparisons $p>0.05$).

Table 35: Anti Saccade Task (AST) outcomes explored across SNP genotypes in all participants

Gene	**SNP**	**geno- type (n=)**	**AST error Mean (SD)**	***F***	***p***	**AST latency Mean (SD)**	***F***	***p***
DAOA	rs142129	T/T (15)	24.29 (24.23)	1.01	0.37	335.34 (62.68)	0.94	0.40
		T/A (25)	32.27 (31.10)			299.28 (81.51)		
		A/A (8)	17.68 (11.76)			307.65 (84.93)		
COMT	rs737865	T/T (27)	20.64 (19.45)	1.87	0.16	318.09 (67.80)	0.29	0.75
		T/C (13)	36.92 (31.40)			305.34 (106.38)		
		C/C (8)	31.85 (35.98)			296.78 (37.49)		
COMT	rs4680	G/G (19)	30.33 (26.39)	0.40	0.68	316.75 (75.75)	1.32	0.28
		G/A (15)	22.66 (23.22)			322.63 (74.50)		
		A/A (9)	25.88 (28.76)			274.88 (62.27)		
COMT	rs165599	G/G (9)	33.37 (25.79)	1.40	0.26	301.97 (72.6)	0.02	0.98
		G/A (22)	33.65 (30.35)			303.34 (82.12)		
		A/A (10)	16.95 (19.20)			298.30 (55.16)		
CNR1	rs1049353	G/G (43)	27.44 (25.59)	0.00	0.99	314.96 (74.83)	1.21	0.28
		G/A (4)	27.25 (41.63)			271.13 (93.71)		
		A/A (0)						
FAAH	rs324420	C/C (31)	25.75 (25.47)	0.25	0.78	312 (70)	0.03	0.97
		C/A (11)	31.90 (28.86)			312 (89)		
		A/A (6)	23.83 (32.02)			303.37 (90)		
NRG1	rs221533	T/T (34)	26.78 (28.0)	0.277	0.601	301.70 (65.73)	1.04	0.36
		T/C (13)	22.42 (16.29)			336.88 (97.51)		
		C/C (1)	90(-)					

There was no evidence of any significant influence of the SNP genotypes for DAOA, COMT, CNR1, FAAH and NRG1 in relation to IGT performance (see Table 36; all comparisons $p > 0.05$).

Table 36: Iowa Gambling Task (IGT) outcomes explored across SNP genotypes in all participants

Gene	**SNP**	**geno- type (n=)**	**IGT Mean (SD)**	***F***	***p***
DAOA	rs142129	T/T (19)	1293.42 (1002.35)	0.764	0.471
		T/A (29)	1522.41 (949.82)		
		A/A (10)	1110.00 (1009.75)		
COMT	rs737865	T/T (34)	1286.02 (1032.59)	0.656	0.523
		T/C (16)	1400.00 (1000.74)		
		C/C (10)	1682.50 (559.02)		
COMT	rs4680	G/G (26)	1473.07 (1119.48)	0.188	0.829
		G/A (17)	1389 (797.06)		
		A/A (11)	1256.81 (902.02)		
COMT	rs165599	G/G (13)	1140.38 (1089.65)	0.808	0.452
		G/A (25)	1543 (873.2)		
		A/A (15)	1270 (1084.39)		
CNR1	rs1049353	G/G (54)	1347.69 (990.00)	0.935	0.338
		G/A (5)	1785 (570.25)		
		A/A (0)	-		
FAAH	rs324420	C/C (35)	1267.85 (1042.5)	1.11	0.337
		C/A (16)	1398.44 (908.86)		
		A/A (9)	1800.00 (199.52)		
NRG1	rs221533	T/T (34)	1408.70 (943.7)	0.012	0.912
		T/C (13)	1375 (1037.2)		
		C/C (0)	-		

4.9.6 IGT, CPT and AST: study 2 (cannabis users and non-cannabis users)

Selective/sustained attention/executive control assessed by the CPT (see Table 39), executive control assessed by the AST (see Table 38), and decision-making assessed by the IGT (see Table 36) were explored across SNP genotypes in the cannabis users and non-cannabis users. No significant differences were for outcomes on the IGT in relation to variation in each of the genes –see Table 37. Table 38 below indicates no main effect was found between cannabis users but a trend for an interaction was found between cannabis users and SNP 4680 (F (2, 37) =2.865, p = 0.07). Cannabis users with the SNPrs4680 A/A had higher AST errors than non-cannabis group with the A/A genotypes, whereas cannabis users with G/A genotypes had higher AST error relative to G/A carriers in non-cannabis users. There was a significant finding between variation in the CNR1 gene and AST error. A trend was found between AST latency and CNR1 rs1049353 for the SNP (F (1, 43) = 3.017, p = 0.09), with the A/A carriers responding more quickly on the task.

There were two significant findings for the CNR1 genotypes on CPT outcomes. Firstly, those with the CNR1 rs1049353 G/G genotypes were faster to respond on the CPT (F (1, 58) = 5.4, p = 0.045) compared to the G/A genotypes. Secondly, those with the G/A genotypes had higher commission errors on the CPT (F (1, 58) = 25.63, p < 0.001). Although these effects may both skewed by the inclusion of the single cannabis user with the G/A allele. A trend existed for significance of SNP rs165599 in the COMT gene for CPT ME (F (2, 46) = 2.511, p= 0.09) with G/G carriers having more motor errors on the task than G/A and A/A.

Table 37: Iowa Gambling Task (IGT) outcomes explored across SNP genotypes in cannabis users and non-cannabis users

		(n = CU; NCU)	**Non-cannabis users**	**Cannabis users**		
Gene	**SNP**	**genotype**	**IGT Score Mean (SD)**	**IGT Score Mean (SD)**	***F***	***p***
DAOA	rs142129	T/T (7; 12)	1071.43 (874.93)	1422 (1084.84)	0.300	0.610
		T/A (15; 14)	1495 (877.25)	1531.79 (1034.77)		
		A/A (7; 3)	850 (1099.9)	1716.66 (412.56)		
COMT	rs737865	G/G (16; 18)	937.5 (873.26)	1595.8 (1086.7)	0.756	0.474
		G/A (9; 7)	1408.3 (1042.6)	1389.29 (1026.7)		
		A/A (5; 5)	2015 (320.4)	1350 (569.26)		
COMT	rs4680	G/G (11; 15)	1436.36 (1072.46)	1500 (1189.33)	0.157	0.885
		G/A (7; 10)	1210.71 (667)	1515 (889)		
		A/A (7; 4)	1232.14 (1144.98)	1300 (293.68)		
COMT	rs165599	G/G (6; 7)	1883.33 (464)	503.57 (1081.7)	0.568	0.571
		G/A (11; 14)	1111.36 (791.78)	1882.14 (802.4)		
		A/A (8; 7)	837.50 (1284.94)	1764.28 (532)		
CNR1	rs1049353	G/G (29; 25)	1240.3 (944.5)	1472.7 (1047.7)	0.570	0.453
		G/A (1; 4)	1775 (-)	1787 (658.4)		
		A/A (-)	-	-		
FAAH	rs324420	C/C (18; 17)	1158.3 (982.49)	1383 (1120.79)	1.33	0.273
		C/A (8; 8)	984.37 (752.36)	1812.50 (901.19)		
		A/A (4; 5)	2256.25 (201.45)	1435 (558.06)		
NRG1	rs221533	T/T (22; 24)	1380.68 (889.32)	1434.38 (1009.35)	0.442	0.645
		T/C (7; 6)	1014.28 (1075.2)	1795.83 (894.48)		
		C/C (1; -)				

Table 38: AST outcomes explored across SNP genotypes in cannabis users and non-cannabis users.

			Cannabis user	*Non-cannabis user*	*F or*	*p*		*Cannabis user*	*Non-cannabis user*	*F or*	*p*
Gene	**SNP**	**genotype (n = cu; ncu)**	**AST error Mean (SD)**	**AST error Mean (SD)**			**genotype (n = cu; ncu)**	**AST latency Mean (SD)**	**AST latency Mean (SD)**		
DAOA	rs142129	T/T (6; 7)	33.63 (25.6)	16.28 (21.6)	0.885	0.42	T/T (6;7)	344.61 (55.3)	327.36 (71.71)	0.854	0.433
		T/A (14; 11)	35.31 (31)	28.4 (32)			T/A (14; 11)	285.86 (97)	316.36 (55.77)		
		A/A (3; 3)	18.7 (7.57)	16 (19.05)			A/A (5; 3)	313.65 (107)	297.66 (45.65)		
COMT	rs737865	T/T (14; 13)	22.45 (16.7)	18.96 (22.56)	0.854	0.43	T/T (14;13)	309.28 (68.56)	327.57 (68.3)	0.174	0.841
		T/C (9; 4)	44.94 (31.73)	14.37 (16.69)			T/C (9; 4)	298.74 (127.56)	319.75 (38.43)		
		C/C (3; 3)	25.93 (31.90)	35.4 (41.39)			C/C (3; 5)	314.25 (58.53)	286.30 (19.51)		
COMT	rs4680	G/G (10; 9)	25.51 (18.03)	36.11 (33.68)	0.475	0.63	G/G (10; 9)	311.52 (89.21)	322.55 (62.34)	1.259	0.296
		G/A (7; 8)	30.21 (27.28)	16.06 (18.28)			G/A (7; 8)	319.36 (98.6)	325.50 (52.42)		
		A/A (5; 4)	41.8 (30.39)	6.0 (4.90)			A/A (5; 4)	281.20 (93.1)	267 (18.67)		
COMT	rs165599	G/G (5; 4)	21.06 (16.45)	48.75 (29.00)	1.696	0.20	G/G (5; 4)	270.35 (61)	341.50 (73.30)	0.026	0.974
		G/A (11; 11)	44.40 (31.27)	22.90 (26.98)			G/A (11;11)	291.90 (102.97)	314.77 (57.20)		
		A/A (5; 5)	28.26 (21.63)	5.7 (6.57)			A/A (5; 5)	307.30 (70.2)	289.30 (41.26)		
CNR1	rs1049353	G/G (25; 18)	29.02 (23.8)	25.25 (28.3)	1.876	0.18	G/G (25;18)	311.79 (86.45)	319 (56.98)	3.017	0.09
		G/A (1; 3)	89.0 (-)	6.66 (7.63)			G/A (1; 3)	168 (-)	305.33 (78.4)		
		A/A (0; 0)	-	-			A/A (0; 0)	-	-		
FAAH	rs324420	C/C (17; 14)	25.33 (21.97)	26.25 (30.04)	1.711	0.19	C/C (17;14)	319.91 (79.8)	302.39 (57.8)	0.510	0.604
		C/A (7; 4)	41.28 (29.0)	15.5 (23.04)			C/A (7; 4)	302.43 (107.5)	330.75 (51.37)		
		A/A (2; 4)	47.5 (50.20)	12.0 (17.57)			A/A (2; 4)	203.57 (60.6)	353.12 (51.16)		
NRG1	rs221533	T/T (18; 16)	29.63 (27.6)	23.56 (28.91)	2.810	0.07	T/T (18;16)	293.95 (73.2)	310.40 (57.14)	0.958	0.392
		T/C (7; 6)	27.28 (9.1)	16.75 (21.56)			T/C (7; 6)	339.57 (127.27)	333.75 (57.96)		
		C/C (1; 0)	90 (-)	-			C/C (1; 0)	295 (-)	-		

Table 39: CPT outcomes explored across SNP genotypes in cannabis users and non-cannabis users.

		(n=)	*CU*	*NCU*	*F or*	*p*	*(n =)*	*CU*	*NCU*	*F*	*p*	*(n =)*	*CU*	*NCU*	*F or*	*p*	*(n =)*	*CU*	*NCU*	*F or*	*p*
Gene	**SNP**	**geno-type (CU; NCU**	**CPT accuracy Mean (SD)**	**CPT accuracy Mean (SD)**			**genotype (CU; NCU**	**CPT RT Mean (SD)**	**CPT RT Mean (SD)**			**genotype (CU: NCU**	**CPT ME Mean (SD)**	**CPT ME Mean (SD)**			**genotype (CU: NCU**	**CPT CE Mean (SD)**	**CPT CE Mean (SD)**		
DAOA	rs142129	T/T (7; 12)	18.45 (8.46)	20.3 (3.62)	0.656	0.523	T/T (6; 12)	558.74 (119.98)	565.67 (77.26)	0.268	0.766	T/T (6; 12)	1.5 (1.22)	1.0 (1.12)	1.179	0316	T/T (7; 12)	13.86 (13.73)	9.25 (5.46)	0.930	0.401
		T/A (15; 4)	19.86 (3.68)	18.0 (6.89)			T/A (15; 14)	575.67 (142.69)	506.77 (237.76)			T/A (15; 14)	1.13 (1.35)	2.0 (1.90)			T/A (15; 14)	8.26 (11.3)	8.5 (8.43)		
		A/A (7; 3)	21.57 (2.69)	21.0 (1.73)			A/A (7; 3)	646.78 (153.48)	370.41 (327.44)			A/A (7; 3)	2.0 (2.4)	2.66 (2.08)			A/A (7; 3)	5.0 (5.6)	9.0 (5.57)		
COMT	rs737865	T/T (16; 18)	19.75 (6.21)	20.7 (3.59)	1.145	0.326	T/T (15; 18)	623.05 (137)	533.8 (157.5)	0.40	0.672	T/T (15; 18)	2.0 (2.61)	1.72 (1.8)	0.368	0.694	T/T (16; 18)	6.87 (8.94)	11.4 (7.26	0.190	0.828
		T/C (9; 7)	19.8 (3.29)	16 (7.95)			T/C (9; 7)	545.49 (146.4)	531.1 (254.3)			T/C (9; 7)	1.22 (1.56)	1.83 (1.94)			T/C (9; 7)	11.11 (13.8)	4.85 (3.80)		
		C/C/ (5; 5)	20.0 (3.1)	18.2 (5.4)			C/C (5; 5)	620.19 (170.8)	449.9 (262)			C/C (5; 5)	1.60 (1.51)	1.0 (0.70)			C/C (5; 5)	9.20 (12.5)	5.6 (3.57)		
COMT	rs4680	G/G (11; 15)	22.18 (5.23)	19.33 (4.59)	0.801	0.455	G/G (10; 15)	615.24 (138.5)	510.16 (222.5)	0.015	0.985	G/G (10; 15)	1.1 (0.99)	1.73(1.70)	0.456	0.637	G/G (11; 15)	10.27 (12.4)	9.73 (7.02)	0.181	0.833
		G/A (7; 10)	20.43 (2.99)	18.10 (6.99)			G/A (7; 10)	600.5 (209.6)	521.7 (209.8)			G/A (7; 10)	2.28(3.14)	1.53(1.94)			G/A (7; 10)	9.57 (16.7)	7.1 (5.30)		
		A/A (7; 4)	16.43 (5.38)	20.75 (3.94)			A/A (7; 4)	610.5 (134.8)	536.4 (81.5)			A/A (7; 4)	2.28 (2.16)	1.75 (1.25)			A/A (7; 4)	6.14 (3.43)	10.75 (10.34)		
COMT	rs165599	G/G (6; 7)	19.83 (4.02)	19.57 (4.35)	1.272	0.290	G/G (6; 7)	621.7 (184.9)	478.8 (226.3)	0.287	0.752	G/G (6; 7)	1.16 (1.16)	0.857 (0.69)	2.511	0.092	G/G (6; 7)	8.33 (11.6)	10.4 (9.72)	0.215	0.807
		G/A (11; 14)	19.18 (2.78)	19.36 (4.07)			G/A (11; 14)	612.16 (172.5)	547.5 (184.8)			G/A (11; 14)	2.09 (2.77)	2.64 (1.82)			G/A (11; 14)	8.0 (13.4)	9.9 (6.49)		
		A/A (8; 7)	16.75 (5.17)	17.43 (8.42)			A/A (8; 7)	582.7 (118.85)	487.7 (233.3)			A/A (8; 7)	2.12 (2.16)	0.66 (0.81)			A/A (8; 7)	8.25 (4.16)	6.42 (3.99)		
CNR1	rs1049353	G/G (29; 25)	19.79 (5.01)	19 (5.38)	0.284	0.596	G/G (28; 25)	610.4 (132.6)	525.4 (181.35)	4.3	**0.045**	G/G (28; 23)	1.678 (2.17)	1.70 (1.78)	0.004	0.952	G/G (29; 25)	7.2 (8.3)	8.24 (7.11)	25.63	**<0.001**
		G/A (1; 4)	21 (-)	21 (1.82)			G/A (1; 4)	264.1 (-)	464.5 (320)			G/A (1; 4)	2.0 (-)	1.25 (1.25)			G/A (1; 4)	47 (-)	12.75 (3.2)		
		A/A (0; 0)					A/A (0;0)	-	-			A/A (0; 0)	-	-			A/A (0; 0)	-	-		
FAAH	rs324420	C/C (18; 17)	19.72 (5.87)	18.82 (6.45)	0.143	0.867	C/C (17; 17)	558.31 (147.6)	488.58 (203)	1.66	0.199	C/C (17; 17)	1.35 (2.17)	1.37 (1.75)	0.940	0.397	C/C (18; 17)	9.55 (12.43)	7.58 (6.85)	0.745	0.479
		C/A (8; 8)	20.0 (3.42)	20.25 (3.53)			C/A (8; 8)	658.56 (116.6)	554.3 (231.3)			C/A (8; 8)	2.0 (2.2)	1.875 (1.35)			C/A (8; 8)	5.25 (4.10)	9.12 (5.71)		
		A/A (4; 5)	20.0 (3.56)	19.0 (3.53)			A/A (4; 5)	649.09 (163.6)	567.18 (101.6)			A/A (4; 5)	2.5 (2.08)	2.0 (2.12)			A/A (4; 5)	10.50 (14.0)	13.20 (7.39)		
NRG1	rs221533	T/T (22; 24)	19.95 (5.24)	20.20 (4.05)	2.152	0.126	T/T (21; 24)	597.39 (148.2)	516.4 (182.7)	0.34	0.70	T/T (21; 24)	2.0 (2.39)	1.42 (1.44)	0.657	0.421	T/T (22; 24)	9.45 (12.43)	9.0 (7.17)	0.494	0.62
		T/C (7; 6)	20.42 (3.64)	15.3 (8.04)			T/C (7; 6)	620.45 (147.7)	530.28 (262.6)			T/C (7; 6)	1.0 (1.0)	2.6(2.5)			T/C (7; 6)	5.0 (3.82)	8.6 (5.24)		
		C/C (1; 0)	13.0 (-)	-			C/C (1; 0)	467.69 (-)	-			C/C (1; 0)	0 (-)	-			C/C (1; 0)	13.0 (-)	-		

4.9.7 Cannabis use variables and genotypes

A Chi² analysis was run for genotype for cannabis users only assessing cannabis use variables (e.g. Joints per Week (JPW); Age of Onset (AOO) of cannabis use; and Cannabis Duration (CD) to see whether those with the known risk alleles would also be an indication of riskier drug use – see Appendix xv for Table G7 of the results. There was a significant difference between duration of cannabis use in variation of the NRG1 with the T/C genotypes using the drug for a significantly longer time relative to the T/T genotypes (X^2 (2) = 76.14, $p < 0.001$).

