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The role of cognitive and reward processes in substance use: A cognitive neuroscience approach to understanding drugs of addiction.

Liam Nestor

Trinity Term, 2008

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Department of Psychology & Institute of Neuroscience
Trinity College
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Supervisor: Prof Hugh Caravan
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Liam Nestor
Abstract

The role of cognitive and reward processes in substance use: A cognitive neuroscience approach to understanding drugs of addiction.

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Drugs of addiction are widely considered to induce a pathological state which involves the progression of acute drug use to the development of drug-seeking behaviour, the decreased and slowed ability to respond to naturally rewarding stimuli, compromised impulse control and a vulnerability to drug relapse during abstinence. Therefore, it is believed that drug addiction is a disorder of the brain involving alterations in major neural substrates related to reward, motivation and/or drive, learning and memory and cognitive control. Cogent scientifically based research that focuses on distinct, well-characterized cognitive processes, believed to be the product of these neuronal substrates, affords neuroscience the opportunity to elucidate cognitive operations in drug users, and importantly, how changes in neural functioning related to cognition likely promotes continued drug use and ultimately, addiction.

The excessive use of cannabis has become a growing concern in Western societies, with abuse patterns leading to dependence (8%), on a par with other drugs of addiction such as cocaine (15%) and alcohol (12%). Tobacco use, like cannabis, continues to be a widespread public health problem, and with nearly one billion smokers worldwide, it is estimated that half are likely to die from smoking-related diseases. Importantly, cannabis and nicotine are often co-administered, with abusers of “hard” drugs commonly reporting cannabis and/or nicotine as their first recreational drug of use. Despite an increased understanding concerning the pharmacology of cannabis and nicotine, there is little research which has examined their long-term effects upon learning, memory, reward processing and cognitive control. Furthermore, little research has investigated the effects of long-term drug abstinence on cognition, specifically comparing cognitive neural substrates in current substance users and those who have embarked upon a successful period of drug abstinence. Such comparisons may explicate ways in which cognitive neuronal operations may contribute to avoiding drug relapse, while leading one to query whether it is possible to develop behavioural and/or pharmacological treatments which may facilitate abstinence through improvements in cognition.

The research presented in this thesis uses cognitive functional magnetic resonance imaging (fMRI) in chronic cannabis and nicotine users to demonstrate differences in neural activity related to learning and memory, reward processing, responses to drug-predictive stimuli, inhibitory control and error monitoring processes. The results are discussed in relation to the cognitive neuroscience of addiction.
In memory of my loving father,

Jeremy Michael Nestor
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General Discussion ................................................................. 258-290
Chapter 1 - General Introduction
The acute and chronic use of addictive drugs is believed to impair cognitive functioning, but there still remains considerably debate as to whether specific cognitive deficits are related to specific drugs of abuse. Importantly, recent advances in developing cognitive behavioural assays and the advent of functional magnetic resonance imaging (fMRI) has afforded cognitive neuroscience the opportunity to examine, in depth, the role various drugs of abuse play in affecting cognitive processing, especially executive functioning. Executive functioning refers to a collection of higher order cognitive abilities involving organization and integration, which have been associated with different prefrontal cortical (PFC) neuroanatomical pathways (Roberts et al, 1998). These abilities include anticipating and establishing goals, designing plans, self-regulation/monitoring and inhibitory control (Lezak, 1995). Research has shown that brain circuits involved in these executive abilities may be disrupted by drugs of abuse, eliciting disturbances in reward processing, motivation and/or drive, salience attribution, memory consolidation and inhibitory control, thus facilitating and exacerbating future drug use (Volkow et al, 2003a).

The evolution of substance abuse and dependence is thought to involve a loss of self-control (Baler and Volkow, 2006). Despite their best efforts and expressed preferences, drug-dependent individuals often appear incapable of exerting sufficient control over their drug urges, their drug-seeking and drug-taking behaviour. This appears to implicate the role of cognitive processing in controlling human behaviour, which may be compromised in drug dependent individuals. While much research attests to compromised cognitive abilities in drug abusers, relatively little research has been conducted into the functional neuroanatomy linked to these impairments. Another feature of much of the extant literature is the use of complex tasks that
engage many distinct cognitive processes. As a consequence, it is difficult to infer from compromised performance on such tasks, which specific cognitive process or processes are affected, and what specific brain areas may be altered by drug use.

Drug abuse and addiction still remain poorly defined concepts. While research has attempted to encompass the detrimental effects of drugs on brain and behavioural processes, there still remains a gap in substance abuse research, which attempts to address both the distinct and overlapping consequences of different drugs of abuse. One might argue that sustained exposure to all drugs of abuse is a prerequisite to drug dependence, given the mechanisms of action of all drugs within the mesocorticolimbic system. Evidence regarding the actions of drugs of abuse implicate dopamine (DA) release at the level of the nucleus accumbens (NAcc), whether it involves direct, increased release and reduced uptake of DA by amphetamines, cocaine and MDMA (Koob, 1992), or the indirect actions of drugs such as alcohol, cannabis, nicotine and opiates, which disinhibit the NAcc DA system (Koob, 1992). The way in which different and varying degrees of cognitive impairment are related to a particular drug of abuse, however, is still unknown. Despite no unequivocal classification regarding the cognitive effects of different drugs of abuse, all drugs of abuse do share the ability to induce a gradual transition from recreational use to addiction, resulting in motivational shifts in which behaviours become more reflexive, and consequently, much less amendable to cognitive interference (Volkow et al, 2004, 2007).

The reinforcing actions of drugs of abuse, as stated, rely heavily upon the induction of DA release at the level of the NAcc (Koob, 1992). DA is a multifaceted
neurotransmitter, which is involved in a number of behaviours, including the fine-tuning of motor functioning, the modulation of salience attribution and attention, the regulation of reward and motivation and cognitive control (Tzschentke, 2001). Preclinical and clinical research is now beginning to produce convincing evidence that the repeated administration of drugs of abuse can bring upon changes in the brain at a molecular, cellular and circuit organizational level, which are related to changes in DA functioning (Volkow et al, 2002; Volkow and Li, 2004).

According to one model (Volkow et al, 2003a), the major neural substrates underlying addiction (See Figure 1a below) make up a network of four independent and overlapping circuits in the brain consisting of (i) reward, located within the NAcc, ventral pallidum and hypothalamus; (ii) motivation and/or drive located in the orbitofrontal cortex; (iii) memory and learning, located in the amygdala and hippocampus and (iv) cognitive control, located in the PFC and dorsal anterior cingulate. These four circuits are modulated by DA via direct and indirect pathways, and communicate with one another via glutamatergic and GABAergic projections. Furthermore, changes resulting from prolonged drug use are likely to affect the brain, and ultimately behaviour, given the role of glutamate and GABA in neuroplasticity at these circuits (Hoffman et al, 2003; Liu et al, 2005; Wolf et al, 2003). According to this model (See Figure 1b below), the salience value of a drug (i.e. the degree to which the drug becomes a “wanted” incentive stimulus), and those cues associated with it are enhanced, the salience of natural reinforcers is reduced, and the strength of inhibitory control over reflexive, drug-taking behaviour is diminished as drug addiction develops (Volkow et al, 2003a).
Figure 1. Addiction circuitry. (a) Depicting four circuits that are postulated to play key independent and overlapping roles in addiction: (1) reward prediction and pleasure (red) in the nucleus accumbens (NAcc) and ventral pallidum (VP); (2) memory and learning, the main substrate of conditioning (purple), located in the amygdala (Amyg) and hippocampus (HIP); (3) motivation, drive and salience evaluation (green) located in the orbitofrontal cortex (OFC) and (4) cognitive control (blue), in charge of restraining cravings, located in the prefrontal cortex (PFC) and anterior cingulate gyrus (ACG). (b) Hypothetical model of drug dependence. Compared with the normal brain (left), the salience value of a drug (red) and its associated cues (purple) is enhanced in addiction (right), the strength of inhibitory control is weakened (blue), leading to unrestrained motivation (green) resulting in drug taking behaviour (taken from Baler and Volkow, 2006).
Therefore, it is possible that physiological processes controlled by DA, which contribute to reward, motivation and/or drive, learning and memory, together with cognitive control are likely to be hampered at a cellular and circuit level, which in turn perpetuates drug abuse, and ultimately addiction.