4.9.8 Results for the COMT Haplotype

It was found that 6 (4 cannabis users and 2 non-cannabis users) out of 79 participants were carriers of the C-G-G haplotype (also known as G-G-G in the literature) and this was not enough to make decent statistical comparisons. Please refer to Table H8 in appendix xv for the frequency of all versions of the COMT haplotype (T-G-A, T-A-A; T-G-G; T-A-G; C-G-A; C-A-A; C-G-G and C-A-G) in the whole group and between the cannabis users and non-users. The T-G-G haplotype which is linked to having a protective effect from schizophrenia occurred in 42 out of 79 participants. Table I9 and J10 (in appendix xv) shows that most of the findings reached the level of significance in the whole group for the COMT haplotype in relation to cognitive and trait outcomes (all $p > 0.05$). Trends existed for those with the non-protective haplotype to perform slower on the AST. Trends existed for those in the COMT Haplotype and disorganised thinking – please refer to Table J10 in appendix xv. There was no main effect of cannabis (F (1, 78) = 1.249, $p = 0.207$); no main effect of COMT haplotype (F (1, 78) = 0.469, $p = 0.495$) but an interaction was found for COMT and Cannabis on SPQDT outcomes (F (1, 75) = 4.281, $p = 0.042$). Cannabis users with the protective haplotype had lower SPQDT scores (which may reduce negative symptoms), whereas those cannabis users without protective haplotype had higher SPQDT scores, whereas the opposite effect was found in the non-cannabis group.

4.9.9 Results for the combination of risk markers in relation to trait and cognitive outcomes between the cannabis users and non-users.

The genetics data were analysed to combine the number of risk SNP markers each participant had and these were categorised for those having (0-5) of the known risk SNPs (i.e. T/T or T/A for DAOA SNP rs142129, G/G or G/A for COMT rs4680, G/G for CNR1 rs1049353, A/A for the FAAH SNP rs324420, and C/C for NRG1 SNP rs221533). Please refer to Table K11 (in Appendix xv) for a full table of results of those which did not reach significance or were leaning toward significance.

4.10 Discussion

The current study assessed 5 candidate schizophrenia genes (DAOA, COMT, NRG1, CNR1, and FAAH) in a group of cannabis users and non-cannabis users. The aim was to assess for potential differences in variation of these genes (i.e. SNPs) and possible interactions with cannabis in relation to outcomes on the LI associative learning tests from Study 1 (Latent inhibition only and Study 2 (Iowa Gambling Task, a decision making task; Continuous Performance Test, a measure of attention and impulsivity; and the Anti-saccade task, a measure of attentional control). All of these tests have known sensitivity to schizophrenia. One personality measure was also included from data taken from Study 1 and 2, which assessed for traits linked to schizotypal personality disorder (e.g. SPQ-B, Raine and Benishay, 1995). Below is a discussion of some of the main outcomes as well as findings from the three SNP at-risk haplotypes from the COMT gene. The findings discussed below were either significant with a *p*-value less than 0.05 or were non-significant findings but possibly indicated a trend towards significance (*p*-value greater than 0.05 but less than 0.15).

4.10.1 DAOA gene

One SNP (rs142129) was assessed in the DAOA gene. Although none of the reported findings for this SNP were statistically significant (most likely due to the reasons discussed below - see limitations), there were some interesting differences in the frequencies of genotypes in relation to the cognitive and trait (SPQ-B) outcomes that are worthy of comment.

There was a trend towards significance in that the T/A genotype occurred more frequently in the cannabis users. In previous studies the T/A genotype for SNP rs142129 was positively associated with schizophrenia (e.g. Chumakov *et al*, 2002; Schumacher *et al*, 2004). Though it was only a weak trend, the difference between cannabis users and non-users for T/A genotype may be contributing to differences in performance and trait scores in the cannabis users. In a larger follow up study matched cannabis users with the T/A genotype could be compared to those without this genotype to see if there is a noticeable difference –and so highlight if there is any form of cannabis use and T/A interaction effect.

The T/T genotype was associated with the poorest associative learning on the LI task. Interestingly, it is the T/T genotype within the literature which is linked to poorer cognitive performance, particularly in those diagnosed with schizophrenia (e.g. Goldberg *et al*, 2006). Furthermore, it was this SNP T/T which has previously been reported as conferring the highest risk for susceptibility to methamphetamine psychosis (Kotaka *et al*, 2009). Even though the results for this SNP on the DAOA gene were not statistically significant, the findings were in the predicted direction.

4.10.2 COMT gene

Three SNPs (rs737865; rs4680; rs165599) were assessed in the COMT gene individually and then assessed together as a protective haplotype for carriers of the T-G-G alleles. The COMT gene SNP rs4680 has been widely assessed in the literature with over 250 peer-reviewed studies published since 1996; it is the most studied gene in psychiatry (Haraldsson *et al*, 2009). There was a significant finding in the whole sample with the T/T allele of SNP rs737865 occurring more frequently than the T/A and C/C genotypes. Furthermore, there was a trend for the cannabis users to have the risk G/G allele SNP rs4680 occur much more frequently. There was no significant variation on the risk SNP rs4680 for individual differences in psychosis-like personality traits, which is not in line with previous studies (Swart *et al*, 2011; Ucok *et al*, 2010; Smyrnis *et al*, 2007; Sheldrick *et al*, 2008). There were trends found in SNP rs737865 in that those individuals with the C/C alleles reported higher interpersonal problems compared to the T/T genotypes in the whole group and when the group was broken down the trend existed for the cannabis group, not for the non-cannabis users. In the whole group it was those individuals with SNP rs165599 A/A genotypes who had lower cognitive perceptual and interpersonal sensitivity scores on the SPQ-B. Furthermore, those with the G/G alleles reported higher problems with disorganised thinking in both the cannabis users and non-cannabis users. Most of the literature links rs4680 A/A with better cognitive performance (Bilberg *et al*, 2002; Goldberg *et al*, 2003; Jorber *et al*, 2002; Sheldrick *et al*, 2008) whilst the G allele is associated with the worst cognitive performance (Bruder *et al*, 2003; Bertolino *et al*, 2006; Goldberg *et al*, 2003). None of the findings reached a level of significance or trends for significance for the most widely studied SNP rs4680 and CPT outcomes. A study by Symrnis *et al* (2007) also assessed COMT SNP rs4680 carriers and reported no effect of this gene on CPT outcomes. There has been

inconsistent findings with the COMT gene (SNP rs4680) and this also reflects research published in this area with negative findings (Chen *et al*, 1999; Daniels *et al*, 1996; Rosa *et al*, 2004) and positive findings with G/A (Egan *et al*, 2001; Kunugi *et al*, 1997a; 1997b) and with G/G (e.g. Wanodi *et al*, 2003).

Cannabis use and SNP rs4680 has been widely studied but less is known regarding the other two SNPs (rs737865 and rs165599) on the COMT gene in relation to schizophrenia. Trends for significance were found for the following results. Individuals with SNP rs737865 C/C genotypes (also known as G/G in the literature) performed better on the LI task regardless of whether or not they used cannabis. It was the G/A genotypes of SNP rs165599 who had poorer attentional control as they made more motor errors on the CPT. In the AST, it was the T/T (also known as A/A) genotypes of SNP rs737865 in the whole group who had better attentional control as they made the least errors on the task. Even though these trends for results were not statistically significant it does seem that they were in the direction predicted. However, these findings were taken from the whole group as opposed to these being specific cannabis group effects.

Some of the inconsistencies in the data may be the result of multiple SNPs being involved, as opposed to one single marker. Therefore, one haplotype was investigated in all three SNPs (rs737865, rs4680 and rs165599) on the COMT gene. In the literature the risk haplotype C-G-G is positively associated with schizophrenia (De Rosse *et al*, 2006; Schifman *et al*, 2002; Handoko *et al*, 2004). The T-A-A haplotype has also been linked to schizophrenia (Kotrotsou *et al*, 2012). However, the risk haplotypes T-A-A occurred in 6 participants and the C-G-G haplotypes occurred in 8 participants, so this was not enough to make for viable statistical comparisons. The COMT haplotype data were reviewed without completing any statistical analysis to check if there were any common variables amongst the cannabis group with this haplotype compared to the other COMT haplotypes. Interestingly, the cannabis users with the risk C-G-G haplotype that had the lowest use of drugs such as MDMA, amphetamine, cocaine, alcohol, tobacco, and cannabis, whereas those with the T-A-A risk haplotype had the highest use of all of these aforementioned drugs (x4 times greater use). The T-A-A haplotypes also had higher schizotypal personality traits. Another haplotype T-G-G known to have a protective effect with regards to schizophrenia was identified in 50% of

the sample and was therefore compared against the rest of the sample to assess for potential differences in trait and cognitive outcomes. There were trends for those individuals with the protective haplotype to take longer to respond on the AST. Trends also existed for cannabis users with the protective haplotype to have lower SPQ-DT scores (which may reduce negative symptoms), whereas those cannabis users without protective haplotype had higher SPQ-DT scores, which could be taken to suggest that the haplotype actually is protecting the user from possible negative effects of cannabis on thinking. This is the first study to date to assess the T-G-G haplotype in cannabis users and the SPQ-B disorganised thinking trait data seems to be in line with Kotrotsou *et al*'s (2012) research that this is haplotype has a protective effect.

4.10.3 NRG1

Only three participants in the entire sample had the risk SNP rs221533 C/C allele, with most of the group having the T/T genotype. This, therefore, contravened the criteria for the HWE and also made it difficult to draw comparisons from these data against previous studies (e.g. Stefansson *et al*, 2002; 2004) which link the rs221533 (C/C) allele with schizophrenia. Trends existed within the whole group, with the T/C genotypes reporting higher SPQ-B scores and the T/T genotype reporting the lowest SPQ-B scores. There was a trend for SNP rs221533 (T/C) carriers to have higher scores on the SPQ-CP and SPQ-DT subscales, naturally suggesting problems with cognition, perception and thinking. The effect was there in the overall group data, with no effect of cannabis user/non-user status, or regardless of whether or not individuals were cannabis users. Some behavioural trends also existed for SNP rs221533: cannabis users with the T/C allele made 50% more errors on the AST than non-cannabis users with the T/C genotype, whereas cannabis users and non-cannabis users with the T/T did not differ in AST errors. Thus, in line with previous research, it seems that cannabis users with the C allele was linked to worse cognitive performance and in the whole group was associated with having more psychotic traits relative to the T allele. This is the first known study to demonstrate variation on the NRG1 gene for SNP rs221533 in relation to cannabis use, cognition and trait outcomes.

4.10.4 CNR1 gene and the FAAH gene

No participant had the SNP rs1049353 A/A allele for the CNR1 gene and the majority of the sample had the risk G/G allele (96%), so this contravened the HWE as the result of low participant numbers. Therefore, interpretations of these data are limited. Trends were found in the whole group for those with SNP rs1049353 G/G genotypes reporting the highest SPQ scores and this is supported by previous research which links this allele with schizophrenia (Leroy *et al*, 2001). In Study 2, those with the SNP rs1049353 G/G genotype were faster to respond on the CPT. One would expect that these carriers in the at-risk group G/G would perform worse on this measure of cognition. However the reverse was found in this study, as there was a significant difference between SNP rs1049353 G/G and G/A genotypes, with G/G carriers making significantly fewer commission errors on the CPT. A trend was found in the cannabis group for those individuals with the G/A genotypes in SNP rs1049353 to be slower to respond in the CPT and made significantly more commission errors on the task. The G/A allele has previously been shown to be positively associated with schizophrenia (Costa *et al*, 2012). However, on further investigation only one participant had the G/A genotype in the cannabis group, so this person's poor performance may have skewed these data.

There was no significant variation in the FAAH gene on trait outcomes and no interactions with cannabis use. A significant difference was found between variation in SNP 324420 C/C and C/A for Kamin Blocking outcomes, as those with the C/C genotype had better associative learning performance scores in both the cannabis users and non-users. Interestingly within the literature it is the A allele which is linked more often to a schizophrenia profile. This is the first study to report findings from variations in the CNR1 and FAAH genes in relation to cognitive outcomes for associative learning.

4.10.5 Cannabis use variables

None of the cannabis use variables for the DAOA, FAAH and CNR1 genes showed significant variation on cannabis use variable outcomes for joints per week, duration of use and age of onset. There was a trend for variation in the NRG1 gene; those with the SNP rs221533 T/C genotype reported the longest duration of cannabis use compared to users with the T/T genotype. Interestingly, recent research linked variation in the NRG1 gene to cannabis dependence (Tan *et al*, 2012).

4.10.6 Combined SNP risk markers

It was predicted that those with a profile of multiple risk alleles from the candidate schizophrenia genes may show greater biases on cognitive tests and schizophrenia-like personality symptoms, in the direction of schizophrenia-like deficits and differences. However, none of the findings were statistically significant, which may be due to having low participant numbers across multiple cognitive assessments.

Overall though, genetic research is a complex area and research is moving towards using haplotype data, as opposed to single risk markers (Clarke, 2004). The key finding above was linked to the COMT protective haplotype in cannabis users associated with lower disorganised traits compared to cannabis users without this protective haplotype. This research is useful as it looked at the combination of trait, cognitive and genetic data in the risk model of schizophrenia, which helps to further understand the link between cannabis use and schizophrenia. In this current research, the significant findings for the genetic data were mainly for the whole group, as opposed to these being specific cannabis effects.

4.10.7 Methodological issues

The COMT gene SNP rs737865, CNR1 gene SNP rs1049353 and NRG1 gene SNP rs221533 all contravened the HWE. To overcome this issue a future replication would need more participants to increase the power of the study. The aim of the study was not to assess for gender or ethnic differences but given the link these factors have on genetic expression of

some of these SNPs, this cannot be ruled out of the findings. Further to this, the cannabis users frequently reported lifetime or current use of other drugs, so polydrug use is a factor that needs to be accounted for when interpreting these findings. Due to the already small cell sizes in these data (when divided by cannabis use and genotype) further breakdowns or covariance using other drugs use, gender and/or ethnicity would weaken the statistical validity of tests even further. However, if replicated in much larger cohorts then these would be important additional variables to allow for inter analyses and grouping of data. These limitations alongside others will be explored in more detail in the next chapter (see section 5.5).

Chapter 5: Summary and general discussion

There exists a substantial body of empirical research, alongside anecdotal observations, indicating that cannabis use is a contributory risk factor for the development of psychotic symptoms and schizophrenia in some users (e.g. Smit *et al*. 2004; Semple *et al*, 2004; Moore *et al*, 2007; and see Chapter 1, sections 1.4, 1.4.1 and 1.4.2). Cannabis use related psychosis has been argued to be related to effects of THC on normal endocannabinoid functioning during crucial stages in early and late neurodevelopment (Spear, 2000, Cannon *et al*, 2005), induced release of dopamine in frontal circuits (Szabo & Schlicker, 2005) and to combinations of these effects, alongside an array of psychosocial and genetic vulnerabilities (see section 1.4.2 and 1.5). Implicit in such models are notions of additive or compound effects, and the possibility therefore that THC use may act to push individuals towards a 'pathological tipping point'. If correct, it could be argued that outside of clinical populations of cannabis users (i.e. the larger population of non-clinical/non-pathologised recreational cannabis users) such effects of THC may well push individuals along psychosis-linked continua; perhaps never producing pathology but leading to observable changes in selective psychological parameters relative to non-users, for example.

Nonetheless it is also clear that the majority of cannabis users do not go on to develop this disorder. It is argued that the use of cannabis in some users is causing some of the sub-schizophrenia symptoms, and that prolonged use and/or heavy use of stronger versions of cannabis is linked to the onset of induced symptoms in some people who may have otherwise not developed any of these symptoms. Much of the published research on the cannabis-psychosis link has focussed on schizophrenia-like symptoms, as opposed to looking at these symptoms combined with cognitive performance and genetic susceptibility. Furthermore, research has focussed mainly on clinical samples (i.e. people diagnosed with schizophrenia). These studies are not without their problems as there is heterogeneity in symptoms and behaviours in terms of schizophrenia. Moreover, findings drawn from clinical studies are difficult to fully explain in terms of causality as a confounding factor is linked to clinical status, which is difficult to control. Research using a sample of recreational users may provide a better way of attempting to elucidate the link between cannabis use and psychosis (Verdoux *et al*., 1998; van Os *et al*., 2000; 2001; Verdoux and Van Os, 2002). The first issue is whether cannabis use is possibly producing changes in some aspects of the user's

psychology, in the direction of psychosis, which is important in its own right because most people who use cannabis see it as a harmless drug, and the majority of users will never be diagnosed with schizophrenia. Then secondly, depending on what is found this may then inform our understanding of the link between cannabis use and schizophrenia (in a much smaller cohort of users). Therefore, the general aim of the thesis was to explore a range of cognitive functions, psychotic-like personality traits, and candidate genes for schizophrenia, in a group of recreational cannabis users (free from existing psychopathological disturbance) and a control sample of non-cannabis users. In line with the model that cannabis use may impact on cognitive function, in a direction seen in those diagnosed with schizophrenia and high schizotypal individuals, it was predicted that cannabis users would show more impairment on these measures when compared against a group of non-cannabis users.