An alternative model regarding the development of addiction has been formulated by Bechara (2005); in which an imbalance between two separate, but interacting, neural systems is conceived to develop. Here it has been postulated that an impulsive amygdala system for the signalling of pain or pleasure regarding immediate prospects, together with a reflective prefrontal cortical system, which signals pain or pleasure of future prospects, are the basis for both the development and maintenance of addiction. According to this model, the reflective prefrontal cortical system usually controls the impulsive amygdala system via “top-down” control. This control, however, is not always absolute; with hyperactivity within the impulsive amygdala system overriding these reflective “top-down” executive processes. Drugs of abuse are believed to trigger “bottom-up”, involuntary signals originating from the amygdala, which are thought to commandeer the goal-driven cognitive control required for the normal operations of the reflective system; in which there is normally an exercising of willpower to resist drugs. Anomalies within these two systems are considered to pre-date the addiction state, by facilitating the progress from experimentation to addiction; with the subsequent excessive and chronic use of drugs exacerbating these abnormalities.
Figure 2. A schematic diagram illustrating key structures belonging to the impulsive system (red) and the reflective system (blue). An emergent dominant pattern of affective signalling can modulate activity of several components of the impulsive and reflective systems. These include regions involved in (i) representing patterns of affective states (e.g., the insula and somatosensory cortices); (ii) triggering of affective states (e.g., amygdala (A) and VMPC); (iii) memory, impulse and attention control (e.g., lateral orbitofrontal, inferior frontal gyrus and dorsolateral prefrontal (DLPC), hippocampus (Hip) and anterior cingulate (AC); and (iv) behavioral actions (e.g., striatum and supplementary motor area). 5-HT: serotonin; DA: dopamine (Taken from Bechara, 2005).
There are still gaps in the literature concerning executive components of addiction, particularly relating impaired executive control to prolonged drug abuse and relapse. Studies have shown altered levels of brain activity in cocaine and opiate addicts during inhibitory control tasks (Hester et al, 2004; Kaufman et al, 2003; Forman, 2004), reduced activity in cannabis users during Stroop and working memory tasks (Eldreth et al, 2004) and different degrees of activation in the DA reward system in cigarette smokers and alcoholics during monetary reward and nicotine/alcohol cue paradigms (Martin-Solch et al, 2001; Due et al, 2002; David et al, 2005; McBride et al, 2006; Wrase et al, 2007). There still remains uncertainty, however, regarding cognitive deficits related to specific drugs of abuse, and how the abuse of different substances alters the ability to remain abstinent through cognitive control. Functional neuroimaging studies that focus on distinct, well-characterized cognitive processes that are guided by current theory, thus afford us the best chance to understand the behavioural control processes of drug abusers.

Nicotine is one of the most commonly used substances throughout many societies, and one which has been proven to be highly addictive, and consequently, have potentially detrimental effects upon physical health. Cannabis, commonly administered with nicotine, has become one of the most frequently used substances around the world, with only alcohol and nicotine demonstrating a greater prevalence of use. Based on current theories regarding the effects of drugs on cognitive processing, the current research sought to examine the cognitive consequences arising from regular cannabis and nicotine use, and how changes in learning and memory, reward processing and cognitive control are related to their use, and likely contribute to their continued abuse.
Cannabis

The excessive use of cannabis has become a growing concern in Western societies. While cannabis is often considered to be an innocuous substance, abuse patterns leading to dependence (8%) are on a par with other drugs of addiction such as cocaine (15%) and alcohol (12%) (Ridenour et al, 2003; Wagner et al, 2003), making it the third most common drug of choice in Europe behind alcohol and tobacco (Calafat et al, 1999). Despite an increased understanding concerning the pharmacological effects of cannabis (Herkenham et al, 1991; Tsou et al, 1998; Iversen, 2000, 2003), there is little research examining the long-term effects of its use upon learning, memory and reward processing. The need for further and more specialised research into the long-term effects of cannabis upon cognition is particularly imperative, given that abusers of “hard” drugs commonly report cannabis as their first recreational drug of use (Golub and Johnson, 2001). Therefore, the implications of cannabis use upon cognitive functioning are potentially far reaching.

Various large cross-sectional studies have also demonstrated that over 30% of cannabis users present with DSM-IV criteria for drug dependence (SAMHSA, 1998). Therefore, the use of cannabis can lead to dependency, suggesting that there are changes in the brain, which may facilitate and maintain cannabis use behaviour. Indeed, the neurophysiological changes related to cannabis use also appear to emulate those changes observed in other forms of addiction (Iversen et al, 2003) with alterations to cerebral structure and metabolism resulting from cannabis use (Loeber and Deborah, 1999; O’Leary et al, 2000; Mathew et al, 2002) located within neural mechanisms which are argued to contribute to the progression and maintenance of drug abuse (Goldstein and Volkow, 2002; Volkow et al, 2002). Critically, it has been
proposed that the cognitive processes, which are compromised in chronic cannabis users (Solowij et al 1997; Solowij 1998), may also facilitate the continuation of drug consumption, and may also be compromised acutely following cannabis withdrawal. Taken together, cannabis dependence, like dependence on other drugs such as alcohol, nicotine, stimulants and opiates, may induce a susceptibility to relapse; a core component of which involves a diminished capacity within brain centres associated with learning, reward processing and cognitive control.

**Nicotine**

The number of cigarette smokers worldwide is estimated at 1.3 billion, although there has been an observed decline in the prevalence of smoking in the Western world. Despite some success in reversing the tobacco epidemic in the developed world, the use of tobacco continues to increase in less developed countries, where insufficient resources and education concerning tobacco use, lead to its widespread consumption. The World Health Organization (WHO) has estimated that in the year 2000, nearly 5 million people died as a consequence of tobacco use (Ezzati, 2004). Furthermore, it has been estimated, based on current trends of tobacco use that 70% of the global deaths from tobacco consumption by 2025 will have occurred in developing nations (Mackay and Crofton, 1996). Despite these statistics and the well-documented health consequences, it is extraordinary that people continue to consume tobacco. This continued use in the face of adversity is a sad, but powerful testimony, to the effects of nicotine dependence, clearly demonstrating its addictive properties.

While the majority of cigarette smokers endorse the desire to give up smoking, only 14-49% will achieve full abstinence after 6 months (Holmes et al, 2004; Hughes et al, 1999; Hurt et al, 1997; Jorenby et al, 1999; Killen et al, 2000). Because cigarette
smoking presents with considerable health risks (Bartal, 2001; Mokdad et al, 2004) and induces high costs on health care resources (Leistikow et al, 2000), there is a pressing need to understand, in greater detail, the effects of nicotine addiction in the brain, and how changes in functioning may contribute to its continued use. Laboratory studies in animals demonstrate that DA release at the level of the ventral striatum (VS) underlies the reinforcing properties of nicotine (Koob, 1992; Leshner and Koob, 1999), with DA release in response to nicotine or a cigarette challenge observed in non-human primates and humans (Brody et al, 2002; Dewey et al, 1999; Marenco et al, 2000; Tsukada et al, 2002). Functional MRI (fMRI) and positron emission tomography (PET) studies also demonstrate that the administration of nicotine (or cigarette smoking) increases activity in the prefrontal cortex (PFC) (Nakamura et al 2000; Stein et al 1998), and anterior cingulate (ACC) (Stein et al 1998), two major neural substrates underlying addiction, and which contribute to cognitive control processing (Volkow et al, 2003a).

Importantly, nicotine is often administered in conjunction with other drugs of abuse, which may exacerbate its effects on neural functioning, furthering the progression and maintenance of drug abuse in general (Goldstein and Volkow, 2002; Volkow et al, 2002). This may in turn compromise cognitive control mechanisms which necessitate drug abstinence. Therefore, nicotine addiction presents a serious risk to health, with changes in brain functioning underlying reward, motivation and/or drive, learning and memory, together with cognitive control, central to the aetiology of continued nicotine addiction.
Cannabis Effects

Cannabinoid 1 receptors (CB₁) are seven transmembrane receptors, which mediate the central effects of both Δ⁹-tetrahydrocannabinol (Δ⁹THC) and naturally occurring arachidonic acid derivatives. The naturally occurring derivatives include anandamide, 2-arachidonylglycerol (2-AG) and 2-arachidonylglyceryl ether, which together with Δ⁹THC, act as ligands at CB₁ receptors, where they modulate neurotransmitter release (See Iversen, 2000, 20003 for reviews). CB₁ receptors are predominantly located on the presynaptic nerve terminals of cortical and subcortical structures. In both animals and humans, frontal regions of the cerebral cortex contain a high density of CB₁ receptors, as do the cerebellar and basal ganglia regions. Within limbic forebrain regions, CB₁ receptors are particularly prevalent in the hypothalamus and the anterior cingulate, with the hippocampus also containing high numbers. CB₁ receptors are negatively coupled through Gₛ/ọ proteins to adenylate cyclase, where they modulate the release of various neurotransmitters, such as acetylcholine (ACh), glutamate, gamma-aminobutyric acid (GABA), norepinephrine (NE), dopamine (DA) and serotonin (5-HT) from axon terminals (Schlicker and Kathmann, 2001). The activation of CB₁ receptors inhibits synaptic transmission in the hippocampus (Sullivan, 1999; Hoffman and Lupica, 2000), substantia nigra pars compacta (Chan and Yung, 1998), cerebellum (Levenes et al., 1998), and prefrontal cortex (Auclair et al, 2000).

The pharmacological effects of cannabis, which sustain its use, are believed to be associated with the sense of euphoria and well-being that it produces. Feeling “high” and relaxed are some of the most cited subjective properties reported in humans (Green et al, 2003; Huestis et al, 2007; De Souza et al, 2008; Hunault et al, 2008).
Based on these subjective reports, it is likely that the euphorogenic properties of cannabis, like other drugs of abuse, are related to the way in which it interacts with the brain’s “reward circuitry”. The VS contains a moderately high density of CB\textsubscript{1} receptors (Herkenham et al, 1991; Tsou et al, 1998), with evidence that cannabinoids increase the activity of midbrain DA neurons that project to the VS (French, 1997) elevating DA levels at this structure (Chen et al, 1990). Therefore, CB\textsubscript{1} receptors in the VS represent a potentially critical site for mediating the rewarding and/or addictive properties of cannabis in humans.

While previous proposals suggested that cannabis is not potentially addictive, there is now evidence for dependence in both adolescents and adult users seeking treatment (Crowley et al, 1998; Budney et al, 2003; Dawes et al, 2006) and in frequent users not seeking treatment (Wiesbeck et al, 1996). This dependency and withdrawal syndrome (also observed in animals), includes craving, decreased appetite, disturbances in sleep, weight loss, aggression, increased irritability, restlessness and increased ‘dream’ activity (Budney et al, 2002; Budney et al, 2003). The mesocorticolimbic DA system is also thought to be involved in the addictive properties of cannabis (Hoffman and Lupica, 2001). The chronic administration of cannabinoids is believed to induce adaptive changes in the brain, with acute withdrawal from cannabis resulting in reduced DA transmission in the limbic system, particularly the VS and anterior cingulate (ACC), similar to those observed in other addictive drugs (e.g., cocaine, opiates) (Diana et al, 1998; Tanda et al, 2003). VS reward functioning is central to the current research theme, with neuroadaptive changes at this structure, possibly influencing the way in which cannabis users process cues which are predictive of non-drug rewards. Conversely, one could surmise that differences between cannabis-using
and drug naïve populations preceded and influenced the onset of drug use, more suggestive of a biological propensity to administer drugs of abuse.

The location of CB₁ receptors throughout the brain, and their effects on transmitter systems known to partake in cognition, are therefore, well placed to facilitate the disrupting effects of Δ⁹THC upon learning, memory and reward processes, as well as upon higher order executive functions, such as impulse control and working memory. While some of the disrupting effects of cannabis on learning and memory may arise from its habitual use, adaptations within brain areas responsible for aspects of learning and reward processing, may be responsible for the continued use of cannabis, and quite possibly, the progression to more harmful drugs of abuse.

**Nicotine Effects**

Nicotine exerts its effects within the brain by acting at nicotinic acetylcholine receptors (nAChRs), which are cation-selective, ligand-gated channels. nAChRs are pentameric combinations of 12 genetically distinct homologous subunits (α2 – α10 and β2- β4), with each nAChR assumed to be heterooligomeric (i.e. composed from various combinations of α and β subunits) (McGehee and Role, 1995). Early nicotine binding studies identified two distinct nicotine binding sites in the form of the high affinity α4β2 and the low affinity α7 receptor subtypes (Couturier et al, 1990; McGehee and Role, 1995; Seguela et al, 1993), which have subsequently provided evidence concerning neuronal brain changes related to nicotine use (Corrigall et al, 1994; Marks et al, 1983; Schwartz and Kellar, 1983; Benwell et al, 1988; Wooltorton et al, 2003; McCallum et al, 2006).
The release of ACh onto nAChRs throughout the brain takes place through a series of cholinergic forebrain projections, which include the nucleus basalis of Meynert (NBM) (Johnston et al, 1981; Mesulam et al, 1983); the diagonal band nucleus (Henderson, 1981); nucleus ansa lenticularis (Bigl et al, 1982) and part of the magnocellular preoptic nucleus (Bigl et al, 1982). Approximately two thirds of the NBM neurons projecting to the cortex are cholinergic, with ~30% GABAergic (Fisher et al, 1988), which preferentially connect with GABAergic interneurons (Freund et al, 1992). nAChRs have been shown to modulate presynaptic glutamate release (Gray et al, 1996; McGehee et al, 1999) with further observations of nicotine-induced GABA modulation in multiple brain regions such as the ventral tegmental area (VTA), thalamus, cerebral cortex and hippocampus (Alkondon et al, 1997, 2000; Fisher et al, 1998; Lena and Changeux, 1997; Lena et al, 1993; Mansvelder and McGehee, 2000; Radcliffe et al, 1999). There are also a number of neurotransmitters, which interfere with cortical ACh release, such as DA acting at the NBM (Day et al, 1992); serotonin modulating neuronal activity at the NBM and the release of ACh in the cortex (Hirano et al, 1995) and NE, acting as a tonic inhibitor of cortical ACh release (Telez et al, 1997). Therefore, the distribution of nAChRs is well placed to contribute to the long-term effects of nicotine administration during smoking; particularly upon neuronal networks, which contribute to reward processing and cognitive control.