The cognitive tasks chosen had particular significance to the field of psychosis due to performance being significantly affected by schizophrenia, high schizotypy, and in relatives of those diagnosed with schizophrenia. Study 1 assessed LI, KB and schizotypy (please refer to sections: 2.1.2, 2.1.3 for further details on LI; sections: 2.14, 2.1.5 for KB (due to lack of findings will not be discussed under this final discussion), and section 2.2.2 for materials, and study 2 added further personality analyses (see section 3.41, 3.4.2 for further details on Ambivalence, Mood and Paranoia) and alongside other cognitive tasks (refer to section 3.1.1, 3.2.1, and 3.3.1 for further details on the IGT, CPT, and AST respectively) known to be sensitive to cannabis use and to schizophrenia. It was predicted that cannabis users would show impaired associative learning in Study 1, and in Study 2 show riskier decision making, disrupted attention and poorer executive control. A key difference in this research thesis compared to previous research looking at purely cognitive effects of cannabis, is that schizotypy has been measured alongside cognitive performance, in order to see if there is an interaction here and how this then relates to cannabis use. It is important to measure schizotypy for a number of reasons. Many studies have not done this before but an obvious question is whether any disruptions seen in cognitive performance are due to the use of THC or to underlying personality traits which might interfere in performance of cognitive tasks that have previously been shown to be disrupted by schizophrenia. Another important consideration is the role of genetic risks which may underlie a possible exacerbation of certain traits when cannabis use is added. Therefore, Chapter 4 assessed the relative contribution of 5 candidate genes linked to schizophrenia in relation to outcomes from the

battery of cognitive measures (from Chapters 1 and 2) along with one trait measure, schizotypy, assessed across both studies (SPQ-B, Raine & Benishay, 1995). Tables 40 and 41 below summarise the key findings of the research presented in this thesis: the main behavioural and trait outcomes (Table 40) and the genetic data and interactions with measures (Table 41).

Table 40: A summary of the key behavioural and trait measure findings

Measure	**Key Significant findings**	***Test statistic***		***p***
Study 1: Primary analysis – LI	LI was abolished in cannabis users	***F***	10.3	**0.003**
Study 1: Primary analysis – LI & SPQ-B	In the whole group low SPQ-B scorers were better at associative learning under the NPE condition compared to high SPQ-B scorers.	***T***	4.567	**<0.001**
Study 1: Primary analysis – LI & SPQ-B	In the whole group high SPQ-B scorers showed disrupted LI under the PE condition relative to low SPQ-B scorers	***T***	3.198	**0.005**
Study 1: Secondary analysis – LI	LI was abolished in cannabis users under the PE condition.	***F***	5.83	**0.02**
Study 1: Secondary analysis – LI	In the whole group, LI was abolished under the PE condition in high SPQ-B scorers.	***F***	6.32	**0.017**
Study 1: Secondary analysis – SPQ-B and LI	In the whole group, poorer performance on LI task was correlated with higher SPQ-B scores.	***R***	0.336	**<0.05**
Study 1 – Cannabis Dependency and SPQ-B	Cannabis dependency was higher scores on the SPQ-B subscale of Interpersonal Thinking	***R***	0.409	**<0.05**
Study 1- Cannabis use and SPQ-B	Heavy use of cannabis was associated with higher dependency,	***R***	0.370	**<0.05**
Study 1- Cannabis use and SPQ-B	Heavy cannabis use was associated with higher scores on all three SPQ-B subscales for interpersonal thinking, cognitive perceptual; disorganised thinking	***R***	0.561; 0.28; 0.416 (respectively)	**0.01; < 0.05. <0.05 (respectively)**
Study 1- Cannabis use and SPQ-B	Earlier onset of cannabis was associated with higher scores on the SPQ-B subscale for disorganised thinking.	***R***	0.468	**0.01**
Study 2: IGT	Cannabis users demonstrated riskier based decision-making	***F***	3.391	**0.05**
Study 2: IGT and heavy cannabis use	Heavier use of cannabis was association with riskier based decision making	***R***	0.326	**0.03**
Study 2: AST and SPQ-B	In the whole group, high SPQ-B scorers demonstrated poorer executive control	***F***	9.143	**0.004**
Study 2: CPT and SPQ-B	In the whole group, high SPQ-B scorers were faster to react during the CPT	***F***	4.689	**0.035**
Study 2: CPT and SPQ-B	In the whole group, higher scores on the SPQ-B subscale of 'interpersonal' deficits were associated with slower performance on the CPT.	***R***	0.39	**<0.001**
Study 2: AST and SPQ-B	In the whole group, higher scores on the SPQ-B subscale of 'interpersonal' deficits were associated with slower performance on the AST.	***R***	0.37	**<0.05**
Study 2: AST and SPQ-B	In the whole group, those reporting more traits linked to the SPQ-B subscale of interpersonal were associated with poorer executive control.	***R***	0.48	**<0.001**
Study 2: AST and BIS-2nd.	In the whole group, those with higher reporting more impulsive traits were associated with poorer executive control.	***R***	0.46	**<0.001**
Study 2: IGT and SPQ-B	Cannabis users with higher SPQ-B scores were associated with riskier based decision making.	***R***	0.326	**<0.05**
Study 2: AST and SPQ-B	Cannabis users with higher SPQ-B scores were associated with poorer executive control.	***R***	0.325	**0.01**
Study 2: Cannabis use and BIS-1st	Heavy use of cannabis was associated with having more impulsive traits.	***R***	0.435	**0.01**
Study 2: Cannabis use and clarity	Longer duration of cannabis use was associated with being less clear in thinking.	***R***	0.389	**<0.05**
Study 2: Trait data	Cannabis users reported more schizotypal traits linked to cognitive perceptual, disorganised thinking, and ambivalence, as well as more social reference to paranoia, ideas of persecution, impulsivity, and less emotional clarity.	***F***	ranged from 4.99-13.8	**all ≤ 0.03**

Key:

AST: Anti-saccade task BIS-2nd: Barratt Impulsivity Scale -2nd order factor CPT: Continuous performance test

IGT: Iowa Gambling Task LI: Latent inhibition SPQ-B: Schizotypal Personality Questionnaire-Brief

Table 41: A summary of the key genetic findings in groups and across measures

Gene and SNP	Key Significant findings	*Test statistic*		*p*
DAOA rs142129	T/A genotype occurred more frequently in cannabis users.	X^2	7.231	0.055
NRG1 rs221533 and SPQ-B	In the whole group, T/T genotypes scored higher on the SPQ-B subscale of interpersonal compared to the T/C genotypes	*F*	3.183	**0.046**
COMT rs165599 and SPQ-B	In the whole group, the G/G genotypes experienced more disorganised thinking problems	*F*	2.623	0.078
NRG1 rs2215533 and SPQ-B	In the whole group, the T/T genotypes reported less disorganised thinking problems.	*F*	2.468	0.09
NRG1 rs221533 and SPQ-B	In the whole group, the T/C genotypes scored higher on having cognitive perceptual problems.	*F*	2.509	0.08
COMT rs737865 and LI	In the whole group, the C/C genotypes took less time to find the paired association for the LI task.	*F*	2.526	0.09
COMT rs737865 and LI	Cannabis users with the T/T and T/C alleles took less time to find the paired association in the LI task compared to non-users with the same alleles.	*F*	3.89	**0.026**
CNR1 rs1049353 and CPT	In the whole group, those individuals with the G/A genotypes had higher commission errors relative to G/G genotypes.	*F*	8.96	**0.004**
CNR1 rs1049353 and CPT	In the whole group, those individuals with the G/A genotypes were slower to perform on the CPT relative to G/G genotypes.	*F*	0.32	0.08
COMT rs165599 and CPT	In the whole group, those individuals with G/A genotypes showed increased motor errors relative to G/G and A/A genotypes.	*F*	2.59	0.08
COMT rs4680 and AST	An interaction was found between variation in SNP rs4680 and AST performance. Cannabis users with the G/A genotypes showed impaired executive control relative to the non-cannabis users with the G/A genotypes. Cannabis users with the A/A genotypes showed improved executive control relative to non-cannabis users with the A/A genotype.	*F*	2.865	0.07
CNR1 rs1049353 and AST	In the whole group, those individuals with the A/A genotypes were faster to respond on the AST.	*F*	3.017	0.09
CNR1 rs1049353 and CPT	In the whole group, those individuals with the G/G genotypes were faster to respond on the CPT compared to the G/A genotypes.	*F*	5.4	**0.045**
CNR1 rs1049353 and CPT	In the whole group, those individuals with the G/A genotypes made more commission errors on the CPT.	*F*	25.63	**<0.001**
COMT rs165599 and CPT	In the whole group, those individuals with the G/G genotypes made more motor errors on the CPT.	*F*	2.511	0.09
NRG1 rs221533 and cannabis	The T/C genotypes had used cannabis for a longer duration than the T/T genotypes	X^2	76.14	**<0.001**
COMT haplotypes (T-G-G) and SPQ-B	Those cannabis users without the TGG protective haplotype had higher disorganised thinking traits, whereas cannabis users with the TGG protective haplotype had lower disorganised traits, whereas the opposite effect was found in the non-cannabis group.	*F*	4.281	**0.042**

Key:

AST: Anti-saccade task BIS-2nd: Barratt Impulsivity Scale -2nd order factor CNR1: Cannabinoid Receptor 1

COMT: The Catechol-O-methyl transferase CPT: Continuous performance test DAOA: D-amino Acid Oxidase Activator

FAAH: Fatty Acid Amide Hydrolase NRG1: Neuregulin IGT: Iowa Gambling Task LI: Latent inhibition

SPQ-B: Schizotypal Personality Questionnaire-Brief

5.1 Neuropsychological assessments – study one

The purpose of study one was to assess the performance of a group of cannabis users relative to non-cannabis users, through the use of two associative learning tasks with known sensitivity to schizophrenia: the latent inhibition and kamin blocking paradigms (see sections 2.1.1 and 2.1.4). Both tasks have also been shown to be disrupted in first degree relatives of people with schizophrenia, and by drugs which affect dopamine. It was predicted that cannabis users would show disruption on the LI and KB tasks due to the effect of cannabis on the dopaminergic and other key systems underpinning attention and associative learning. The first study also assessed schizotypal personality traits as measured by the SPQ-B.

5.1.1 Associative Learning – Latent inhibition

Cannabis users appeared to show performance akin to a schizophrenic-like profile on the LI task, as normal LI was abolished in the PE condition, with no significant difference found in LI scores between the task conditions (PE and NPE) for the cannabis users. In the primary analysis, the cannabis users took less time overall to find the paired association between the white noise and the counter incrementing under the PE condition compared to the cannabis group in the NPE condition. There was a LI effect in the non-cannabis users but in the opposite direction to what is expected (i.e. faster learning in the PE condition). There were no clear explanations as to why the controls performed badly. It was 5 participants in the non-cannabis group who were excluded for not reaching the learning criterion (e.g. did not successfully learn the paired association) and those in particular who reported cannabis use in the past. A secondary set of analyses were carried out, which also included 5 new participants: four new participants allocated to the NPE condition and one participant to the PE condition. There was a significant difference between the PE versus NPE conditions for the non-cannabis group; thus indicating a normal LI effect (i.e. slower learning in the PE condition). Furthermore, there was a significant difference between cannabis users and non-cannabis users in the PE condition but not under the NPE condition, indicating that cannabis users overall were less affected by the pre-exposure to the white noise during the masking task (of the PE condition), thus indicating a trend for abolition of normal LI in the secondary analyses. It could be argued that these LI findings fit the idea that use of cannabis may be disrupting associative learning as also seen in psychotic populations, first degree relatives of schizophrenia sufferers (Serra *et al*, 2001) and following amphetamine use (Soloman *et al*,

1981). Cannabis has been independently associated with deficits in PFC activity (Block *et al*, 2002; Lundquist *et al*, 2001; Solowij *et al*, 2002) linked to attentional dysfunction (Weinberger *et al*, 2001) and increased mesolimbic dopamine transmission in the brain (Tanda *et al*, 1997; Voruganti *et al*, 2001). This parallels to the finding that dopamine is critical for LI performance (Soloman *et al*, 1981; Weiner *et al*, 1981; 1984) and appears to be central in some forms of attentional dysfunction (Matthysse, 1978; Swerdlow & Koob, 1987; Swerdlow *et al*, 2003). Further to this, CB1 receptors have a known role in associative learning in animals (Gruart *et al*, 2012) and cannabis has been shown to disrupt associative learning in humans (Jager *et al*, 2007; Skosnik *et al*, 2007). Disrupted associative learning (as demonstrated by the LI task) is therefore argued to be due to the failure of inhibiting attention to the irrelevant stimuli during the pre-exposure stage of the task, which may be linked to cannabis increasing DA transmission.

5.1.2 Individual Differences – study one

Individual differences in psychotic-like personality traits were assessed using the SPQ-B (Raine & Benishay, 1995) but no significant differences were found between the cannabis and non-cannabis users in Study 1. This does not support previous research (Skosnik *et al*, 2001; Barkus *et al*. 2008; Friedberg *et al*. 2010). These data may indicate that if cannabis use is in some way pushing users in a psychosis-linked direction, then this effect is clearly not so profound as to be causing significant personality change in everyday recreational users. Additionally in Study 1, this data indicates that the controls and users were matched effectively on underlying schizotypy traits –thus any cognitive differences observed cannot easily be attributable to existing psychosis-linked differences or as secondary effects to a more profound shift towards schizotypy/psychosis due to cannabis use. Or it could be argued that the lack of findings could be due to the low participant numbers within study one (n=40), or alternatively it could be due to a lack of sensitivity in the SPQ-B as a measure. The SPQ-B requires a binary Yes/No response and does not allow for any recognition for levels of this trait. The owner (Adrian Raine) was contacted via email in 2008 to ask if the SPQ-B measure can be modified to adopt a Likert-scale responding for Study 2 of this thesis, but he refused the request. Then a study published by Cohen *et al.* (2010) adjusted the SPQ-B measure using a likert-scale and reported that the brief revised SPQ was much more sensitive than the original version for uncovering a psychosis-proneness personality profile. This

adaption was not made for Study 2, but instead a range of additional psychotic-like measures which used likert scale responding were added (see section 3.5.2).

5.1.3 Individual differences and associative learning

In the primary analysis, when collapsing data across groups (combining cannabis users and controls) there were no significant differences were found in the PE condition for high and low SPQ scorers, whereas low SPQ scorers took significantly less time than high SPQ scorers under the NPE condition to make the association. This agrees with Wuthrich and Bates (2001) who found that when levels of schizotypy increase, learning of the paired association in the LI task decreased. Baruch *et al*'s (1988a) research also reported that LI is attenuated in people with a high-schizotypy relative to low schizotypy scorers. In the secondary analysis, low SPQ scorers took longer to find the paired association in the PE condition versus the NPE condition, thus highlighting a normal LI effect. This was contrasted by high SPQ scorer's performing significantly better at finding the paired association in the PE condition relative to the NPE condition, thus indicating abolishment of normal LI in these high SPQ scorers. When the data was separated for cannabis users and non users, the correlation data also highlighted that for the non-cannabis group in the secondary analysis, scoring higher on the SPQ-B measure and its three subscales was positively associated with higher scores on the LI task, but this was not the case for the cannabis group. Therefore the findings strongly support the argument that the LI abolition effects in this current study, under the PE condition, are drug specific, as opposed to being linked to personality traits.

5.2 Neuropsychological assessment – study two

This study aimed to further look at this issue of cognitive disruption in cannabis users which seems to parallel to schizophrenia. Thus far from Study 1 only the LI task had shown some effects in the predicted direction. It was important therefore, given the lack of an effect in KB, to explore whether this effect was limited to LI or whether other domains typically affected by schizophrenia were also impacted upon by cannabis use at recreational (non-dependent) levels. It was important to again look at schizotypy as a possible driver or confound in any cognitive effects. The possible limitations of the SPQ-B were also addressed by using additional psychosis-linked trait measures. The cognitive tasks assessed

decision-making using the IGT, attention using the CPT and executive control using the AST (see sections 3.1, 3.2 and 3.3 for a full review of the tests and 3.7 for a full discussion of the key findings). In line with the key predictions of the thesis, decision-making impairments were found in cannabis users for IGT performance, in that they selected the decks which had immediate higher gains but also higher losses overall (see Figure 7) and made a significant loss in comparison to the non-cannabis users. This finding is supported by previous research (Hermann *et al*, 2009; Bolla *et al*, 2003; Wesley *et al*, 2011). Other factors may have been involved in these decision-making strategies, for example, emotional and motivational processes may have played a part. It may be that the cannabis users have an increased sensitivity to rewards and insensitivity to losses, or are generally less risk-averse individuals because they have specifically chosen to use an illegal substance for personal gratification and to discount the possible risks associated with this psychoactive drug. Previous research found evidence to suggest that drug users reduce the value of a reward when there is a delay in receiving this (e.g. Coffey *et al*, 2003).

Cannabis users made more errors on the AST and were faster to respond relative to the non-cannabis group, but these differences were not statistically significant. No clear differences were found between cannabis users and non-cannabis users on the CPT for accuracy, speed and number of motor and commission errors. Research in the area of performance on the CPT and AST in schizophrenic patients tends to show that schizophrenic patients perform less well compared to controls, whereas the research on cannabis use in clinical samples and in non-clinical samples has been less clear. This may help to explain why no differences were found between the cannabis users and non-cannabis users. For example, Chung *et al* (2010) looked at adolescents with and without cannabis use disorder and found no difference in performance on the AST. Rodríguez-Sánchez *et al* (2010) found no difference on CPT performance between schizophrenics who used cannabis versus those schizophrenics that did not use cannabis. Jockers-Scherübl *et al* (2007) found that performance on the CPT was impaired in healthy controls if they used cannabis before the age of 17, but performance was improved in a group of patients that started cannabis before the age 17, after a 28-day abstinent period.

5.2.1 Individual differences – study two

Study 2 used the SPQ-B measure and also explored a range of other psychotic-like traits such as ambivalence, emotional processing, and paranoid thinking, using scales which adopted likert responding. Cannabis users reported experiencing more Paranoia (part A and B of the Green *et al* 2008, Paranoid Thought Scale), Ambivalence (SAS) and Impulsivity (first and second order factor of the Barratt Impulsivity Scale) and were less clear for their emotions assessed by the trait meta-mood questionnaire subscale for clarity. These findings support previous research that cannabis users experience more psychotic-like traits (Arsenault *et al*, 2004; Henquet *et al*, 2003; van Os *et al*, 2002; Stefanis *et al*, 2004; Skosnik *et al*, 2008; Dumas *et al*, 2004 Kuepper *et al*, 2011) and impulsivity (Schmid *et al*, 2004; Barkus *et al*, 2008). Unlike Study 1, cannabis users scored significantly higher on the SPQ-B and all sub factors, and this may be the result of having a higher number of participants in the total group (n=60). A review of other drugs used between cannabis users in Study 1 and 2 was explored to see if this could explain these SPQ-B differences. There was higher use of other drugs amongst cannabis users in Study 1 (see Table 3i and Table 3ii) with 15 out 20 cannabis users reporting polydrug use (i.e., two or more drugs as well as current cannabis use), as opposed to 2 out of 30 in Study 2 (see Table 20). There were no apparent differences between amount of cannabis use and type of use between the users in Study 1 and Study 2, whereas cannabis users in Study 2 had a lower mean age of cannabis first use, and a higher mean dependency score. These SPQ-B differences in Study 2 may specific to cannabis as opposed to polydrug use.