Nicotine, like other drugs of abuse, activates the mesocorticolimbic DA system (Buisson and Bertrand, 2002; Samaha and Robinson 2005; Wonnacott et al, 2005). The VS has been implicated in the development of nicotine addiction due to its role in processing the hedonic effects of nicotine and signalling the presence of nicotine-related environmental stimuli (Balfour, 2002; Balfour et al, 1998; Janhunen and Ashtee, 2004; Stein et al, 1998). Rat studies have also demonstrated that addictive
drugs, such as nicotine, stimulate DA neurons in the midbrain VTA, resulting in increased burst firing at the shell of the NAcc (Benwell and Balfour, 1992; Corrigall et al, 1992).

Most studies concerning the reinforcing effects of nicotine, however, only use acute administration procedures. Because smoking is a chronic form of behaviour that likely leads to long-term adaptations in the brain, knowledge concerning changes related to its prolonged use is essential. Indeed, animal studies have documented the long-term effects of nicotine, with research showing increased numbers of $\alpha_4\beta_2$ nAChRs in the brain (Marks et al, 1983; Schwartz and Kellar, 1983; Benwell et al, 1988). Interestingly, striatal DA release is modulated by $\alpha_4\beta_2$ nAChRs in rats (Champtiaux et al, 2003; Salminen et al, 2004), with recent research showing that chronic nicotine treatment significantly increases $\alpha_4\beta_2$ nAChRs in the VS of monkeys, together with a corresponding increase in $\alpha_4\beta_2$ evoked DA release in this region (McCallum et al, 2006). Research has also shown that the VS responds selectively to the presence of reward cues in animals (Schultz et al, 1992), with evidence for VS activation during reward anticipation in humans (Knutson et al, 2001). Therefore, nicotine induces cellular adaptations in a brain region strongly implicated in reward processing and drug addiction, with the current research hypothesis suggesting that the VS in smokers and former smokers will show altered levels of activity during the anticipation and the delivery of monetary rewards.

With respect to the effects of nicotine dependence on behavioural and cortical functioning, smokers do not perform as efficiently as non-smokers on tasks which require working memory (Ernst et al, 2001; Spilich et al, 1992), known to activate the lateral PFC (Braver et al, 2001; Meyer-Lindenberg et al, 2001; Zurowski et al, 2002).
Smokers also exhibit higher rates of impulsivity than the general population (Mitchell, 1999; Waldeck and Miller, 1997) and show neuropsychological evidence for diminished PFC functioning (Brower and Price, 2001). Additionally, smokers demonstrate state-dependent (abstinence vs. satiety) alterations in reaction time (Hatsukami et al, 1989; Pritchard et al, 1992; Shiffman et al, 1995), arousal (Parrott and Kaye, 1999), motivation (Powell et al, 2002) and sustained attention (Rusted et al, 2000); all of which require some degree of functioning within the ACC (Bench et al, 1993; Critchley et al, 2001; Rauch et al, 1999). Furthermore, long-term nicotine use is also associated with reduced prefrontal grey matter volumes (Brody et al, 2004; Gazdzinski et al, 2005; Gallinat et al, 2006), suggesting that behavioural and functional brain changes related to smoking may be enveloped within changes to brain anatomy. Therefore, given the functions of the PFC in behavioural control, a region also thought to play an integral part in the development of drug dependence (Everitt et al, 2001; Robbins and Everitt, 1999; Volkow et al, 2003a), it is imperative that more research is conducted, investigating PFC neuronal networks, which may underlie nicotine addiction.

Learning and Memory – The Effects of Cannabinoids

Presently, there still remains much debate as to whether the effects of chronic cannabinoids are a consequence of alterations in hippocampal neuronal functioning, or deficits related to both the hippocampus and PFC. Given that regions within the medial temporal lobe receive convergent inputs from unimodal and polymodal association cortices in the temporal, frontal and parietal lobes (Van Hoesen and Pandya, 1975; Van Hoesen et al, 1975; Tranel et al, 1988; Suzuki and Amaral, 1994), it is quite possible that deficits in learning and memory observed in chronic cannabis
users (Grant et al, 2003; Pope et al, 2001; Pope, 2002; Rodgers et al, 2001; Solowij et al, 2002) are the result of related neuronal changes within both these structures, given the cortical and hippocampal distribution of CB₁ receptors (Herkenham et al, 1991; Tsou et al, 1998).

Structures within the medial temporal lobe such as the hippocampus and parahippocampal gyrus are thought to be involved in the "consolidation" of information during learning and memory processing. The hippocampal region plays a crucial role in forming new associations or episodic memories (Sperling et al, 2001; Crane and Milner, 2002; Zeineh et al, 2003), with its extensive connections with the entorhinal cortex thought to be involved in the translation of temporary hippocampal information storage (Rolls, 2000). CB₁ receptors are also expressed at particularly high densities in the dentate gyrus (DG) and cornu amonis (CA) 3 regions of the hippocampus (Herkenham et al, 1991; Tsou et al, 1998) and it is thought that these regions of the hippocampus are imperative to learning. Furthermore, research has found that greater activity in the parahippocampal region during learning predicts more efficient subsequent recall, suggesting a strong association between parahippocampal neuronal activity and memory encoding (Fernandez et al, 1998; Brewer et al, 1998; Wagner et al, 1998).

Presently, much research is being directed at understanding the functional role of CB₁ receptors within various brain areas, especially the hippocampus, and how learning and memory processes relate to the effects of cannabinoids at these receptors. Early studies have revealed that Δ⁶THC reduces the uptake of ACh in the hippocampus, appearing to restrict ACh synthesis (Lindamood et al, 1980, 1981); with more recent
evidence that cannabinoids inhibit the release of ACh through CB₁ receptors on cholinergic nerve terminals (Gifford et al, 1996, 1997, 2000; Kathmann et al, 2001; Carta et al, 1998; Gessa et al, 1997, 1998). Given that the cholinergic septohippocampal pathway is important for learning and memory (Gifford and Ashby, 1996), it is reasonable that impairments in regular and chronic users of cannabis are, in some way, linked to the effects of Δ⁶-THC on ACh functioning.

Research has shown that the stimulation of CB₁ receptors on presynaptic glutamate nerve terminals inhibits N-Q type calcium channels via an inhibitory G-protein (Shen et al, 1996; Shen and Thayer, 1998; Sullivan, 1999). Synapses at the CA3-CA1 region of the hippocampus appear to be involved in the facilitation of memory encoding within the endocannabinoid system. The synthesis of 2-arachidonylglycerol in the dendritic spines of hippocampal pyramidal neurons leads to the long-term depression of neighbouring GABA terminals, such that adjacent excitatory synapses are primed as a result of a reduction in the threshold for long-term potentiation (LTP) (Zhu and Lovinger, 2007; Chevaleyre and Castillo, 2003, 2004; Carlson et al, 2002). Cannabinoid receptor activation has been shown to inhibit LTP in the hippocampus (Nowicky et al, 1987; Collins et al, 1994; Collins et al, 1995; Terranova et al, 1995; Misner and Sullivan, 1999) providing potential support for the memory disrupting effects of cannabinoids on glutamatergic functioning. Therefore, there appears to be evidence for a potential cannabinoid-induced disruption of synaptic plasticity following the stimulation of CB₁ receptors, providing an additional mechanism by which hippocampal learning and memory impairments may arise in chronic cannabis users. There is also evidence that the stimulation of CB₁ receptors by cannabinoids influence GABAergic activity in the hippocampus. Activation of presynaptic CB₁
receptors has been shown to reduce the release of GABA (Katona et al, 1999; Katona et al, 2000; Hoffman et al, 2000; Wilson et al, 2001), with reductions thought to increase non-specific excitatory firing (noise) in the hippocampus, reducing normal neuronal functioning at this structure.