5.2.3 Individual differences and decision-making/executive control/attention

In the entire sample when the data was collapsed across users and non users for high/low scores on the total SPQ-B there was no impact on IGT performance. Although, risky decision-making (on the IGT) was associated with positive schizotypy (as assessed by the SPQ-CP). This finding links to Wout and Stanfley (2010) research who found that higher scores on the SPQ-CP were predictive of poorer bargaining behaviours in game theory. Therefore, positive symptoms could influence normal everyday decision making. Participants with higher total SPQ-B scores made twice as many errors on the AST, which supports previous research in that increased psychotic like traits are associated with greater deficits on this task (Holahan & O'Driscoll, 2005; Ettinger *et al*, 2005; Ettinger *et al*, 2006;

Ettinger *et al* (in press); Larrison *et al*'s (2000). Interestingly, deficits in AST performance were linked more to psychotic traits than cannabis use *per se*. CPT response time was affected in those with high SPQ scores as they had significantly faster reaction times. CPT performance was also linked to Interpersonal Deficits on the SPQ-B, but was not correlated with the SPQ-B subscales for positive symptomology and disorganised thinking. Interestingly, Bedwell, Kamath and Compton (2009) found that CPT was associated with severity scores obtained from a structured interview of schizotypal symptoms, but not with those scores from a self-report psychometric assessment of schizotypal personality dimensions. Therefore, the binary method of assessing psychotic traits may be limited in assessing the breadth and subtleties of psychosis-like experiences.

There was a lack of findings for the AST and CPT between the cannabis users and non-cannabis users. As previously discussed, there are conflicting CPT outcomes in clinical samples and non-clinical samples, and this may be due to variations in the task used across studies. Also, CPT RT was linked to the negative symptomology (for Interpersonal Deficits) as measured by the SPQ-B, and so it may be that outcomes are more pronounced in people with greater negative symptoms, as these have been more closely linked to genetic components of schizophrenia (Bassett *et al*, 1993). The AST has been less widely assessed in cannabis users. Overall, AST performance was predicted by psychosis linked personality scores, and particularly so in the cannabis using group. This suggests that these traits may constitute a liability for some of the cognitive disruption seen in schizophrenia, a finding backed up by previous research (e.g. Ettinger *et al*, 2000; Larrison *et al*, 2002).

5.3 Cannabis use variables – study one and study two

Throughout the thesis, a correlation between earlier onset of cannabis use, frequency and duration of use was assessed in relation to neuropsychological performance. In study one, the amount of cannabis used was explored and it was found that heavier users of cannabis scored higher on SPQ-B measures for positive (SPQ-CP), negative (SPQ-IP) and disorganised thinking (SPQ-DT), which supports previous research (e.g. Compton *et al*, 2009). It is well documented that cannabis use is linked to increased positive symptoms (Negrete *et al*, 1996; Skosnik *et al*, 2001; Verdoux *et al*, 2002; Skosnik *et al*, 2008) and disorganised thinking in

schizotypy (e.g. Skosnik *et al*, 2001; Dumas *et al*, 2002; Bailey and Swallow, 2004). Whereas, the findings have been less clear for the negative symptoms with some significant (e.g. Bailey and Swallow, 2004; Compton *et al*, 2009) and non significant findings (Skosnik *et al*, 2001). It is unclear whether an increase in schizotypal traits is a causal influence or consequence of cannabis use. It may be that people are predisposed to use cannabis, or that use of cannabis results in eliciting positive (i.e., hallucinations) or negative (i.e., flat affect) psychotic symptoms in heavy users (Verdoux *et al*, 2002). Age of onset of cannabis use was negatively associated with scores on SPQ-DT, with earlier onset of the drug related to higher scores on this third subscale of disorganised thinking. This differs from Compton *et al*'s (2009) study as early age of first use of cannabis was associated with interpersonal schizotypy symptoms. Compton's research, however, used two distinct testing groups (first-degree relatives of patients and non-psychiatric controls) and this therefore makes it difficult to generalise these findings against a sample of recreational cannabis users on these SPQ traits. What is clear is that earlier use of cannabis and higher frequency of use is linked to experiencing more of these psychotic-like traits, which arguably may account for why some people at are a higher risk for the development of schizophrenia (Bossong & Niesink, 2010).

In Study 2, heavy cannabis use was also correlated with poorer decision-making on the IGT, which suggests that heavier use of cannabis was linked to riskier decision making, and may help to explain why people continue to use the drug despite its potentially negative effects. Previous research by Whitlow *et al* (2004) and Hermann *et al* (2009) support this as they found significantly poorer outcomes on the IGT in those that had used cannabis heavily relative to those with lighter more partial use.

Clarity of thoughts on the trait-meta mood scale was negatively correlated with duration of cannabis use, and the first order factor of the BIS was positively correlated with joints per week (JPW). Thus longer use of cannabis was linked to having more emotional confusion and increased use of cannabis was linked to impulsivity (across the spectrum of motor, attention, cognitive complexity, perseverance, and cognitive instability). Trends existed for heavy use of cannabis to be linked with greater deficits on the AST and IGT as well as with people experiencing more psychotic traits such as ambivalence, loss of clarity of thoughts, and impulsivity. An increased amount of cannabis used per week and length of use was

positively correlated with reaction time on the AST, with heavier use of cannabis and longer duration of use both being associated with faster responding. Longer duration and heavier use were both linked to increased cognitive disruption on the decision making and inhibition tasks, as well as increased scores on psychotic-like personality traits. Taken together, this does support the general notion that more problems are seen in the heavier users and those who have taken the drug for longer periods of time (Solowij *et al*, 1998; Fletcher *et al*, 1996; Pope *et al*, 1996). Furthermore, cognitive dysfunction associated with long term or heavy use of cannabis has been proposed as possible vulnerability markers for schizophrenia (Solowij & Michie, 2007; Pope *et al*, 2001). Cannabis use and duration of use varied across the group, so it may be that if heavier cannabis users and those categorised as short-term versus long-term users were assessed as separate groups, then deficits may be more prominent for this drug group.

5.4 Summary findings of the SNP markers (DAOA, COMT, CNR1, FAAH and NRG1) in relation to study one and study two

As previously stated, numerous genetic markers have been explored in the field of schizophrenia research and increasingly alongside other risk factors such as cannabis use. Genetic susceptibility to schizophrenia is widely accepted as underpinning this illness and as acting in combination with other causative factors in the aetiology of the pathology (e.g. Van Os *et al*, 2008). As explored in sections 1.4 and 1.5, cannabis use has been clearly linked to some instances of psychotic illness and may be a possible driver of psychosis-linked changes in recreational user populations (as supported by some of the findings here). Taken together with the genetic research in this field, therefore, it is likely that any psychosis-like changes linked to cannabis use in non-pathologised populations, will be more pronounced where one or more schizophrenia-linked (psychosis-risk) genetic markers are present. With this model in mind, all participants were screened for several genetic risk markers linked to schizophrenia. A final 5 candidate schizophrenia genes (DAOA, COMT, CNR1, FAAH, NRG1 – please refer to section 4.2 for a background to these genes) and three SNP marker haplotypes from the COMT gene were assessed. The aim was to assess for potential differences in variation of these genes (i.e. SNPs) in relation to outcomes on cognitive tests from study one and two. One personality measure was also included in these analyses which assessed traits linked to schizotypal personality disorder (SPQ-B, Raine & Benishay, 1995).

In addition to allowing some evaluation of these markers in relation to possible compound effects of cannabis use and genetic risk on task/measure outcomes, the genetic analyses (presented in Chapter 4) also explored the possible contributions of these risk-markers to outcomes more generally (regardless of drug use). All the key genetic findings for Chapter 4 were discussed in detail under sections 4.8 and 4.10, and are presented above in Table 41. A short summary of some of the key findings mainly for the cannabis users, which were either significant ($p<0.05$) or leaning towards significance ($p\leq 0.15$) are discussed below.

DAOA gene

One SNP (rs142129) was assessed for the DAOA gene. In the literature T/A genotypes are more commonly found in patients with schizophrenia (Chumakov *et al*, 2012, Schumacher *et al*, 2004), and T/T genotypes more closely linked to cognitive disruption in schizophrenia (Goldberg *et al*, 2006) and methamphetamine psychosis (Kotaka *et al*, 2009). In the current study a trend for significance was found in that the T/A genotype occurred more frequently in the cannabis users, demonstrating a degree of overrepresentation of this genotype in the users which could be influencing findings across measures. Further, in the entire group (users and controls together) the T/T genotype was associated with the poorest associative learning on the LI task.

COMT

The most widely studied COMT SNP rs4680 was assessed alongside two other COMT SNPs: rs737865 and rs165599. A trend existed for the risk G/G allele in SNP rs4680 to occur much more frequently in cannabis users compared to the G/A and A/A alleles. There was no significant effect of variation of the risk SNP rs4680 across all participants with regards to scores on individual differences in psychotic-like personality traits, which does not align with previous studies (Swart *et al*, 2011; Ucok *et al*, 2010; Smyrnis *et al*, 2007; Sheldrick *et al*, 2008). Most of the literature links the rs4680 A/A allele with better cognitive performance (Bilberg *et al*, 2002; Goldberg *et al*, 2003; Jorber *et al*, 2002; Sheldrick *et al*, 2008) and the G allele with the worst cognitive performance (Bruder *et al*, 2003; Bertolino *et al*, 2006; Goldberg *et al*, 2003). SNP rs4680 has now been widely studied (see Williams *et al*, 2007) but less is known regarding the other two SNPs (rs737865 and rs165599) on the COMT gene

in relation to schizophrenia. The T/T genotypes (also known as A/A in the literature) of SNP rs737865 occurred significantly more in the cannabis group compared to the C/C genotypes (also referred to the G/G in the literature). A trend was found in the cannabis users with SNP rs737865 C/C genotypes to report higher interpersonal problems as measured on the SPQ-IP subscale compared to the T/T genotypes. Those individuals with SNP rs165599 A/A genotypes had lower SPQ reported cognitive perceptual and interpersonal problems, and those with the G/G alleles reported more problems with disorganised thinking in both the cannabis users and non-cannabis users. Cannabis users with SNP rs737865 C/C genotypes performed better on the LI task. Other trends were found in the whole group with the A allele on the COMT gene linked more to improved cognitive performance and the G allele more closely linked to cognitive disruption similar to that seen in people diagnosed with schizophrenia. For example, it was the G/A genotypes of SNP rs165599 who had poorer attentional control as they made more motor errors on the CPT. It was the T/T genotypes of SNP rs737865 who demonstrated better attentional control as they made the least errors on the AST.

Three SNPs (rs737865, rs4680, rs165599) were then assessed together as a haplotype marker. In the literature the risk haplotype (C-G-G) is positively associated with schizophrenia (De Rosse *et al*, 2006; Schifman *et al*, 2002; Handoko *et al*, 2004) and the T-A-A haplotype has also been positively associated with schizophrenia (Kotrotsou *et al*, 2012), so both are deemed to be risk markers for schizophrenia. However, in the current study the risk haplotypes T-A-A occurred in 6 participants and the C-G-G haplotypes occurred in 8 participants, so the numbers were too small to allow clearly viable statistical comparisons. Common variables amongst the cannabis group with this haplotype compared to the other COMT haplotypes were explored. The cannabis users with the risk C-G-G haplotype had the lowest use of drugs such as MDMA, amphetamine, cocaine, alcohol, tobacco, and cannabis, whereas those with the T-A-A risk haplotype had the highest use of all of these aforementioned drugs (x4 times greater use). The T-A-A risk haplotypes also had a higher number of schizotypal personality traits. Another known haplotype T-G-G was identified in 50% of the sample and compared against the rest of the sample to assess for potential differences in trait and cognitive outcomes. The T-G-G appears to be a protective haplotype with regards to psychosis (Kotrotsou *et al*, 2012). Trends existed for cannabis users with the protective haplotype to have lower disorganised thinking scores as measured by the SPQ-DT,

compared to those cannabis users without the protective haplotype. No other study has assessed this T-G-G haplotype in cannabis users, but the SPQ-DT data in the current study seems to be in line with Kotrotsou *et al*'s (2012) research, further suggesting that this haplotype has a somewhat protective effect.

NRG1

Only 3 participants in the entire sample had the risk SNP rs221533 C/C allele, with most of the group having the T/T genotype. This, therefore, contravened the criteria for the HWE and also made it difficult to draw comparisons from these data against previous studies. Please refer to Chapter 4 (see section 4.10.3) for a speculative discussion of these findings.

CNR1 gene and the FAAH gene

The majority of the entire sample had the risk SNP rs1049353 G/G allele (96%) in the CNR1 gene, so this contravened the HWE and interpretation of these data may be limited. Please refer to Chapter 4 (section 4.10.4) for speculative discussion of these findings.

There was no significant variation in the FAAH gene SNP 324420 on trait outcomes. In the whole sample, a significant difference was found between the C/C and C/A genotypes for Kamin Blocking outcomes. Those with the C/A genotype had worse associative learning compared to those with the C/C genotypes. Interestingly, the 'A' allele is linked more to a schizophrenia profile (Arias *et al*, 2010).

SNP markers and cannabis use variables

There was a trend for variation in the COMT gene SNP rs4680, with those individuals with A/A genotypes reporting the highest use of cannabis. Interestingly, this SNP has links with drug reward/addiction and related differences in the metabolism and breakdown of DA (Chen *et al*, 2004). The low enzymatic A/A genotype has previously been linked to drug dependence (Lohoff *et al*, 2008), sensation seeking in females (Lang *et al*, 2008) and a greater responsiveness to reward (Lancaster *et al*, 2012).

The ‘C’ allele is known as the schizophrenia risk allele in the NRG1 gene and in the current study those individuals with SNP rs221533 T/C genotypes reported the longest duration of cannabis use compared to the T/T genotypes. The NRG1 has recently also been put forward as a gene linked to cannabis dependence (Tan *et al*, 2012). Taken together, these findings warrant further investigation of the COMT gene and NRG1 gene for possible links to drug dependence.

Combined SNP marker effect

It was predicted that those with a profile of multiple risk SNP markers from the candidate schizophrenia genes may show greater cognitive disruption and personality symptoms in the direction towards schizophrenia-like deficits, but none of the findings reached the level of statistical significance. This lack of finding may be due to the low participant numbers when assessing cognitive performance with different tasks in Study 1 and Study 2, as opposed to using at least one similar cognitive task across both studies.

5.5 General limitations

The general limitations for this thesis were summarised in sections 2.4.5, 3.7.6 and 4.10.7 respectively. In Study 1, the non-cannabis group did not demonstrate a normal LI effect as they achieved fewer trials to criterion in the pre-exposed condition versus the non pre-exposed condition in the primary analysis. Further, the cannabis group also demonstrated better associative learning skills under the NPE condition compared to the non-cannabis group, with 35% of the non-cannabis users achieving the learning criterion compared to 80% of the cannabis users. Thus, the non-cannabis group overall had poorer performance. There may be a range of reasons for this unpredicted effect,

- No intelligence tests were administered to check for possible differences between the groups, and it would have been useful to use a measure such as the NART or Ravens matrices as a pre-test screen to match groups.
- The non-cannabis group had 3 participants who reported heavy lifetime use. Therefore, a new group of 5 non-cannabis users were tested (1 in the PE and 4 in the NPE conditions) and

this revealed a true LI effect, as the non-cannabis users were faster under the NPE condition compared to the PE condition, with 60% of the sample achieving the learning criterion.

- Most of the non-cannabis user controls were internal candidates from the UEL in the primary analysis, so it could be argued that their motivation to take part may not have been as high as those willing to travel to the university to take part in the research. However, counter to this argument is that in the secondary analysis the 5 new participants were internal candidates and this actually increased performance for the non-cannabis group, as opposed to decreasing or not affecting performance.
- The cannabis users were self-selected and the study may have created a bias in attracting higher functioning and more motivated individuals; which may have helped to widen the performance gap between users and controls. Those users with poorer cognitive functioning may have been less likely to take part for various reasons (e.g. more disorganised thought, poorer planning etc. would work against interest in and attention to volunteering), and other issues such as paranoia could be problematic for this recruitment method as well. Future alternative recruitment strategies could be explored, such as online interest groups and a direct approach.

There was a gender imbalance in study one with more males in the cannabis using group in both the primary and secondary analysis. This gender difference may have impacted on the findings, as previous research by Kaplan & Lubow (2010) indicated that low schizotypal healthy males, but not females, exhibited LI. In Study 2, there were a significantly greater number of males in the cannabis group. However the regression models were adjusted for sex and this factor did not predict performance on any of the cognitive assessments.

The COMT gene SNP rs737865, CNR1 gene SNP rs1049353 and NRG1 gene SNP rs221533 all contravened the HWE. To overcome this issue a future replication would need more participants to increase the power of the study. The aim of the genetic analysis was not to assess for gender or ethnic differences but given the link these factors have on genetic expression of some of these SNPs, this cannot be ruled out of the findings. Due to the already small cell sizes in these data (when divided by cannabis use and genotype) further breakdowns or covariance using other variables such as gender and/or ethnicity would weaken the statistical validity of tests even further. However, if the genetic research would

be replicated in much larger cohorts, then these would be important additional variables to allow for inter analyses and grouping of data.