Evidence now suggests that cannabis impairs learning and memory in rodents (Heyser et al, 1993; Terranova et al, 1996; Nava et al, 2001) and non-human primates (Evans, 1992), which may be related to disruptions in hippocampal neurotransmission and/or the neurotoxicity potential of cannabis on hippocampal integrity. As stated, the effects of cannabis are mediated through the CB₁ receptor in the brain (Matsuda et al, 1990; Herkenham et al, 1991; Tsou et al, 1998), with evidence that chronic cannabinoid administration in rats (Scallet, 1991; Landfield et al, 1988), and monkeys (Harper et al, 1977; Heath et al, 1980) results in hippocampal neuronal damage. While there is still inconclusive evidence that cannabis is neurotoxic in humans, Matochik et al (2005) observed that frequent cannabis users had lower grey matter tissue densities in the right parahippocampal gyrus, with Arnone et al (2008) providing some evidence for the damaging effects of cannabis on the corpus callosum.

Animal studies have additionally shown that cannabis exerts some of its intoxicating effects upon learning via the hippocampus (Carta et al, 1998; Collins et al, 1995; Gessa et al, 1997, 1998; Nava et al, 2001) consistent with hippocampal endocannabinoid receptor density (Herkenham et al, 1991; Tsou et al, 1998). Impairments to learning and memory have been observed in chronic cannabis users (Grant et al, 2003; Pope et al, 2001; Pope, 2002; Rodgers et al, 2001; Solowij et al, 2002), with neuroimaging studies demonstrating decreased memory-related blood

To date, most studies examining many of the underlying neural effects of cannabis use on learning and memory have focussed upon the hippocampal system, where as already stated, there is strong evidence that deficits related to the effects of Δ⁹THC are due to disruptions in hippocampal neural transmission (Carta et al, 1998; Collins et al, 1995; Gessa et al, 1997, 1998; Nava et al, 2001; Herkenham et al, 1991; Tsou et al, 1998). In recent years, evidence regarding the contribution of prefrontal areas to learning and memory, and the effects of cannabis on cortical CB₁ receptors, has shown that prefrontal functionality and cognitive ability may be impaired due to cannabis use (Amen and Waugh, 1998; Yurgelun-Todd et al, 1999; Lundqvist et al, 2001; Eldreth et al, 2004; Bolla et al, 2005; Pillay et al, 2004; Gruber and Yurgelun-Todd, 2005; Chang et al, 2006). Moreover, adaptations in neurochemistry and receptor functioning within prefrontal areas following the chronic use of cannabis may not only contribute to impairments in learning and memory, but more importantly, develop into problems regarding prefrontal-related cognitive control, with memory-related prefrontal deficits an indication of potential impairments to executive functioning.
Reward Processing – Alterations in Reward Circuitry Related to Drug Use

Long-term changes in synaptic transmission as a consequence of prior drug use, represents an appealing mechanism to explain some of the changes in neural circuits that may occur during recreational and compulsive drug consumption (Gerdeman et al, 2003). Drugs of abuse are conceived to commandeer brain mechanisms that have evolved to support beneficial forms of synaptic plasticity, such as those that occur during learning and memory (Gerdeman et al, 2003). In keeping with this hypothesis, several recent studies have demonstrated that synaptic plasticity in brain reward circuits can be initiated or modified by commonly abused drugs (Thomas et al, 2001; Robbe et al, 2002; Saal et al, 2003).

The VS represents a critical site for mediating the rewarding and/or addictive properties of several classes of abused drugs, including cannabis and nicotine (Koob, 1992; Koob et al, 1998; Robbe et al, 2002). Research has demonstrated that chronic nicotine treatment alters VS α4β2 nAChRs (McCallum et al, 2006), and that the inhibitory effects of Δ⁹THC on glutamate and GABA at the VS are greatly diminished following chronic cannabinoid treatment (Hoffman et al, 2003). In human smokers, there are reductions in D₁ and D₂ receptor density in the VS, suggesting the induction of a hypodopaminergic state due to prolonged tobacco use (Dagher et al, 2001; Fehr et al, 2008). There are also reductions in CB₁ receptor density in the VS in humans (Villares, 2007) and evidence to suggest an enduring form of neuronal adaptation in VS DA neurons after subchronic cannabinoid intake at a young age in animals (Pistis et al, 2004). Only one study to date, however, has examined the effects of cannabis on human striatal DA integrity (Sevy et al, 2008), failing to observe any deficit in DA D₂ receptor density. Therefore, potential changes in receptor functioning and synaptic
transmission, resulting from chronic prior nicotine and cannabis use, represent a mechanism by which these drugs may differentially alter reward processing in the brain (Gerdeman et al, 2003). By the same token, pre-existing differences in VS reward processing may be a core pre-requisite to substance use, invoking cannabis and nicotine self-administration.

In contrast to increases in DA transmission observed during acute drug intake, human imaging studies have shown that in drug-addicted individuals, DA levels in the VS are acutely diminished (Volkow et al, 1997). Also, long-lasting reductions in DA D\textsubscript{2} receptors have been observed in drug abusers (Volkow et al, 1990; 1993; 1996; Fehr et al, 2008), an observation which lends credence to the supposition that as drug associated experiences gain in significance, the threshold required for natural reinforcers to activate DA at the VS, increases (Martin-Solch et al, 2001b). This observation of reduced DA functioning in the VS of drug users, poses an important question concerning the processing of non-drug rewards within the mesocortical limbic system. How, for example, do alterations in VS-dependent reward processing relate to the long-term use of drugs such as cannabis and nicotine, and are there differences or similarities in activation patterns between these groups? Furthermore, how may reward processing in the VS in cannabis and nicotine users relate to drug use history, and more importantly, the number of successful periods of previous drug abstinence?
There are different theoretical positions regarding the role of DA, which may have implications for the way in which it contributes to addiction. Berridge and Robinson (1998), for example, have suggested that DA systems mediate the incentive salience of rewards, modulating their motivational value in a manner separable to that of hedonia and reward learning. These systems may be more important to incentive salience attributions to the neural representations of reward-related stimuli. Incentive salience, it has been suggested, is a distinct component of motivation and reward, in which DA systems are necessary for "wanting" incentives, but not for "liking" them or for learning new "likes" and "dislikes". The "dopaminergic reward prediction error" (DRPE) hypothesis has also become a standard model in neuroscience regarding the role of DA in learning. According to this hypothesis, the release of DA is proportional to the difference between the "predicted reward" and the "experienced reward" of a particular event. This model was developed by Schultz et al (1993) and Mirenowicz and Schultz (1994), who showed that DA release occurs in the presence of a clear prior cue to reward, in which the release of DA shifts forward in time to coincide with the cue rather than during reward receipt.

Therefore, in most natural situations where learned associations accompany a repeatedly encountered motivational event, DA is likely to be released as part of the overall experience. Essentially, DA can be viewed as alerting the organism to the appearance of novel, salient stimuli (Berridge and Robinson 1998), thereby, promoting neuroplasticity (learning). Moreover, alerting the organism to the pending appearance of a familiar and motivationally relevant event is based on learned associations made with environmental stimuli predicting the event (Schultz, 1993, 1998; Mirenowicz and Schultz (1994; Keitz et al, 2003).
Numerous imaging studies have assessed neural processing of financial rewards in substance dependence and have found dysfunctional activity within mesocorticolimbic areas. Wrase et al (2007) studied detoxified alcoholics using a monetary incentive delay task (MID) and observed reduced activation in the VS during the anticipation of monetary gain, and increased VS activation in response to alcohol-associated cues. Goldstein et al (2007) assessed cocaine abusers during their performance on a forced-choice task under three monetary value conditions. Results here showed that cocaine abusers demonstrated an overall reduced regional brain response to differences between monetary value conditions. In another study, higher activations in the lateral orbitofrontal cortex/inferior frontal gyrus and amygdala, together with lower activations in the middle frontal gyrus, were associated with degradations of high money amount evaluation in cocaine addicts, appearing to provide evidence for restricted subjective sensitivity to gradients of reward, possibly related to deficits in frontolimbic circuitry (Goldstein et al, 2007). Using feedback to assess responses in mesostriatal and mesocorticolimbic circuits has shown that while healthy controls respond to both monetary and non-monetary reward feedback, opiate addicts only elicit a response in these areas during monetary feedback (Martin-Solch et al, 2001a). Additionally, Martin-Solch and colleagues have found that monetary reinforcement fails to activate typical DA regions, such as the VS, in long-term cigarette smokers, suggestive of neuroadaptive changes in brain centres in nicotine addiction, which are responsible for reward processing (Martin-Solch et al, 2001c).
Based on this small literature concerning non-drug reward processing in substance-dependence, there appear to be variations in the neural responses of different drug-using groups. Alcohol and nicotine users appear to show blunted responses to financial reward (Martin-Solch et al, 2001c; Wrase et al, 2007), whereas users of more illicit, and potentially more deleterious substances show more aberrant activations in limbic forebrain regions (Martin-Solch et al, 2001a; Goldstein et al, 2007). Animal research suggests that the anticipation of reward can elicit DA release at the VS (Schultz et al, 1997), with human imaging studies demonstrating that reward anticipation increases the VS BOLD signal (Knutson et al, 2001). Furthermore, accumulating evidence suggests that the release of DA in response to the anticipation of reward activates DA D₁ receptors at the VS, increasing the BOLD signal in this area (Dixon et al, 2005; Choi et al, 2006). Therefore, neural responses to potential rewards and losses can be successfully modelled through the use of fMRI, thus elucidating the long-term role different drugs may play in modulating the VS DA system and its associated neural circuitry.

Cognitive Control – Response Inhibition and Error Monitoring

Executive functioning is an important and yet poorly understood construct that is invoked within current theoretical models of human cognition. One aspect related to executive functioning and particularly pertinent to the effects of drug use, is that of cognitive control. Cognitive control can be viewed as flexible goal-directed behavior, which requires an adaptive cognitive control system to decide upon contextually relevant information, and for organizing and optimizing processing pathways (Ridderinkhof et al, 2004). Emerging evidence from cognitive neuroscience is now beginning to converge on the different contributions of the PFC in the service of
cognitive control. Furthermore, this convergence of evidence regarding the role of prefrontal functioning in cognitive control is well placed to explicate why certain processes may be compromised in various psychiatric disorders, especially drug dependence, where a loss of cognitive control may be a central component of continual drug abuse.

Alterations in PFC activity in drug addiction are to be expected, given the existing evidence of differences between drug users and controls in brain metabolism, brain morphology, and brain functional activation (Bolla et al, 2004; Liu et al, 1998; Matohichik et al, 2003; Brody et al, 2004; Gazdzinski et al, 2005; Gallinat et al, 2006; Stapleton et al, 1995; Volkow et al, 1992). Importantly, the PFC and ACC regions have been implicated in response inhibition and action monitoring functions (Garavan et al, 1999, 2002; Aron et al, 2003; Watanabe et al, 2002; Carter et al, 1998; Ullsperger and von Cramon, 2001), with both the PFC and ACC known to be affected by drugs of abuse, such as nicotine (Stein et al, 1998). The functioning of the ACC, particularly, has been implicated in addiction and its cognitive sequelae (Goldstein and Volkow, 2002; Peoples et al, 2002; Volkow et al, 2002) and has been found to be hypoactive in drug users during attention switching (Kubler et al, 2005), Stroop (Bolla et al, 2004; Eldreth et al, 2004) and inhibitory control tasks (Hester et al, 2004; Kaufman et al, 2003). The ACC, through its functioning as an action monitoring system in response to errors (Carter et al, 1998; Ullsperger and von Cramon, 2001) has been found to be particularly hypoactive during a GO/NOGO paradigms in cocaine (Kaufman et al, 2003) and opiate addicts (Forman et al, 2004), thereby suggesting the potentially disrupting effects of abused drugs on ACC circuitry.
Diminished ACC activity in response to errors has also been observed on a Stroop task following alcohol administration (Ridderinkhof et al, 2002), in both cocaine (Bolla et al, 2004) and cannabis users (Eldreth et al, 2004), and in schizophrenic and attention deficit hyperactivity disorder (ADHD) groups, in whom monitoring abilities and behavioural control are compromised (Alain et al, 2002; Fallgatter et al, 2004). Evidence also points to the role of DA in error-related processing in the ACC, consistent with the high concentration of DA D2 receptors in this region (Khan et al, 1998; Volkow et al, 1999). Because of the relevance of DA to drug abuse and reward systems, a neural system, such as the ACC might be expected to exhibit deficient error processing following prolonged nicotine exposure. Indeed, Holroyd and Coles (2002) have proposed that the ACC error-related signal is driven by the same mesocorticolimbic DA system that generates VS responses related to expected and unexpected rewards and losses, as reported by Schultz (1998) and Schultz et al (1997). Supporting the role of DA in the ACC, increased error-related negativity (ERN - an electrophysiological component observed for errors) has been observed following amphetamine administration in non-dependent controls (de Bruijn et al, 2003). Given that amphetamine increases synaptic levels of DA, and ERN has been localized to the ACC (Dehaene et al, 1994), this result is consistent with a role for ACC DA in error processing, with the prolonged use and abuse of drugs such as nicotine, potentially impinging upon ACC functioning, and consequently, error monitoring.
Compromised inhibitory control in drug users (Fillmore and Rush, 2002) is an important area of investigation with respect to drug-related executive dysfunction, given the role of the ACC in cognitive control (Garavan et al, 1999, 2002; Aron et al, 2003; Watanabe et al, 2002; Carter et al, 1998; Ullsperger and von Cramon, 2001), the deficits which develop as a consequence of abuse (Kaufman et al, 2003; Forman et al, 2004) and the role these deficits may play in the progression and maintenance of addiction (Goldstein and Volkow, 2002; Peoples et al, 2002; Volkow et al, 2002). Smoking leads to changes in behavioural states associated with ACC functioning, such as improvements in reaction time (Hatsukami et al, 1989; Pritchard et al, 1992; Shiffman et al, 1995), arousal (Parrott and Kaye, 1999), motivation (Powell et al, 2002) and sustained attention (Rusted et al, 2000). Furthermore, smaller grey matter volumes in the dorsal ACC have been found in smokers (Brody et al, 2003), an area believed to play an important role in attention and executive functioning due to its extensive connections with the dorsolateral PFC (Paus, 2001).

Therefore, the assessment of ACC functioning, and its connection to inhibitory control and error monitoring in nicotine addiction, is a key component of the research below. If prolonged nicotine exposure is potentially responsible for deleterious effects on DA functioning in the ACC, then reductions in ACC activity may be expected in the presence of poorer inhibitory and action monitoring in nicotine dependence. Furthermore, given the ACC structural differences previously observed in smokers (Brody et al, 2004; Gazdzinski et al, 2005; Gallinat et al, 2006), tasks which exploit inhibitory functioning and action monitoring related to this structure may reveal whether latent functional impairments exist in former cigarette smokers as a consequence of prolonged nicotine exposure. Moreover, examining the role of the
ACC in former cigarette smokers, successful in remaining abstinent from nicotine, may allow one to address neural mechanisms which have evolved to facilitate smoking abstinence.