The cannabis users in this current study abstained for at least two days (at least 48h). Those cannabis users scoring in the range for dependency was not linked to cognitive performance, both in Study 1 and 2 (see sections 2.3.9 and 3.6.5). However, it could be argued that differences found between the cannabis users and non users on the trait and cognitive data could be residual effects from cannabis. Residual effects are difficult to assess (Whitlow *et al*, 2004) and Pope *et al* (1995) argue that such effects may be due to the presence of drug residue, either a dopamine agonist, or metabolites, which continue to have an intoxication effect for several hours (Grotenhermen, 2003; Crean *et al*, 2012) after peak acute effects. A residual effect can also indicate that even after complete elimination of the dopamine agonist-like effect of THC there are still changes that can persist, which indicate that there may be ongoing neuroadaptions (e.g. persistent changes that remain and are caused by the continued drug use). Or alternatively differences found could be due to withdrawal effects, such as irritability, negative affect and/or aggressiveness (Kouri *et al*, 1999). Withdrawal symptoms are seen to first appear after 24h abstinence from cannabis use (Budney *et al*, 2003), but the current drug users in both studies were able to abstain for two days without any difficulty; and those unwilling (or unable) to abstain made this known to the researcher and were not included in the study. Most studies have an abstinence period of 12-72h and therefore it is difficult to make firm conclusions about residual or withdrawal effects, because of this variability in research protocols.

It is clear that many cannabis users experiment with other drugs and in this current study they reported a greater degree of past and current polydrug use. Cannabis users frequently reported use of other party drugs such as cocaine, amphetamine and high use of MDMA, all which are neurotoxic and impact on cognition (e.g. Rogers & Robbins, 2001; Gouzoulis-Mayfrank & Daumann, 2006). Therefore, it is difficult to draw conclusions about the sole impact of cannabis, especially as elevated psychopathology is associated with polydrug use in general (e.g. Parrott et al, 2001), and as such it is possible that other drug use or polydrug use *per se* may be a key reason for cognitive disruption, as opposed to cannabis use alone (e.g. Croft *et al*, 2001). For example, Soar *et al* (2015) have very recently demonstrated that LI is

disrupted by recreational cocaine use; although cocaine use levels were higher and generally much more recent in this sample than the cohorts studied for this thesis. It should also be stressed that in the current cohorts cannabis was the most commonly used drug, and polydrug use was far more varied, such that the impact on the data of any single drug other than cannabis was likely to be minimal. Whilst some of the cannabis users here had used cocaine, all of the cocaine users in Soar *et al*'s study were also cannabis users.

Moreover, there are limitations to having just a two-group comparisons (between the cannabis users and non- cannabis users), which makes it difficult to assess the true impact of cannabis use, especially as cannabis use is varied within this drug group as well high polydrug use. A way to overcome this for future research would be to use a three-group comparator: 1) cannabis use and high polydrug use, 2) cannabis use with low polydrug use, and 3) a non-drug using sample (with little party drug history).

5.6 Interpretation of the findings

Regular cannabis use (particularly heavier use) was linked to higher schizotypal personality traits and cognitive dysfunction which parallels some problems seen in people diagnosed with schizophrenia. A cursory comparison with extant data was carried out to briefly examine the mean scores on those tasks in the current study which were affected by cannabis use (namely LI, the IGT and SPQ-B data). The cannabis users' and non-cannabis users' mean scores for the SPQ-B data were lower than those from the original SPQ-B data from Raine & Benishay (1995) for non clinical samples. The means scores were however similar to Compton *et al's* (2009) large study of non clinical samples. Studies with clinical samples do show higher mean scores across different psychiatric groups, namely substance use disorder and personality disorder (e.g. Axelrod *et al*, 2001) in comparison to the current cohorts.

Normative data does not exist for the IGT (Evans *et al*, 2004) and a comparison to other studies proved to be difficult due to the variations in testing procedures, such as different instructions to participants, number of trials, analysis by gender, analysis of performance in terms of percentage of choosing advantageous cards versus a net score, or total winnings,

analysis by deck, use of real versus virtual cards, use of real versus virtual money (Fernie & Tunnet, 2006; Overman *et al*, 2013). Studies which assessed decision making on the IGT in people diagnosed with schizophrenia generally indicate that decision making is poor when an overall average loss has been made (Sevy *et al*, 2007; Ritter *et al*, 2003). In the current study cannabis users made significantly more risk based decisions on the IGT compared to non-cannabis users, and their performance was akin to the performance of data taken from clinical samples, such as those with schizophrenia (e.g. Yang-Tae *et al*, 2012) and psychopathy (e.g. Morgan *et al*, 2011). The cannabis users' decision-making on the IGT was poorer than other research studies assessing the difference between cannabis users and controls (see Gonzalez *et al*, 2012). It is notable that the controls also demonstrated poorer decision making on the IGT, and their performance did not match that of healthy controls in previous research (e.g. Kim *et al*, 2012; Penolazzi *et al*, 2013).

Comparing the mean scores from the LI data to other research yielded some difficulty, as previous researchers have used different versions of the LI task, such as within-participants designs (e.g. Lubow *et al*, 2007), and others using between participants (e.g. Serra *et al*, 2001); some have used the median value in the data analysis (e.g. Gal *et al*, 2009) as opposed to the mean for correct number of trials. In the current LI study, cannabis users were less affected by the white noise and performed better (i.e. lower mean for correct number of trials) in the PE condition than clinical samples and controls when compared to Serra *et al's* 2001 research. The controls in Serra *et al's* study performed better than the cannabis users in the current study for basic associative learning under the NPE condition. The cannabis users performance seemed to be better than most of the published research in clinical samples (e.g. Baruch *et al*, 1988; Granger *et al*, 2012*;* Gray *et al*, 1992), healthy controls (e.g. Kaplan & Lubow, 2011) and in those with schizotypal traits (e.g. Baruch *et al*, 1988b) for those placed under the PE condition where it should normally take longer to find the association between the white noise and counter incrementing. One argument to explain this would be a self-selection bias in the cannabis users, in that this cohort would be very relaxed, intelligent and open minded. The cohort might be very motivated to take part in this study and analytical enough to be trying to figure out what the study is about whilst taking part. Post-test interviews would uncover such an effect perhaps, such as self-rated motivation scales pre and post testing (to see if levels are maintained and how these compare to controls) or an intelligence baseline measure to control for this. Cannabis was the user's first drug of choice

and this would distinguish this type of drug users from other drug users (cocaine, MDMA etc.) in that they may have be more relaxed individuals (as they prefer to stay at home to get stoned as opposed to using party drugs) and/or have a distinct personality type (e.g. highly creative). Interestingly, the performance in cannabis users mimic the performance seen in those deemed to be high in creativity, with decreased LI seen in those deemed high in creative achievement (Carson *et al*, 2003), which also ties into the previous argument about possible cohort characteristic differences. There is growing evidence of a link between creativity and schizophrenia; a recently published study by Power *et al* (2015) found that people in a creative profession were 25% more likely to carry DNA variants linked to schizophrenia.

The precise biological explanations as to why cannabis use might increase risk for schizophrenia is yet to be established. A key argument from the review of the literature presented here (see Chapter 1) is that THC (a partial agonist) acts on CB_1 receptors in the areas of the brain which have been described as a 'neural circuitry of psychosis', including frontal regions, basal ganglia, hippocampus, anterior cingulate and cerebellum. Cannabinoids modulate the release of a number of neurotransmitters implicated in psychosis, namely DA, glutamate and GABA. Cannabis users may experience more schizotypal traits and cognitive disruption due to the effect cannabinoids have on these transmitter systems (see section 1.1.1). DA has been linked to positive symptoms in schizophrenia (Davis *et al*, 1991) and DA, glutamate and GABA have all been linked to normal and dysfunctional higher order cognitive processes (Goldman-Rakic, 1996; Robbins, 2000). The interaction between cannabinoids and DA, GABA and glutamate may help to explain the acute effects cannabis has on acute positive, negative and cognitive symptoms of schizophrenia. Less is clear is the mechanism of how exposure to cannabis may exacerbate the risk of developing schizophrenia (Radhakirshnan *et al*, 2014). One of the key arguments is that schizophrenia is a neurodevelopmental disorder (Weinberger, 1996, Rapoport *et al*, 2005) and that exposure to cannabinoids during adolescence and young adulthood during critical phases for cerebral development (up to 25 years) increases the risk for psychotic-like behaviours, as has been demonstrated in animal (O'Shea *et al*, 2004; Cha *et al*, 2006; Schneider *et al*, 2008) and human epidemiological studies (Andreasson *et al*, 1987; Arsenault *et al*, 2002; Zammit *et al*, 2002; Caspi *et al*, 2005; De Forti *et al*, 2015). In the current research, the average age of cannabis first use (onset) was 15. Therefore, it could be argued that the individual

differences in psychotic-like personality traits and cognitive disruption in cannabis users and non users are a result of early exposure to cannabis and the continued use of the drug, which results in being closer on the schizophrenia-spectrum compared to those who do not use the drug, or those who may have started use much later in life (though some additional work would be needed to explore this later notion in more detail). Alternatively, or additionally, it could be argued that the cognitive decline from cannabis use may enhance the vulnerability for schizophrenia.

It may be that smoking cannabis elevates schizotypy over time, which would then alone or in combination with other cognitive effects of THC, produce alterations in task performance. It is clear that those areas rich in CB_1 receptors correlate to increased cognitive dysfunction (e.g. hippocampal regions = short/long term memory problems; frontal regions = executive dysfunction; Solowij & Michie, 2007), so naturally one would expect more cognitive problems in heavy users compared to non drug using controls. The issue of whether the cognitive effects observed here are linked to psychosis-like changes rather than more general cannabis-related cognitive changes is difficult to disentangle. Cognitive disruption in normal cannabis users for most of the cannabis–psychosis studies presented in Chapter 1 is under researched. More research needs to be done to look at convergence in the data that might more clearly disambiguate cognitive impact more attributable to direct effects of cannabis on general cognitive functioning from effects more linked to a specific schizogenic process. The most prominent effects of cannabis on cognition are those associated with acute intoxication (Hart *et al,* 2001), with chronic effects less consistently proven in all but the heaviest of users and polydrug cannabis users (Solowij & Battisti, 2008; Thames *et al,* 2014). Therefore if there is a convergence in the data towards long term effects of cannabis being seen more selectively in cognitive domains that overlap schizophrenia/psychosis spectrums or symptoms, then this would suggest the cognitive effects might be secondary or most closely aligned to some form of schizogenic process.

5.7 Suggestions for future research

Clearly longitudinal data would be needed to monitor which comes first, cannabis use, schizotypy score changes or task changes; and thus would disentangle some of the complexities discussed above. New repeat studies are needed, controlling for a range of additional variables (gender, IQ, personality traits and possibly motivation) and using bigger samples, and focussing on the most interesting gene marker outcomes (e.g. the protective haplotype data which, as a first finding, needs replication). To help address the issue of cognitive disruption representing more schizophrenia-like responses as opposed to general cognitive deficits, it would be important to administer a more thorough battery of cognitive assessments, to see if the users are more generally impaired. The findings in this thesis generally, but also this issue of cognitive confounds, also suggest a need for neuroimaging work to see whether cannabis use is more preferentially impacting upon the neural systems currently known to be affected by schizophrenia.

The multicomponent model of cannabis use and schizophrenia presented in Chapter 1 (see Figure 1) is partially supported by the data in thesis (see summary of data Table 40 & 41). Particularly in support of this would be the research on cannabis use variables (e.g. early use and heavy use of cannabis) is linked to an increase in psychotic personality traits and greater cognitive disruption, which is pushing individuals higher up the spectrum of risk of subclinical symptoms compared to the non-cannabis users with a similar genetic make-up. For example, cannabis users reported more schizophrenia-like traits on measures of: paranoia, ambivalence, schizotypy, lacking emotion clarity. Further to this, heavy cannabis use was associated with higher schizotypal traits and impulsive traits and riskier decision-making (as measured by the IGT). Earlier onset of cannabis use was associated with higher scores on the disorganised thinking subscale of the SPQ-B measure, Cannabis users also demonstrated schizophrenia-like behaviours in that they showed abolished Latent Inhibition and riskier decision making compared to the non-cannabis users, The COMT haplotype data was interesting in relation disorganised thinking traits (the symptoms which are seen as being more closely linked to the genetic component of schizophrenia, Bakker *et al,* 2004); the cannabis users with the protective T-G-G haplotype had lower disorganised traits compared to the cannabis users without this protective haplotype. It is not clear what these protective factors would represent, but for the COMT haplotype, it may have an involvement in how

people react to their external environment. The COMT gene is involved in the breakdown of dopamine, so this haplotype and the known impact dopamine has on brain functions (in frontal cortex) may be linked to motivation, how people react to stressful situations, positive negative drug reinforcement, inhibition and self-regulation (Luciana *et al*, 2012). The multicomponent model does not address protective factors in the model, as it represents the negative factors in risk for developing schizophrenia, as opposed to also looking at what protects individuals. The psychosocial factors were not addressed in this thesis, but future research could be explored (in conjunction with this protective haplotype) to see what social factors help to protect individuals from risk of subclinical to full blown schizophrenia. One idea would be to explore additional factors such as: lifetime and current social stressors, positive and negative life experiences, assess coping strategies, diet and nutrition; all of this would be explored in relation to genetics, individual differences in personality traits (namely schizotypy and impulsivity), cognition and drug use history (using a 3-group comparison of: controls – no cannabis or club drug history, cannabis users with little polydrug use, and polydrug users) to further explore the link between cannabis use and schizophrenia in relation to the known risks and potential new protective factors.

From a clinical perspective research is progressing in this area and it is unlikely that there is a single cause for schizophrenia as it is made up of an array of positive, negative and disorganised symptoms as well as cognitive disruption. In line with Radhakrishnan *et al* (2014), it is likely that cannabis-induced psychotic disorder may emerge as a sub-type of what is currently being diagnosed broadly as schizophrenia. Within a clinical setting, those individuals seeking treatment for acute schizophrenia are generally assessed on improving symptoms, as opposed to also viewing cognitive disruption as a treatment target. If cannabis-induced psychotic disorder was treated as a sub-type, then more specific treatment strategies could be used to reduce the positive, negative and disorganised traits. Sofuoglu *et al*'s (2013) research model is an example of emerging research looking to improve cognition as a treatment goal for drug addiction/problems. Therefore, in parallel to drugs education and prevention for harm reduction, particularly amongst those at-risk individuals aged between 14-25 years, future cannabis research should also assess factors which help improve cognition to reduce psychotic symptoms (or vice versa), as a preventative measure against psychosis.

5.8 Summary

This current study is unique in its attempt to explore the cannabis-schizophrenia link using non-clinical recreational cannabis users, assessing cognitive functioning in psychosis-sensitive tasks, exploring trait markers that have been strongly linked to psychosis and additionally looking at schizophrenia-linked genetic markers. Furthermore, these genetic profiles were explored in relation to psychotic-like traits and behavioural outcomes for levels of attention, decision making, associative learning, and executive control; all of which are risk markers for schizophrenia. The overall aim of the thesis was to explore sub-schizophrenia like psychology in a group of cannabis users versus non-cannabis users. Some of the key findings of the thesis were highlighted in Table 40 & 41 above.

In sum, it seems that cannabis users showed abolished Latent Inhibition which resembles the performance of those in the acute stages of schizophrenia. Cannabis users demonstrated riskier decision making strategies on the IGT. Heavier use of cannabis was associated with poorer decision making and higher schizotypal traits. Cannabis users scored significantly higher on a range of psychotic-like trait measures assessing three subscales of the SPQ-B measure for positive, negative and disorganised traits, as well as higher paranoia, ambivalence, and impulsivity. Longer duration and heavier use of cannabis also exacerbated these traits. Genetics research exploring the cannabis-psychosis link is a relatively new area but some of the findings here with SNPs deemed as risk markers were in line with previous research linking these with cognitive disruption and also linking to individual differences in psychotic-like traits (see section 5.4 above). Of particular interest was the T-G-G protective haplotype in the COMT gene. Cannabis users with the protective haplotype had lower SPQ-DT scores (suggesting this haplotype may reduce negative symptoms) compared to non-cannabis users. This is the first study to demonstrate this T-G-G haplotype protective effect in cannabis users.

Taken together the work in this thesis supports existing evidence that cannabis use, in non-dependent and non-pathologised users, may be having a significant impact on everyday psychological functioning. These effects show some overlap with symptoms associated with

psychosis/schizophrenia and could be argued to support the growing evidence of a link between this drug and this disorder. More profoundly these data lend some support to the central hypothesis of this thesis, that recreational cannabis use whilst unlikely to produce schizophrenic disorder in the vast majority of users, may nonetheless contribute to psychosis-like changes; the impact of which, on daily living, is largely so far unexplored. As such the current findings demand further research to inform the literature, clinical practitioners, communities, policy and most importantly recreational cannabis users themselves.

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APPENDICES

Appendix i: Information sheet - Study 1

Principal Investigator	Director of Studies	Research Supervisor	Research Supervisor
Stephanie Lynch School of Psychology University of East London, Romford Road, London, E15 4LZ	Dr John Turner School of Psychology University of East London	Dr Kirstie Soar School of Psychology University of East London	Dr Lynne Dawkins School of Psychology University of East London
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Latent Inhibition, Kamin Blocking and Cannabis

The study you have been asked to contribute to aims to assess whether drug users' show a similar pattern of results to those found in people who have developed psychosis. The study involves volunteering to provide a small sample of your DNA, which is done through the use of a standard cheek-swab to extract a saliva sample. Furthermore, you will be asked to complete two cognitive based associative-learning tasks; a number of different questionnaires assessing demographic details, personal and family psychiatric history; your patterns of recreational drug use; your general health and different aspects of psychotic personality traits. The study aims to assess potential differences in those who use certain recreational drugs such as cannabis compared with those that don't, therefore you do not have to have used recreational drugs to contribute to the research.

Confidentiality of the Data

Confidentiality will be ensured, all personal information and questionnaire data will be anonymous and only identifiable by a unique participant code kept separately from your consent form. Upon completion of the study all data and contact details will be shredded and disposed of as confidential waste.

Disclaimer

Participants are not obliged to take part in this study and are free to withdraw at any stage. Should they choose to withdraw from the programme they may do so without personal disadvantage and without any obligation to give a reason. Please note that the nature of the study in no way implies that the University of East London condones the use of recreational drug.

University Research Ethics Committee

If you have any queries regarding the conduct of the programme in which you are being asked to participate please contact the Secretary of the University Research Ethics Committee: Debbie Dada, Graduate School, University of East London, Docklands Campus, 4-6 University Way, London E16 2RD (telephone 0208 223 2976, e-mail d.dada@uel.ac.uk)

Appendix ii: Consent Form – Study 1

Latent Inhibition, Kamin Blocking and Cannabis

I confirm that I have read and understand the information sheet for the above study, understand the nature and purpose of the research and have had the opportunity to ask questions.
Please tick box ☐

I understand that my participation is voluntary and I am free to withdraw at any time without disadvantage to myself and without being obliged to give any reason.
Please tick box ☐

I understand that I will volunteer for a cheek-swab to be administered which extracts a saliva-sample of my DNA.
Please tick box ☐

I will perform two separate cognitive tests, and will be asked to answer a number of questions concerning ones personal history, including mental health issues, levels of drug use and questions assessing aspects of ones personality.
Please tick box ☐

I understand that my involvement in this study, and particular data from this research, will remain strictly confidential and any data will only be identifiable by a unique participant code.
Please tick box ☐

I understand that this consent form will not be linked with my data
Please tick box ☐

I understand that the research team will not be able to provide feedback on my DNA information, cognitive assessment performance and questionnaire scores.
Please tick box ☐

I hereby fully and freely consent to participate in this study which has been fully explained to me:

Signature of Participant _____ Date _____

Signature of researcher _____ Date _____

Appendix iii: Debriefing Sheet - Study One

Latent Inhibition, Kamin Blocking and Cannabis

Thank you for taking the time to participate in our study. Your time and effort is very much appreciated.
If participation in the study has raised concerns for you about your drug use or about your mood you might find it helpful to discuss these concerns your general practitioner or you can also contact NHS Direct on 0845 4647 or www.nhsdirect.nhs.org.uk
There are also a number of voluntary agencies that may be able to help you, including:

Addaction (www.addaction.org.uk)

Drugscope (www.drugscope.org.uk)

Drugsline (www.Drugsline.org) 0808 1 606 606

MIND (www.mind.org.uk) 0845 766 0163 open Monday to Friday 9.15 am – 5.15 pm

Sane (www.sane.org.uk) 0845 767 8000

The Samaritans (www.samaritans.org) 08457 90 90 90 - open 24 hours

PsychNet-UK(www.psychnet-uk.com): 0845 122 8622

The National Drugs Helpline (www.talktofrank.com): 0800 776600 Free help and advice 24 hours a day, seven days a week.

Appendix iv: Severity of Dependence Scale Modified for Cannabis Use

SEVERITY OF DEPENDENCE SCALE FOR CANNABIS USE

Please circle the answer which is most relevant to you!

1. Did you ever think your use of cannabis was out of control?

Never or almost never	0	Sometimes	1
Often	2	Always or nearly always	3

2. Did the prospect of missing a smoke make you very anxious or worried?

Never or almost never	0	Sometimes	1
Often	2	Always or nearly always	3

3. Did you worry about your use of cannabis?

Not at all	0	A little	1
Quite a lot	2	A great deal	3

4. Did you wish you could stop?

Never or almost never	0	Sometimes	1
Often	2	Always or nearly always	3

5. How difficult would you find it to stop or go without cannabis?

Not difficult	0	Quite difficult	1
Very difficult	2	Impossible	3

Appendix v: Schizotypal Personality Questionnaire – B

SPQ-B

NAME ☐ SEX ☐ AGE ☐

Please answer each item by clicking Y (Yes) or N (No). Answer all items even if unsure of your answer. When you have finished, check over each one to make sure you have answered them all.

1. People sometimes find me aloof and distant. Y ☐ N ☐

2. Have you ever had the sense that some person or force is around you, even though you cannot see anyone? Y ☐ N ☐

3. People sometimes comment on my unusual mannerisms and habits. Y ☐ N ☐

4. Are you sometimes sure that other people can tell what you are thinking? Y ☐ N ☐

5. Have you ever noticed a common event or object that seemed to be a special sign for you? Y ☐ N ☐

6. Some people think that I am a very bizarre person. Y ☐ N ☐

7. I feel I have to be on my guard even with friends. Y ☐ N ☐

8. Some people find me a bit vague and elusive during a conversation. Y ☐ N ☐

9. Do you often pick up hidden threats or put-downs from what people say or do? Y ☐ N ☐

10. When shopping do you get the feeling that other people are taking notice of you? Y ☐ N ☐

11. I feel very uncomfortable in social situations involving unfamiliar people. Y ☐ N ☐

12. Have you had experiences with astrology, seeing the future, UFOs, ESP or a sixth sense? Y ☐ N ☐

13. I sometimes use words in unusual ways. Y☐ N☐

14. Have you found that it is best not to let other people know too much about you? Y☐ N☐

15. I tend to keep in the background on social occasions. Y☐ N☐

16. Do you ever suddenly feel distracted by distant sounds that you are not normally aware of? Y☐ N☐

17. Do you often have to keep an eye out to stop people from taking advantage of you?

Y☐ N☐

18. Do you feel that you are unable to get "close" to people? Y☐ N☐

19. I am an odd, unusual person. Y☐ N☐

20. I find it hard to communicate clearly what I want to say to people. Y☐ N☐

21. I feel very uneasy talking to people I do not know well. Y☐ N☐

22. I tend to keep my feelings to myself. Y☐ N☐

Appendix vi: UEL - Personal/ Familial health questionnaire and current/lifetime drug use

PERSONAL HISTORY

Age_____ **Gender**_____

Age left education _____ **Nationality**_____

Occupation _____ **Ethnicity** _____

	Bad	Moderate	Fine	Good
Current rating of health	1	2	3	4

Have you ever been clinically diagnosed (by a Doctor) with any of the following?

Anxiety	Yes ☐	No ☐
Depression	Yes ☐	No ☐
OCD (Obsessive Compulsive Disorder)	Yes ☐	No ☐
Schizophrenia or Paranoia	Yes ☐	No ☐
Phobia	Yes ☐	No ☐
Panic attacks	Yes ☐	No ☐
Eating disorders	Yes ☐	No ☐
Alcohol or Drug dependency	Yes ☐	No ☐

Have any member of your immediate family ever been diagnosed (by a Doctor) with any of the following?

Anxiety	Yes ☐	No ☐
Depression	Yes ☐	No ☐
OCD (Obsessive Compulsive disorder)	Yes ☐	No ☐
Schizophrenia or Paranoia	Yes ☐	No ☐
Phobia	Yes ☐	No ☐
Panic attacks	Yes ☐	No ☐
Eating disorders	Yes ☐	No ☐
Alcohol or Drug dependency	Yes ☐	No ☐

Have you ever been hospitalised for any brain injury?

Yes ☐ No ☐

Are you on any current medication?

Yes ☐ No ☐

If yes, what is this medication prescribed for?

_____
_____
_____
_____
_____

Have you taken the following substances?

Ecstasy/MDMA No ☐ Yes ☐

If yes,

Approximately what age where you when you first tried this substance? ☐☐ years

Approximately how many times have you taken it? _____

Approximately how long ago was the last time you took it? _____

Amphetamine No ☐ Yes ☐

If yes,

Approximately what age where you when you first tried this substance? ☐☐ years

Approximately how many times have you taken it? _____

Approximately how long ago was the last time you took it? _____

Cocaine No ☐ Yes ☐

If yes,

Approximately what age where you when you first tried this substance? ☐☐ years

Approximately how many times have you taken it? _____

Approximately how long ago was the last time you took it? _____

LSD No ☐ Yes ☐

If yes,

Approximately what age where you when you first tried this substance? ☐☐ years

Approximately how many times have you taken it? _____

Approximately how long ago was the last time you took it? _____

Magic Mushrooms No ☐ Yes ☐

If yes,

Approximately what age where you when you first tried this substance? ☐☐ years

Approximately how many times have you taken it? _____

Approximately how long ago was the last time you took it? _____

Poppers No ☐ Yes ☐

If yes,

Approximately what age where you when you first tried this substance? ☐☐ years

Approximately how many times have you taken it? _____

Approximately how long ago was the last time you took it? _____

Ketamine No ☐ Yes ☐

If yes,

Approximately what age where you when you first tried this substance? ☐☐ years

Approximately how many times have you taken it? _____

Approximately how long ago was the last time you took it? _____

GHB (liquid ecstasy) No ☐ Yes ☐

If yes,

Approximately what age where you when you first tried this substance? ☐☐ years

Approximately how many times have you taken it? _____

Approximately how long ago was the last time you took it? _____

Prozac (not prescribed)

No ☐ Yes ☐

If yes,

Approximately what age where you when you first tried this substance? ☐☐ years

Approximately how many times have you taken it? _____

Approximately how long ago was the last time you took it? _____

Crack

No ☐ Yes ☐

If yes,

Approximately what age where you when you first tried this substance? ☐☐ years

Approximately how many times have you taken it? _____

Approximately how long ago was the last time you took it? _____

Opiates (e.g. (Heroin morphine)

No ☐ Yes ☐

If yes,

Approximately what age where you when you first tried this substance? ☐☐ years

Approximately how many times have you taken it? _____

Approximately how long ago was the last time you took it? _____

Benzodiazepines (e.g. Valium)

No ☐ Yes ☐

If yes,

Approximately what age where you when you first tried this substance? ☐☐ years

Approximately how many times have you taken it? _____

Approximately how long ago was the last time you took it? _____

	No	Yes	
Anabolic Steroids	☐	☐	If yes, Approximately what age where you when you first tried this substance? ☐☐ years Approximately how many times have you taken it? _____ Approximately how long ago was the last time you took it? _____
Solvents	☐	☐	If yes, Approximately what age where you when you first tried this substance? ☐☐ years Approximately how many times have you taken it? _____ Approximately how long ago was the last time you took it? _____

NOTES:

Any unusual experiences from using the substances listed thus far. If yes, please specify:

_____
_____
_____
_____
_____
_____
_____
_____
_____

OTHERS DRUGS USED.

Please specify any other substances you may have used recreationally which are not listed above and indicate how many times you have taken them and how long it is since your last use of them:

1. _____

If yes,

Approximately what age where you when you first tried this substance?
☐☐ years

Approximately how many times have you taken it? _____

Approximately how long ago was the last time you took it? _____

2. _____

If yes,

Approximately what age where you when you first tried this substance?
☐☐ years

Approximately how many times have you taken it? _____

Approximately how long ago was the last time you took it? _____

Cannabis

Have you smoked cannabis? No ☐ Yes ☐
If no, please go to the next section.

Which type(s) of cannabis do you smoke?
Start in the order of the type you use most frequently:
(a)_____ (b) _____

(c) _____ (d) _____

HOW MANY JOINTS DO YOU SMOKE PER DAY? _____
How many days in the week do you smoke cannabis?

☐ everyday ☐ almost everyday ☐ 3 - 4 times per week

☐ 1 - 2 times per week ☐ 2 - 3 times per month ☐ once a month

☐ less than once a month

Age when you first smoked cannabis:☐☐ years

Who introduced you to using the drug? _____

HOW MANY YEARS HAVE YOU SMOKED CANNABIS FOR: ☐☐ YEARS

HOW LONG IS IT SINCE YOU LAST SMOKED IT?_____
Any acute psychological / health problems from using higher doses of cannabis? If yes, please specify**:**
_____
_____
_____
_____
_____
_____

Tobacco

Do you smoke tobacco? No ☐ Yes ☐

IF YES, HOW MANY CIGARETTES DO YOU SMOKE PER DAY ON AVERAGE?_____
What age did you first start smoking regularly: ☐☐ years
(daily smoking, or smoking regularly per week)

Which brand do you smoke most often? _____

Approximately when is the last time you smoked tobacco? _____

Alcohol

Do you drink alcohol? No ☐ Yes ☐

IF YES, HOW MANY UNITS OF ALCOHOL DO YOU DRINK IN A TYPICAL WEEK?_____
What age did you first start drinking regularly: ☐☐ years
(typically consuming 2 or more units per week)

Which type(s) of drink do you consume most often?

(a) _____
(b) _____
(c) _____
(d) _____

Approximately when is the last time you consumed alcohol? _____

THANK YOU

Appendix vii: Green et al's Paranoid Thoughts Scale (Part A & B)

Please read each of the statements carefully. They refer to thoughts and feelings you may have had about others over the last month.
Think about the last month and indicate the extent of these feelings from
1 (Not at all) to 5 (Totally).
Please complete both Part A and Part B.
(N.B. Please do not rate items according to any experiences you may have had under the influence of drugs.)

Part A	**Not at all**	**Somewhat**	**Totally**
1. I spent time thinking about friends gossiping about me	**1**	**2 3 4**	**5**
2. I often heard people referring to me	**1**	**2 3 4**	**5**
3. I have been upset by friends and colleagues judging me critically	**1**	**2 3 4**	**5**
4. People definitely laughed at me behind my back	**1**	**2 3 4**	**5**
5. I have been thinking a lot about people avoiding me	**1**	**2 3 4**	**5**
6. People have been dropping hints for me	**1**	**2 3 4**	**5**
7. I believed that certain people were not what they seemed	**1**	**2 3 4**	**5**
8. People talking about me behind my back upset me	**1**	**2 3 4**	**5**
9. I was convinced that people were singling me out	**1**	**2 3 4**	**5**
10. I was certain that people have followed me	**1**	**2 3 4**	**5**
11. Certain people were hostile towards me personally	**1**	**2 3 4**	**5**
12. People have been checking up on me	**1**	**2 3 4**	**5**
13. I was stressed out by people watching me	**1**	**2 3 4**	**5**
14. I was frustrated by people laughing at me	**1**	**2 3 4**	**5**
15. I was worried by people's undue interest in me	**1**	**2 3 4**	**5**
16. It was hard to stop thinking about people talking about me behind my back	**1**	**2 3 4**	**5**

Part B	Not at all	Somewhat			Totally
1. Certain individuals have had it in for me	1	2	3	4	5
2. I have definitely been persecuted	1	2	3	4	5
3. People have intended me harm	1	2	3	4	5
4. People wanted me to feel threatened, so they stared at me	1	2	3	4	5
5. I was sure certain people did things in order to annoy me	1	2	3	4	5
6. I was convinced there was a conspiracy against me	1	2	3	4	5
7. I was sure someone wanted to hurt me	1	2	3	4	5
8. I was distressed by people wanting to harm me in some way	1	2	3	4	5
9. I was preoccupied with thoughts of people trying to upset me deliberately	1	2	3	4	5
10. I couldn't stop thinking about people wanting to confuse	1	2	3	4	5
11. I was distressed by being persecuted	1	2	3	4	5
12. I was annoyed because others wanted to deliberately upset me	1	2	3	4	5
13. The thought that people were persecuting me played on my mind	1	2	3	4	5
14. It was difficult to stop thinking about people wanting to make me feel bad	1	2	3	4	5
15. People have been hostile towards me on purpose	1	2	3	4	5
16. I was angry that someone wanted to hurt me	1	2	3	4	5

Appendix viii: Trait Meta Mood Scale

Please read each statement and decide whether or not you agree with it by adding a tick (✓) to one box					
	5 = Strongly agree	4 = Somewhat agree	3 = Neither agree nor disagree	2 = Somewhat disagree	1 = Strongly disagree
1. I try to think good thoughts now matter how badly I feel.					
2. People would be better off if they felt less and thought more.					
3. I don't think it's worth paying attention to your emotions or moods.					
4. I don't usually care much about what I'm feeling.					
5. Sometimes I can't tell what my feelings are.					
6. I am rarely confused about what my feelings are.					
7. Feelings give direction to life.					
8. Although I am sometimes sad, I have a mostly optimistic outlook.					
9. When I am upset I realize that the "good things in life" are illusions.					
10. I believe in acting from the heart.					
11. I can never tell how I feel.					
12. The best way for me to handle my feelings is to experience them to the fullest.					
13. When I become upset I remind myself of all the pleasures in life.					
14. My belief and opinions always seem to change depending on how I feel.					
15. I am often aware of my feelings on a matter.					
16. I am usually confused about how I feel.					
17. One should never be guided by emotions.					
18. I never give into my emotions.					
19. Although I am sometimes happy, I have a mostly pessimistic outlook.					
20. I feel at ease about my emotions.					
21. I pay a lot of attention to how I feel.					
22. I can't make sense out of my feelings.					
23. I don't pay much attention to my feelings.					
24. I often think about my feelings.					
25. I am usually very clear about my feelings.					
26. No matter how badly I feel, I try to think about pleasant things.					
27. Feelings are a weakness humans have.					
28. I usually know my feelings about a matter.					
29. It is usually a waste of time to think about your emotions.					
30. I almost always know exactly how I am feeling					

Appendix ix: Schizotypal Ambivalence Scale

Please answer each item by circling T (True) or F (False). Answer all items even if unsure of your answer. When you have finished, check over each one to make sure you have answered them all.

1)	T	F	Often I feel like I hate even my favorite activities.
2)	T	F	My thoughts and feelings always seem to be contradictory.
3)	T	F	My feelings about my worth as a person are constantly changing back and forth.
4)	T	F	Very often when I feel like doing something, at the same time I don't feel like doing it.
5)	T	F	When I am trying to make a decision, it almost feels like I am physically switching from side to side.
6)	T	F	It's impossible to know how you feel because the people around you are constantly changing.
7)	T	F	I always seem to be the most unsure of myself at the same time that I am most confident of myself.
8)	T	F	I always seem to have difficulty deciding what I would like to do.
9)	T	F	Most people seem to know what they're feeling more easily than I do.
10)	T	F	Love and hate tend to go together.
11)	T	F	Love never seems to last very long.
12)	T	F	The closer I get to people, the more I am annoyed by their faults.
13)	T	F	Everyone has a lot of hidden resentment toward his or her loved ones.
14)	T	F	I have noticed that feelings of tenderness often turn into feelings of anger.
15)	T	F	My experiences with love have always been mixed with great frustrations.
16)	T	F	I usually find that feelings of hate will interfere when I have grown to love someone.
17)	T	F	A sense of shame has often made it difficult to accept complements from others.
18)	T	F	I usually experience doubt when I finish something that I have worked on for a long time.
19)	T	F	I doubt if I can ever be sure exactly what my true interests are.

Appendix x: Barratt Impulsivity Scale –II

DIRECTIONS: People differ in the ways they act and think in different situations.
This is a test to measure some of the ways in which you act and think. Read each statement and put an X on the appropriate circle on the right side of this page.
Do not spend too much time on any statement. Answer quickly and honestly.