**Cognitive Control – Attentional Bias to Drug-Related Stimuli**

Recently, interest has emerged regarding the idea that drug-users show biased attention toward drug-related stimuli. Conditioned responses to drug-related stimuli are known to motivate drug taking, with the notion that a biased attention toward these cues may play an important role in continued drug use and relapse following treatment. There have been a number of behavioural studies which have demonstrated an attentional bias towards drug-related stimuli in cannabis (Field et al, 2004; Field, 2005), cocaine (Hester et al, 2006; Vadhan et al, 2007), heroin (Franken et al, 2000, 2004; Lubman et al, 2000) and heavy alcohol users (Townsend and Duka, 2001). Functional neuroimaging (fMRI) has also demonstrated that cues responsible for evoking drug-craving activate an integrated network of brain regions involved in the motivational and appetitive processes of addiction (Breiter et al, 1999; Koob and Le Moal, 2001; Volkow et al, 2003b). Indeed, fMRI studies have consistently shown drug-related versus neutral stimuli activity in the medial PFC and ACC in alcohol (Grusser et al, 2004; Tapert et al, 2003, 2004), cocaine (Maas et al, 1998; Garavan et al, 2000; Goldstein et al, 2007) and nicotine dependence (David et al, 2005; Smolka et al, 2006; McClernon et al, 2007), suggesting a pivotal role for medial prefrontal areas in response to drug cues.
As stated above, the major neural substrates underlying addiction are thought to be made up of an independent and overlapping circuit network in the brain consisting of (i) reward, located within the NAcc, ventral pallidum and hypothalamus; (ii) motivation and/or drive located in the orbitofrontal cortex; (iii) memory and learning, located in the amygdala and hippocampus and (iv) cognitive control, located in the PFC and dorsal ACC. According to one model put forward by Volkow et al (2003a), drug addiction and the strength of inhibitory control over drug-taking behaviour is reduced as the saliency value of a drug, and those cues associated with it, is strengthened. This model of addiction, implicating neuroadaptive processes in these four independent, but reciprocally connected circuits of the brain, provides an appealing base from which to employ cognitive emotional tasks which potentially exploit interactions between salience attribution in the VS and cognitive control in the ACC. Also, the model postulated by Bechara (2005), suggesting that drugs of abuse trigger “bottom-up” involuntary signals originating in the amygdala, commandeering the “top-down” cognitive control required for the normal operations, represents an equally appealing model in which to test hypotheses regarding attentional bias and executive functioning in addiction.

The VS has been implicated in both the rewarding effects of nicotine and in the development of nicotine craving (Brody et al, 2004; Heinz et al, 2004). The NAcc component of the VS contains two functionally distinct subcompartments, in the form of the shell and core (Kelley, 2004). Reciprocal DA innervation from the VTA to the NAcc shell plays an important role in modulating motivational salience and contributes to establishing learned associations between motivational events and concurrent environmental perceptions (Bassareo and Chiara, 1999; Sellings and
Clark, 2003). The core compartment of the NAcc is anatomically connected with the ACC and orbitofrontal cortex, and appears to be a primary site mediating the expression of learned behaviors in response to stimuli predicting motivationally relevant events (Kelley, 2004; Di Ciano and Everitt, 2001). As stated there is evidence that chronic nicotine treatment alters VS (i.e. NAcc) α4β2 nAChRs (McCallum et al, 2006), and reduces D1 and D2 receptor density in the VS (Dagher et al, 2001; Fehr et al, 2008). Furthermore, there is also evidence that the core component of the NAcc becomes sensitized following prolonged exposure to addictive drugs such as nicotine (Balfour et al, 1998). Here it is proposed that this sensitization is related to an enhanced burst firing of mesoaccumbens neurons, which results in an increase in DA release into the extracellular space between the cells where it acts upon putative extrasynaptic dopamine receptors.

Sustained cue-induced changes in DA discharge may code reward uncertainty and elicit a selective form of attention or arousal (Fiorillo et al, 2003) in drug addiction. Cue-induced activation of the ACC and the adjacent medial prefrontal cortex may mediate an attentional response to drug cues (Breiter et al, 1997; Tzschentke, 1998); while cue-induced DA release in the dorsal striatum may trigger relapse to drug-taking behaviour (Ito et al, 2002). Importantly, smokers treated with bupropion hydrochloride have been shown to elicit diminished cue-induced cigarette craving and cigarette cue-induced ACC activation (Brody et al, 2004) following treatment, an observation which may further endorse the role of DA functioning at the ACC in nicotine addiction, particularly with respect to potential drug relapse. This latter finding may also bestow credence upon the incentive sensitization model set out by Robinson and Berridge (1993, 2001), suggesting that neuroadaptations within the VS
DA system mediate an excessive attribution of incentive salience to drug-related stimuli.

Therefore, potential alterations in DA functioning within the NAcc, as a consequence of prolonged nicotine exposure, may interfere with the normal role of DA neurotransmission in the error-detection signal, which would normally indicate the availability of reward (Schultz et al, 1997). Experimental subjects’ who have a history of nicotine use may, therefore, lack a DA-dependent striatal gating function (O’Reilly et al, 2002), which filters out stimuli that are not followed by reward deliveries (Contreras-Vidal and Schultz, 1999). Consequently, nicotine-associated stimuli that are not followed by nicotine intake might elicit an excessive activation of the medial prefrontal cortex and ACC. This notion of ACC “hyperactivity” in nicotine users in response to drug-related stimuli is diametrically opposite to the aforementioned concept of ACC “hypoactivity” during inhibitory functioning and action monitoring in nicotine addiction, thus suggesting potential discrepancies in the integrity of the same neural anatomical system.

While past studies in nicotine users have explored the impact of nicotine-related cues on NAcc, medial prefrontal cortex and ACC functioning (David et al, 2005; Smolka et al, 2006; McClernon et al, 2007), a perennial issue within nicotine addiction research concerns the prevention of relapse and continued recovery. The “cognitive” dorsal ACC has previously been implicated in demanding tasks that involve stimulus-response selection in the face of competing streams of information including Stroop-like tasks (Bush et al, 2000), and particularly in conflict resolution (Carter et al, 1998) encompassing emotional valence (Davis et al, 2005). Using a paradigm, in which
subjects' are required to make stimulus-response selections concerning neutral, evocative and nicotine-related stimuli, the current research additionally sought to replicate the extant literature concerning VS, medial prefrontal cortex and ACC functioning in current smokers with respect to salience attribution and attentional bias; as well as explore the behavioural and neural responses of former smokers who have undergone a protracted period of nicotine abstinence.

Executive Summary

As stated above, research has shown that brain circuits involved in cognition may be disrupted by drugs of addiction such as cannabis and nicotine, eliciting disturbances in reward processing, salience attribution, memory consolidation, together with inhibitory control and action monitoring. While previous research findings attest to the potentially deleterious long-term effects of various addictive drugs on brain functioning, there still remains uncertainty with respect to how different substances affect different cognitive processes. The major neural substrates underlying addiction, it is believed, are made up of a series of independent and overlapping circuits related to reward, motivation and/or drive, memory and learning and cognitive control; all of which are targeted by drugs such as cannabis and nicotine. Functional neuroimaging studies that focus on distinct, well-characterized cognitive processes believed to be related to these circuits, afford neuroscience the chance to understand the behavioural-brain processes of drug users.
The excessive use of cannabis has become a growing concern in Western societies. Cannabis is often considered to be a benign substance, making it a commonly abused drug, often administered in conjunction with alcohol and tobacco. Despite an increased understanding concerning the pharmacological effects of cannabis, there has been little research examining the long-term effects of its use upon learning, memory and reward processing. There is a particular need for further and more specialised research into the long-term effects of cannabis upon cognition, given that abusers of “hard” drugs commonly report cannabis as their first recreational drug of use. Given the implications of drug use upon learning, memory and reward functioning, it is imperative that further studies investigate how alterations in such processes may contribute to further drug use behaviour and particularly, the transition from a commonly abused drug such as cannabis to more malevolent substances. The aim of the current research reported below, therefore, is to examine alterations in brain areas related to these functions in chronic recreational cannabis users who have had little or no exposure to harder drugs of abuse.