	Rarely/Never	Occasionally	Often	Almost always/ Always
1 I plan tasks carefully.	O	O	O	O
2 I do things without thinking.	O	O	O	O
3 I make-up my mind quickly.	O	O	O	O
4 I am happy-go-lucky.	O	O	O	O
5 I don't "pay attention."	O	O	O	O
6 I have "racing" thoughts.	O	O	O	O
7 I plan trips well ahead of time.	O	O	O	O
8 I am self controlled.	O	O	O	O
9 I concentrate easily.	O	O	O	O
10 I save regularly.	O	O	O	O
11 I "squirm" at plays or lectures.	O	O	O	O
12 I am a careful thinker.	O	O	O	O
13 I plan for job security.	O	O	O	O
14 I say things without thinking.	O	O	O	O
15 I like to think about complex problems.	O	O	O	O
16 I change jobs.	O	O	O	O
17 I act "on impulse."	O	O	O	O
18 I get easily bored when solving thought problems.	O	O	O	O
19 I act on the spur of the moment.	O	O	O	O
20 I am a steady thinker.	O	O	O	O
21 I change residences.	O	O	O	O
22 I buy things on impulse.	O	O	O	O
23 I can only think about one thing at a time.	O	O	O	O
24 I change hobbies.	O	O	O	O
25 I spend or charge more than I earn.	O	O	O	O
26 I often have extraneous thoughts when thinking.	O	O	O	O
27 I am more interested in the present than the future.	O	O	O	O
28 I am restless at the theatre or lectures.	O	O	O	O
29 I like puzzles.	O	O	O	O
30 I am future oriented.	O	O	O	O

Appendix xi: Information sheet - Study 2

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Eye-tracking performance, schizotypy and emotional decision-making in regular cannabis users.

The study you have been asked to contribute to aims to assess whether drug users' show a similar pattern of results to those found in people who have developed psychosis. You will be asked to complete a number of computer based assessments: 1) Anti-saccade task involves the tracking and recording of tiny eye movements while watching movable objects on a computer screen; 2) Iowa gambling task requires participants to choose from selected cards on a computer screen and the aim is to 'win as much money as you can'; 3) Continuous Performance Test is an attention test and you will be asked to press the computer keypad once you have seen a consecutive patterns of numbers (e.g. 3, 5 7) from a continuous presentation of numbers on a computer screen. Further to this, a number of different questionnaires will be administered to assess demographic details, personal and family psychiatric history; your patterns of recreational drug (lifetime and current use); your general health; different aspects of perceived mood and psychotic personality traits.

The study aims to assess potential differences in those who use certain recreational drugs such as cannabis compared with those that don't; therefore you do not have to have used recreational drugs to contribute to the research. A final assessment is to screen for five candidate genes which have been implicated in the development of schizophrenia, thus therefore taking part in this study also involves volunteering to provide a small sample of your DNA, which is done through the non-invasive procedure of using a sterile cotton swab to extract some cheek cells.

Exclusion criteria: 1. Current psychopathology, head trauma, 2. Drug dependency and/or drugs for epilepsy; 3. Recreational cannabis users who have not abstained for at least two days.

Confidentiality of the Data

Confidentiality will be ensured, all personal information and questionnaire and computer-based data will be anonymous and only identifiable by a unique participant code kept separately from your consent form. Upon completion of the study all raw data and contact details will be shredded and disposed of as confidential waste.

Disclaimer

Participants are not obliged to take part in this study and are free to withdraw at any stage. Should they choose to withdraw from the programme they may do so without personal disadvantage and without any obligation to give a reason. Please note that the nature of the study in no way implies that the University of East London condones the use of recreational drug.

University Research Ethics Committee

If you have any queries regarding the conduct of the programme in which you are being asked to participate please contact the Secretary of the University Research Ethics Committee: Debbie Dada, Graduate School, University of East London, Docklands Campus, 4-6 University Way, London E16 2RD (telephone 0208 223 2976, e-mail d.dada@uel.ac.uk

Apendix xii: Consent form – Study 2

Eye-tracking performance, schizotypy and emotional decision-making in regular cannabis users.

I confirm that I have read and understand the information sheet for the above study, understand the nature and purpose of the research and have had the opportunity to ask questions.
Please tick box ☐

I understand that my participation is voluntary and I am free to withdraw at any time without disadvantage to myself and without being obliged to give any reason.
Please tick box ☐

I understand that I will volunteer for a cheek-swab to be administered which extracts a sample of my DNA.
Please tick box ☐

I will perform three computer-based tests, and will be asked to answer a number of questions concerning one's personal history, including mental health issues, levels of drug use and questions assessing aspects of one's personality.
Please tick box ☐

I understand that my involvement in this study, and particular data from this research, will remain strictly confidential and any data will only be identifiable by a unique participant code.
Please tick box ☐

I understand that this consent form will not be linked with my data
Please tick box ☐

I understand that the research team will not be able to provide feedback on my DNA information, computer based assessment performance and questionnaire scores.
Please tick box ☐
I understand that by signing this consent form I am agreeing to have my data used for publishing purposes.
Please tick box ☐

Cannabis users only: I have abstained from cannabis use for at least two days
Please tick box ☐

I hereby fully and freely consent to participate in this study which has been fully explained to me:

Signature of Participant _____ Date _____

Signature of researcher _____ Date _____

Appendix xiii: Debriefing sheet – Study 2.

Eye-tracking performance, schizotypy and emotional decision-making in regular cannabis users.

Thank you for taking the time to participate in our study. Your time and effort is very much appreciated.
If participation in the study has raised concerns for you about your drug use or about your mood you might find it helpful to discuss these concerns your general practitioner or you can also contact NHS Direct on 0845 4647 or www.nhsdirect.nhs.org.uk
There are also a number of voluntary agencies that may be able to help you, including:

Addaction (www.addaction.org.uk)

Drugscope (www.drugscope.org.uk)

Drugsline (www.Drugsline.org) 0808 1 606 606

MIND (www.mind.org.uk) 0845 766 0163 - open Monday to Friday
9.15 am – 5.15 pm

Sane (www.sane.org.uk) 0845 767 8000

The Samaritans (www.samaritans.org) 08457 90 90 90 - open 24 hours

PsychNet-UK (www.psychnet-uk.com): 0845 122 8622

The National Drugs Helpline (www.talktofrank.com): 0800 776600
Free help and advice 24 hours a day, seven days a week.

Appendix xiv: Laboratory work for DNA screening and analysis

PCR primers design protocol

In order to amplify the region of interest in the specific genes to look for the SNPs, different primer sets and PCR programmes were used. All primers were found using the Primer Blast website: http://www.ncbi.nlm.nih.gov/tools/primer-blast/ Each primer was 20bp in length, and one forward and one reverse primer for each SNP was ordered from and synthesised by MWG-Biotech, UK.

Table A1 highlights 20 SNPs that were used in the initial investigation and these were then used for PCR primer testing. Testing was conducted on three SNPs with different amounts of DNA, primer, magnesium chloride, PCR master mix and water to achieve the optimal outcomes. The protocol which worked best was: 0.5 µl of DNA, 1µl of forward and reverse primer combined; 2 µl of MgCl and 1 µl of water and was used for all SNP PCR primers.

The washed and prepared PCR products for each of the SNPs were confirmed that DNA is present in each of the samples from electrophoresis (via the gel capture by appearance of the florescent rings); the product was stored in the 96 well plates, at -20 degrees. Cyber safe was added to the agarose gel during early investigations but this was not strong enough to capture the DNA, so ethidium bromide was used in subsequent agarose gels (see PCR results below for Figure B2). Figure A1 below highlights the standard KB+ ladder to refer to as a guide for the number of base pairs in the PCR results to check that the correct SNP has been captured.

The next procedure was to prepare the PCR samples for multiplexing in the genotyping machine; this was done through the SNP start primer extension reaction. Table B2 highlights the standard protocol for using the PCR machine to suit individual primers.

Table A1: List of different forward and reverse primer used in the early PCR experiments along with their respective annealing temperature (TM)

SNP	Gene Forward (F) and Reverse (R) Primer	TM
1	DTNP1rs2619539b F GGCAAAATGATGTACTGCCA	59.55
	DTNP1rs2619539bR GCCTAGCTCTTAACCCGTCC	60.23
2	DTNP1rs3213207cF ATTGGCCAGTTTCCTCAAAA	59.55
	DTNP1rs3213207cR ATTCAGTGCAGGAAACCTCC	59.14
3	DTNP1rs2619538dF GGATGAGGCCAGTGAGGTAA	60.07
	DTNP1rs2619538dR AAGAGTGGGGAAGAGGTGGT	59.97
4	DTNBP1rs1011313aF AAGCCATCCATGAGGGTTG	52
	DTNBP1rs1011313aR TGCATGGCTTATATGTGTCCA	51
5	DA2rs6277F AGGAGTCTTCAGAGGGGGAA	60.19
	DA2rs6277R GGAATGGGACCTTTCACAGA	59.9
6	DISC1rs1322783aF CAGGCCTCTTCAGCAGTGT	60
	DISC1rs1322783aR ACCCCAGAAACCTTGACCTT	59.83
7	DISC1rs3737597bF AAAGGTGGCATATCACTGGG	59.81
	DISC1rs3737597bR GTGAAGGAAACTCTGCAGGC	60
8	DAOArs3918342aF TGGGAAGCAGAATAACCAGG	60.07
	DAOArs3918342aR TTGCCTTATGGGAACCTCAG	60.07
9	DAOArs1421292bF CACTCCACTCCCCCGTAGTA	59.98
	DAOArs1421292bR TCATGGCTTCGAACAACAAA	60.23
10	COMTrs737865aF GCCAGCTTTTTCTCATGTTG	50
	COMTrs737865aR CAGAGGGCCTTGGTGACTT	51
11	COMTrs4680bF ACCAGGGAGGTGAAATACCC	60.05
	COMTrs4680bR CTTGGCAGTTTACCCAGAGC	59.88
12	COMTrs165599cF GACGGACGCTAACGCTAAG	50
	COMTrs165599cR AGGGAGGCAACTACAGGGA	51
13	CNR1rs1049353aF ATCAACTGGGACCCGATACA	60.2
	CNR1rs1049353aR AATCCTCTGCCCCTTTTCC	60.39
14	CNR1rs324420bF TGTTGCTGGTTACCCCTCTC	60.11
	CNR1rs324420bR AGGGTCCACTCCAACAACTG	60
15	CNR1rs2023239cF TTGAATCCAACCACAGGTCA	59.94
	CNR1rs2023239cR CCCTCTGTGCCTTTCTTCTG	59.98
16	NRG1rs373597aF GGTGGCTTCCAAAAGAAGTG	59.71
	NRG1rs373597aR CCCATTTCACAGATTGCAGA	59.65
17	NRG1rs221132bF CAGTCTTTTCCCATTGGAACT	50
	NRG1rs221132bR AAAATAGCGAGCGTTGGTG	51
18	NRG1rs221533cF TAAGACCACGTGGCATTGAA	60.11
	NRG1rs221533cR GTTTGGTGCTTGGTCAACCT	60.01
19	NRG1rs241930dFCCTGCTTTTGAAGGAGAGAAG	50
	NRG1rs241930dR AATGGGCTTTAGCATG	55
20	NRG1rs243177eF AGAAGGCAAAGGGGGAGCA	56
	NRG1rs243177eR CAAATTCAAATGCCCACAGG	53

Table B2: Standard protocols for using the PCR machine with the exception of the primer annealing temperature to suit individual primers (n = 20)

Step	Temp (°C)	Time (mins)	
1	**95**	**5**	
2	**95**	**1**	**35 cycles**
3	**50**	**1**	**35 cycles**
4	**72**	**2**	**35 cycles**
5	**72**	**5**	
6	**4**	**Holding temperature**	

Preparing for the SNP Start Primer Extension Reaction

The SNP extension primer was carried out using the Beckman Coulter Primer Extension Kit for the Genome Lab ™ and AB gene ready mix. The SNP primers were created using the Primer 3 blast function and these were tested initially on my DNA (see Figure D4 for result). Successful primers should be in the range of 60 degrees – 75 degrees and a Poly (T) tail was added to the 5' (please refer to table C3 for a list of SNP primers and table D4 for the primers with their Poly (T) tails. Two multiplexes were created due to only a maximum of 10 SNPs can be used in one reaction (see Table E5). The order of the SNPs were done in relation to the base pair (bp) length and the first multiplex SNP started with lowest bp (e.g. SNP 5) and thereafter 6 bp in length were required between each SNP length.

Table C3: List of different SNP primer sets used in the experiment along with their respective annealing temperature

SNP	SNP Primer	Allele	TM	BP
1	TCAGCTCATTCTGTTATAACTAGTCTGACATGGTCT	G or C	68.2	36
2	TCTAAATGTATTAGGGAACTTTTCTTTGAAGACTTC	G or A	65.5	36
3	CAGTGAGGTAAGTAGCACAAGTACAGGCCC	A or T	69.3	30
4	CCTTAATTCACAGGCTACAGAATGGATGTTGC	G or A	70.8	32
5	CCCACCACGGTCTCCACAGCACTCC	T or C	75.6	25
6	TTACTGCTGCTAGAAATGCCAGAAAATGTAA	T or C	67.3	31
7	GCTGAGATGAAACTATTCTCAAATCCTGTGGAAGA	T or C	71.3	35
8	AAATCTGAGTTAGAAAAATTTGAGCATCAGCACCTT	T or C	70.5	36
9	CCAGTCCTTGCATTTTGACTTCATCAAGTG	A or T	70.8	30
10	ACGGTCCCTCAGGCTTGGAGGGTCACTTTAA	G or A	76.2	31
11	GCGGATGGTGGATTTCGCTGGC	G or A	73.7	22
12	AGCCACAGTGGTGCAGAGGTCAGCCCT	G or A	76.1	27
13	TTTNCCCTCAATGAAAAGGGCTGAGGAA	G or A	71.9	28
14	GCTGACTGTGAGACTCAGCTGTCTCAGGCC	A or C	74.6	30
15	AGCTAGGTTTGTGGATGTGCCAGGACCA	T or C	73.5	28
16	AACTTGATGACCACTTCAAAGACAAACTTCTTACT	G or A	67.5	35
17	TACTGTCCCAGGATCCAATCCAGGGTACCA	G or C	80	37
18	TCCAACACAATTAAACATTATGCAGCTATTAAAAGA	T or C	67.8	36
19	CAAGACAAGCAGGGGGAGGAGACCCAA	G or A	75	27
20	GAGGTTTCCCCATATCGTCCAGGCTGGTCTCA	G or A	77.8	32

Table D4: SNP Primer with tail extension for use in multiplexing

SNP	SNP Primer with tail extension
1	**TTTTTTTTTTTTTTTTTTTTTTTTTTTTTCAGCTCATTCTGTTATAACTAGTCTGACATGGTCT**
2	**TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCTAAATGTATTAGGGAACTTTTCTTTGAAGACTTC**
3	**TTTTTTTTTTTTTTTTTTTTTTTTTTTT CAGTGAGGTAAGTAGCACAAGTACAGGCCC**
4	**TTTTTTTTTCCTTAATTCACAGGCTACAGAATGGATGTTGC**
5	**TTCCCACCACGGTCTCCACAGCACTCC**
6	**TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTACTGCTGCTAGAAATGCCAGAA AATGTAA**
7	**TTTTTTTTTTTTTTTTTTGCTGAGATGAAACTATTCTCAAATCCTGTGGAAGA**
8	**TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAAATCTGAGTTAGAAAAATTTGAGCATCAGCAC CTT**
9	**TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCCAGTCCTTGCATTTTGACTTCATCAAGTG**
10	**TTTTACGGTCCCTCAGGCTTGGAGGGTCACTTTAA**
11	**TTTTTTTTTTTGCGGATGGTGGATTTCGCTGGC**
12	**TTTTTTTTTTTTAGCCACAGTGGTGCAGAGGTCAGCCCT**
13	**TTTTTTTTTTTTTTTTTTTTTTTTTTTTNCCCTCAATGAAAAGGGCTGAGGAA**
14	**TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCTGACTGTGAGACTCAGCTGTCTCAGGCC**
15	**TAGCTAGGTTTGTGGATGTGCCAGGACCA**
16	**TTTTTTTTTTTTTTTTTTTTTTTTTAACTTGATGACCACTTCAAAGACAAACTTCTTACT**
17	**TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTACTGTCCCAGGATCCAATCCAGGGTACC A**
18	**TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCCAACACAATTAAACATTATGCAGCT ATTAAAAGA**
19	**TTTTTTTTTTTTTTTTTTCAAGACAAGCAGGGGGAGGAGACCCAA**
20	**TTTTTTTTTTTTTTTGAGGTTTCCCCATATCGTCCAGGCTGGTCTCA**

Table E5: Two multiplex set-ups for SNP primers

Multiplex 1	SNP	bp	Multiplex 2	SNP	bp
1	5	28	1	15	28+1=29
2	11	22+11 = 33	2	10	31+4=35
3	12	27+12=39	3	4	32+9=41
4	19	27+18=45	4	20	32+15=47
5	13	28+23=51	5	7	35+18=53
6	3	30+27=57	6	16	35+24=59
7	9	30+33=63	7	1	36+29=65
8	14	30+39=69	8	2	36+35=71
9	17	30+45=75	9	8	36+41=77
10	6	31+50=81	10	18	36+47=84

The nanodrop 2000c spectrophotometer machine was used to check for the concentration of DNA that is present. The nanodrop result gave an indication for the amount of DNA to use for the SNP analysis. The PCR product can be between 1-100fmoles and the Beckmann manual for the SNP extension primer provides set information on the exact quantities to use.

Genotyping

The SNP analysis was run according the Beckmann and Coulter manual for the CEO 8000 software. 10 separate multiplexing analyses were conducted and some of these worked and others did not (see figure E5 and F6). Due to timing issues, it was decided that the SNP work would be conducted externally to be more time and cost effective. Therefore all of the PCR samples (n=100) were washed using the Qiagen kit and then run through the PCR, using the same protocol as earlier, but for only 7 out of the 20 SNPs were selected to be sent externally to K-Biosciences (see table F6). The first batch of analysis (which included the SNP primer PCR products) had a poor success rate at K-Biosciences (under 60%). Therefore a secondary batch was sent which including all of the original DNA samples and were cleaned to increase the % of purified DNA. All 100 samples were then re-sent to K-Biosciences for a final attempt to genotype the DNA without any PCR or SNP primers, and it was much more successful with a hit rate of (89%-99%).

Table F6: List of SNP final SNP markers used for the DNA analysis.

Gene	SNP ID	Allele Y	Allele X	Sequence
DAOA	RS142129_SNP9	T	A	TGACTTCATCAAGTG[A/T]GCTTATGTAGTTAAG
COMT	RS737865_SNP10	G	A	AACAGGACACAAAAA[C/T]CCCTGGCTGGAAAAA
COMT	RS4680_SNP11	G	A	GTGGATTTCGCTGGC[A/G]TGAAGGACAAGGTGT
COMT	RS165599_SNP12	G	A	ATGGGGACGACTGCC[A/G]GCCTGGGAAACGAAG
CNR1	RS1049353_SNP13	G	A	AAAAGGGCTGAGGAA[A/G]TCCTCCAAAATGTGG
FAAH	RS324420_SNP14	C	A	CAGCTGTCTCAGGCC[A/C]CAAGGCAGGGCCTGC
NRG1	RS221533_SNP18	T	C	TAAACTTTTAAAATA[C/T]GTCAATACAGAGAAA

PCR Results

Cybersafe was initially used to act as a florescent for the gel capture but this yielded poor outcomes, therefore ethidium bromide was used instead and achieved positive results – see figure B2 below for SNP1 trialled on my own DNA. The percentage of agarose gel for running the electrophoresis was changed from 3% to 1.5% which also yielded better results - see figure C3 below for an illustration of SNP 1 run with 1.5% agarose.

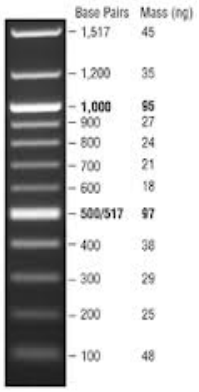

Figure A1. KB+ ladder.

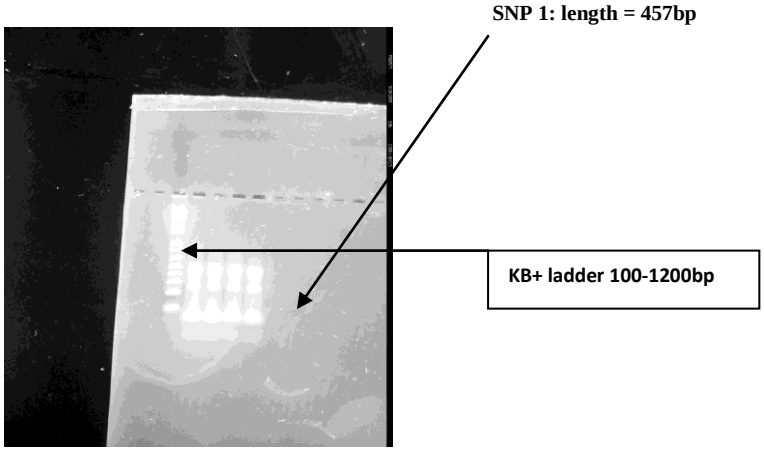

Figure B2: Represents SNP 1 for my DNA using ethidium bromide

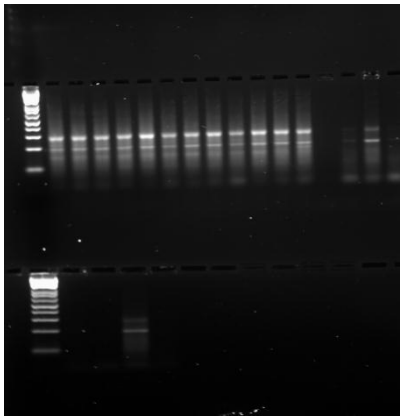

Fig C3: SNP 1 (1.5% agarose gel).

The primers were assessed to make sure the right concentrations were present prior to genotyping. PCRs were run on each primer using my DNA. Figure D4 below highlights the gel capture for testing the primers on my own DNA for 8 SNPs.

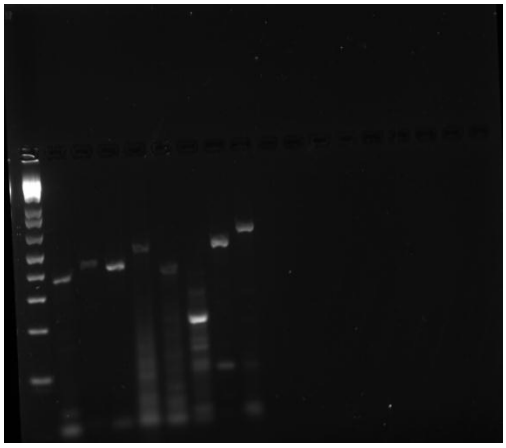

Fig D4: Gel capture from testing primers on my DNA for SNP 1, 2, 3, 4, 5, 10, 11 and 12

10 separate multiplexing analyses were conducted using the genotyping machine and some of these worked and others did not (see figure E5 and F6). Figure E5 highlights that the size standards in red were present, but not many SNPs. Whereas in figure F6 the SNPs are represented by the peaks and the number corresponds to their pre-set base pair size.

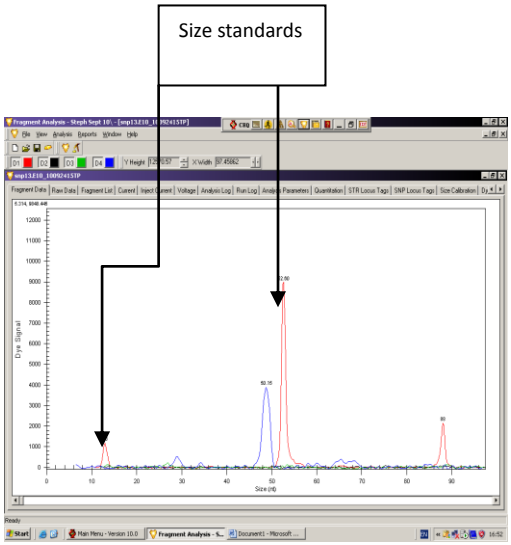

Fig E5: Unsuccessful output from multiplexing

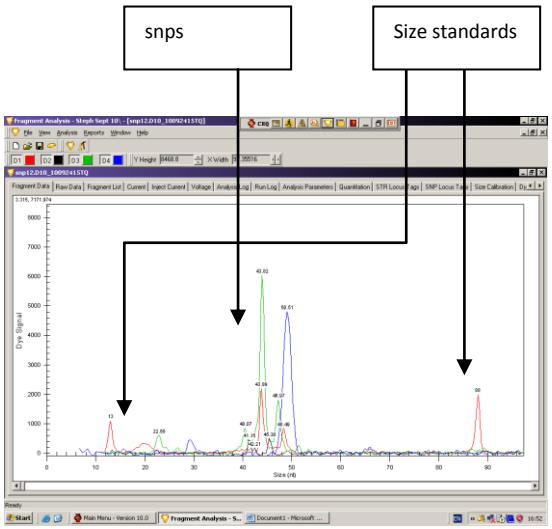

Fig F6: Successful output from multiplexing

Appendix xv: Chapter 4 result tables for cannabis use variables and SNP genotypes and the COMT Haplotype analyses.

Table G7: Cannabis use variables explored across SNP genotypes in cannabis users

Gene	**SNP**	**Genotype (n =)**	**Joints Per Week Mean (SD) [KW]**	**X**	***p***	**Age of Onset Mean (SD) [KW]**	**X**	**p**	**Duration**	**X**	***p***
DAOA	rs142129	T/T (6)	23.4 (10.4)	66.27	0.270	16.33 (3.83)	20.34	0.562	7.5 (4.76)	32.24	0.804
		T/A (16)	13.01 (12.33)			16.61 (4.46)			12.39 (9.86)		
		A/A (6)	10.75 (11.36)			17.0 (2.90)			11.00 (10.32)		
COMT	rs737865	T/T (16)	17.28 (13.2)	54.58	0.673	16.47 (3.48)	21.54	0.487	13.11 (8.07)	39.88	0.476
		T/C (8)	10.03 (10.65)			17.11 (5.63)			8.55 (8.08)		
		C/C (4)	14.15 (11.08)			16.25 (1.26)			8.5 (5.26)		
	rs4680	G/G (12)	16.10 (12.74)	64.15	0.162	16.5 (4.85)	21.03	0.519	8.16 (5.85)	40.43	0.363
		G/A (9)	8.0 (8.7)			17.5 (3.17)			12.8 (9.0)		
		A/A (7)	21.14 (12.5)			15.75 (4.9)			13.5 (12.66)		
	rs165599	G/G (6)	11.5 (10.8)	58.27	0.392	14.83 (2.2)	22.94	0.405	10.1 (7.14)	43.46	0.326
		G/A (13)	12.75 (12.3)			17.5 (4.9)			9.0 (7.44)		
		A/A (9)	19.83 (12.7)			16.5 (3.0)			14.7 (11.7)		
CNR1	rs1049353	G/G (26)	15.6 (12.2)	32.64	0.338	16.57 (4.07)	10.32	0.502	11.75 (9.11)	9.85	0.971
		G/A (2)	3.25 (0.35)			17.5 (2.12)			2.5 (3.0)		
		A/A (0)	-			-			-		
FAAH	rs324420	C/C (17)	12.89 (12.45)	54.94	0.515	17.0 (4.65)	20.33	0.562	9.11 (9.03)	41.67	0.398
		C/A (8)	16.37 (13.00)			16.0 (3.16)			16.4 (8.53)		
		A/A (3)	21.0 (9.64)			16.3 (0.57)			7.33 (5.77)		
Nrg1	rs221533	T/T (21)	15.2 (11.87)	40.69	0.973	16.59 (4.06)	16.31	0.80	10.31 (9.27)	76.137	**<0.001**
		T/C (7)	13.42 (14.28)			16.75 (3.91)			13.37 (8.83)		
		C/C (0)	-			-			-		

Table H8: Frequency of all COMT haplotypes (737865-4680-165599) in whole group and cannabis users and non cannabis users.

COMT haplotype	Whole Group	Cannabis user	Non-cannabis user
TGA	17	5	12
TAA	8	6	2
TGG	40	16	24
TAG	6	3	3
CGA	1	0	1
CAA	1	0	1
CGG	6	4	2
CAG	0	**0**	0

Table I9: Cognitive and trait outcomes explored in carriers of the COMT protective haplotype 737865-4680-165599 (T-G-G) in all participants.

737865-4680-165599 (T-G-G)	Protective Haplotype	Protective haplotype (n =)	Non protective haplotype	Non protective haplotype (n =)	F	*p*
SPQ total	6.26 (4.7)	41	6.07 (4.1)	41	0.04	0.842
SPQ CP	2.51 (2.03)	41	1.95 (1.65)	41	1.869	0.175
SPQ IP	2.41 (2.07)	41	2.48 (2.16)	41	0.025	0.875
SPQ DT	1.46 (1.72)	41	1.63 (1.64)	41	0.212	0.647
CPT accuracy	19.03 (3.79)	27	18.5 (5.94)	22	0.101	0.752
CPT response time	572.45 (177.6)	27	546.44 (211.35)	22	0.219	0.642
CPT ME	2.07 (2.14)	27	1.57 (1.77)	22	0.750	0.391
CPT CE	9.59 (9.8)	27	7.77 (7.89)	22	0.495	0.485
AST error	31.3 (23.7)	21	26.8 (27.2)	17	0.268	0.608
AST Latency	316.26 (78.63)	21	275.98 (61.7)	17	2.972	0.093
Iowa Gambling Task	1387 (1040.99)	27	1384 (982)	22	0.00	0.989
Latent inhibition	12.57 (9)	14	29.2 (9.9)	19	0.49	0.488

Table J10: Cognitive and trait outcomes explored in carriers of the COMT protective haplotype 737865-4680-165599 (T-G-G) in cannabis users and non-cannabis users.

Measurement	Participant Group	Protective Haplotype	Protective haplotype (n =)	Non protective haplotype	Non protective haplotype (n =)	F	*p*
SPQ total	Cannabis user	6.0 (3.86)	16	7.16 (3.9)	19	0.005	0.941
	Non-cannabis user	6.94 (5.26)	25	5.14 (4.12)	22		
SPQ CP	Cannabis user	2.68 (2.02)	16	2.57 (1.8)	19	1.798	0.154
	Non-cannabis user	2.4 (2.08)	25	1.4 (1.3)	22		
SPQ IP	Cannabis user	2.37 (1.54)	16	2.3 (2.5)	19	0.021	0.885
	Non-cannabis user	2.44 (2.3)	25	2.64 (2.13)	22		
SPQ DT	Cannabis user	1.25 (1.40)	16	2.26 (1.52)	19	0.469	0.495
	Non-cannabis user	1.60 (1.87)	25	1.09 (1.57)	22		
CPT accuracy	Cannabis user	19.2 (3.07)	9	18.3 (4.9)	12	0.100	0.753
	Non-cannabis user	18.9 (4.59)	18	18.9 (7.2)	10		
CPT response time	Cannabis user	608 (200)	9	630 (135)	12	0.630	0.432
	Non-cannabis user	553 (168)	18	446.08 (247)	10		
CPT ME	Cannabis user	2.11 (2.75)	9	1.91 (2.15)	12	0.880	0.353
	Non-cannabis user	2.05 (1.86)	18	1.15 (1.05)	10		
CPT CE	Cannabis user	8.66 (14.74)	9	7.81 (8.59)	12	0.348	0.558
	Non-cannabis user	10 (6.63)	18	7.6 (9.4)	10		
AST error	Cannabis user	32.6 (25)	9	38.3 (26)	9	0.399	0.522
	Non-cannabis user	30.3 (28.5)	8	13.9 (23.5)	12		
AST Latency	Cannabis user	290.7 (96)	9	271 (80.9)	9	1.356	0.126
	Non-cannabis user	335 (60)	8	281 (33)	12		
Iowa Gambling Task	Cannabis user	1261 (60)	9	1195 (1257)	18	0.136	0.714
	Non-cannabis user	1451 (1186)	12	1610 (523)	10		
Latent inhibition	Cannabis user	8.42 (8.97)	7	3.28 (2.56)	7	1.841	0.185
	Non-cannabis user	16.7 (7.45)	7	14.5 (8.57)	12		

Table K11: Represents the combination of total number of risk markers in the cannabis users and non cannabis users in relation to trait and cognitive outcomes.

	1 Risk marker Mean (SD)	**n=**	**2 Risk markers Mean (SD)**	**n=**	**3 Risk markers Mean (SD)**	**n=**	**4 Risk markers Mean (SD)**	**n=**	***F***	***p***
SPQ_total – cannabis user	9.0 (4.0)	3	7.25 (3.94)	20	6.35(3.7)	26	8.0	1	0.284	0.837
SPQ_total – non cannabis user	5.5 (3.69)	4	6.0 (3.3)	8	5.84(5.08)	37	3.0	1		
SPQ_CP – cannabis user	3.33(3.21)	3	2.75(1.8)	20	2.65(1.89)	26	1.0	1	0.342	0.795
SPQ_CP – non cannabis user	1.25(1.25)	4	2.0(2.07)	8	2.05(1.8)	37	1.0	1		
SPQ_IP – cannabis user	2.0 (2.0)	3	2.5 (2.13)	20	2.42(1.67)	26	3.0	1	0.002	1.0
SPQ_IP – non cannabis user	3.0 (1.8)	4	2.37 (1.5)	8	2.48(2.37)	37	2.0	1		
SPQ_DT – cannabis user	3.66(0.57)	3	2.0(1.45)	20	1.5 (1.36)	26	4.0	1	1.1.96	0.316
SPQ_DT – non cannabis user	1.25(1.5)	4	1.62(1.76)	8	1.29(1.76)	37	0	1		
AST error – cannabis user	-	-	32.9(28.68)	14	29.5(24.02)	12	-	-	0.695	0.505
AST error – non cannabis user	4.0 (1.41)	2	6.66(7.63)	3	26.4(28.79)	17	-	-		
IGT – cannabis user	1512.5(724)	2	830(984)	15	1654.16 (676.37)	12	2425	1	0.672	0.573
IGT – non cannabis user	1425 (247)	2	1493.75 (581.45)	4	1536.95 (1102)	23	1025	1		
CPT accuracy – cannabis user	18.5 (2.12)	2	18.53 (4.79)	15	21.8 (5.21)	12	18.0	1	0.310	0.818
CPT accuracy – non cannabis user	20 (2.82)	2	21.25 (2.2)	4	18.95 (5.88)	23	16.0	1		
LI – cannabis user	21.0	1	8.4 (7.6)	5	6.5 (7.58)	14	-	-	0.708	0.5
LI – non cannabis user	12.0 (12.72)	2	11.75(10.14)	4	17.2 (6.8)	14	-	-		

Appendix xvi: Ethics Approval

UEL
University of East London

Dr John Turner
School of Psychology
Stratford

ETH/08/56

01 May 2008

Dear Dr Turner,

Application to the Research Ethics Committee: Latent Inhibition, Kamin Blocking cannabis (S Lynch)

I advise that Members of the Research Ethics Committee have now approved the above application on the terms previously advised to you. The Research Ethics Committee should be informed of any significant changes that take place after approval has been given. Examples of such changes include any change to the scope, methodology or composition of investigative team. These examples are not exclusive and the person responsible for the programme must exercise proper judgement in determining what should be brought to the attention of the Committee.

In accepting the terms previously advised to you I would be grateful if you could return the declaration form below, duly signed and dated, confirming that you will inform the committee of any changes to your approved programme.

Yours sincerely

Debbie

Debbie Dada
Administrative Officer for Research
d.dada@uel.ac.uk
02082232976

Research Ethics Committee: ETH/08/56

I hereby agree to inform the Research Ethics Committee of any changes to be made to the above approved programme and any adverse incidents that arise during the conduct of the programme.

Signed:.....Date: 01.05.08

Please Print Name:

Appendix xvii: Ethics Approval

UEL
University of East London

John Turner
School of Psychology, Stratford

ETH/11/24
13 October 2009

Dear John,

Application to the Research Ethics Committee: Schizophrenia-linked cognitive, trait and DNA markers in regular cannabis users. (S Lynch).

I advise that Members of the Research Ethics Committee have now approved the above application on the terms previously advised to you. The Research Ethics Committee should be informed of any significant changes that take place after approval has been given. Examples of such changes include any change to the scope, methodology or composition of investigative team. These examples are not exclusive and the person responsible for the programme must exercise proper judgement in determining what should be brought to the attention of the Committee.

In accepting the terms previously advised to you I would be grateful if you could return the declaration form below, duly signed and dated, confirming that you will inform the committee of any changes to your approved programme.

Yours sincerely

Simiso Jubane
Admission and Ethics Officer
s.jubane@uel.ac.uk
02082232976

Research Ethics Committee: ETH/11/24

I hereby agree to inform the Research Ethics Committee of any changes to be made to the above approved programme and any adverse incidents that arise during the conduct of the programme.

Signed:.....Date: 13th October 2009

Please Print Name: JOHN TURNER