The majority of cigarette smokers endorse the desire to give up smoking, but only a small percentage will ever achieve full nicotine abstinence. Despite their best efforts and expressed preferences, nicotine-dependent individuals often appear incapable of exerting sufficient control over their smoking urges and smoking behaviour. This inability to exert control appears to implicate potential disturbances in reward processing, salience attribution, and ultimately, diminished cognitive control. Because cigarette smoking presents with considerable health risks and induces high costs on health care resources, there is a pressing need to understand, in greater detail, the
effects of nicotine addiction on the brain, and how changes in functioning contribute to nicotine abstinence and its continued use.

Investigating alterations in reward processing, salience attribution and cognitive control are perennial issues in drug addiction research, given evidence that drugs such as nicotine potentially alter the integrity of VS, orbitofrontal and PFC/cingulate circuits, which exacerbate addictive behaviour. Particularly important is the functioning of these circuits in individuals who have successfully renounced nicotine use and how differences in former smokers may elucidate neural mechanisms which have developed to facilitate nicotine abstinence. By the same token, exploring neural processes related to reward, salience attribution and cognitive control in former smokers, one may expound upon those neural substrates which are required to initiate and maintain abstinence in current smokers wish to give up. Therefore, gathering evidence that these alterations exist in former nicotine users leads one to query whether it is possible to use behavioural and/or pharmacological treatments to alter reward, attention and cognitive control processes, further complimenting the amelioration of nicotine dependence. The aim of the current research, therefore, is to also examine alterations in brain areas related to nicotine addiction and successful nicotine abstinence, providing evidence for neural differences in reward processing, salience attribution and cognitive control.
Summary of Objectives

1. Investigate behavioural and neural differences in learning and memory in cannabis users and demographically matched controls using a task shown to be sensitive to the integrity of (para)hippocampal functioning.

2. Investigate neural differences in the anticipation of non-drug rewards and losses in chronic recreational cannabis users and demographically matched controls using a task shown to elicit activation in the ventral striatum.

3. Investigate neural differences in the anticipation of non-drug rewards and losses in demographically matched smokers, ex-smokers and controls using a task shown to elicit activation in the ventral striatum.

4. Investigate neural responses related to attenional bias of nicotine-related stimuli in demographically matched smokers, ex-smokers and controls using an attentional bias paradigm.

5. Investigate neural differences related to inhibitory control and action monitoring in demographically matched smokers, ex-smokers and controls using a GO-NOGO task.
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Chapter 2 - Learning and Memory in Cannabis Users
Abstract

The consumption of cannabis has been linked to impairments in human learning and memory, as well as aspects of executive functioning. Cannabis-related impairments in learning and memory in chronic cannabis users, it has been argued, are caused by the effects of cannabis on hippocampal functioning. The current study involved two experiments. Experiment 1 compared 35 current users of cannabis and 38 well matched controls on a face-name task, previously shown to activate the hippocampal region. Based on the results of experiment 1, experiment 2 used fMRI and a modified version of the face-name task, to examine cortical and (para)hippocampal activity during learning and recall in 14 current users of cannabis and 14 controls. Results of experiment 1 showed that cannabis users were significantly worse with respect to learning, short and long-term memory performance. Experiment 2 showed that despite non-significant differences in learning and memory performance, cannabis users had significantly lower levels of BOLD activity in the right superior temporal gyrus, right superior frontal gyrus, right middle frontal gyrus and left superior frontal gyrus compared to controls during learning. Results also showed that cannabis users had significantly higher BOLD activity in the right parahippocampal gyrus during learning. Hypoactivity in frontal and temporal cortices, and relative hyperactivity in the parahippocampus, identify functional deficits and compensatory processes in cannabis users.
Introduction

The consumption of cannabis has been linked to impairments in human learning and memory, as well as aspects of executive functioning (Grant et al, 2003; Pope et al, 2001; Pope, 2002; Rodgers et al, 2001; Solowij et al, 2002; Pope and Yurgelun-Todd, 1996; Bolla et al, 2002). The effects of cannabis in animals are mediated by cannabinoid CB₁ receptors, which are expressed at especially high densities in the dentate gyrus (DG) and cornu amonis (CA) 3 regions of the hippocampus (Herkenham et al, 1991; Tsou et al, 1998). The hippocampus is a brain structure strongly implicated in both declarative and episodic memory (Sperling et al, 2001; Crane and Milner, 2002; Zeineh et al, 2003). Cannabis-related impairments in learning and memory in chronic cannabis users, it has been argued, are caused by the effects of cannabis on the hippocampus via their influence on CB₁ receptors (Herkenham et al, 1991; Tsou et al, 1998). Despite some of the neuropsychological literature indicating learning and memory deficits in chronic cannabis users (Grant et al, 2003; Pope et al, 2001; Pope, 2002; Rodgers et al, 2001; Solowij et al, 2002), the neurobiology underlying these deficits has yet to be fully clarified.

Evidence now suggests that cannabis impairs learning and memory in rodents (Heyser et al, 1993; Terranova et al, 1996; Nava et al, 2001) and non-human primates (Evans, 1992), which may be related to the neurotoxicity potential of cannabis for hippocampal neurons. The effects of cannabis are mediated through the CB₁ receptor in the brain (Matsuda et al, 1990; Herkenham et al, 1991; Tsou et al, 1998), with evidence that chronic cannabinoid administration in rats causes distinct hippocampal morphological changes (Scallet, 1991; Landfield et al, 1988). While there is still inconclusive evidence that cannabis is neurotoxic in humans, Matochik et al (2005)
showed that frequent cannabis users had lower grey matter tissue densities in the hippocampus bilaterally, providing some evidence for the effects of cannabis on human hippocampal integrity.

Animal studies have also shown that cannabis exerts some of its impairing effects via the hippocampus during learning (Carta et al, 1998; Collins et al, 1995; Gessa et al, 1997, 1998; Nava et al, 2001), consistent with the high density of hippocampal endocannabinoid receptors (Herkenham et al, 1991; Tsou et al, 1998). Impairments to learning and memory have been shown in chronic cannabis users (Grant et al, 2003; Pope et al, 2001; Pope, 2002; Rodgers et al, 2001; Solowij et al, 2002), with imaging studies specifically demonstrating decreased memory-related blood flow in the prefrontal cortex and lower activation in the parahippocampus during verbal memory and associative learning tasks (Block et al, 2002; Jager et al, 2007). Studies in animals also suggest that some of the memory-impairing effects of cannabinoids occur in the prefrontal cortex (Diana et al, 1998; Jentsch et al, 1997; 1998; Verrico et al, 2003), with human imaging studies in cannabis users also demonstrating prefrontal hypoactivity (Amen and Waugh, 1998; Yurgelun-Todd et al, 1999; Lundqvist et al, 2001; Eldreth et al, 2004; Bolla et al, 2005; Pillay et al, 2004; Gruber and Yurgelun-Todd, 2005; Chang et al, 2006). Given the effects of cannabis on hippocampal and cortical functioning in animals, together with some of the neuropsychological and imaging evidence, there remains a need to use paradigms which engage cortical and hippocampal-dependent learning and memory in humans, thereby elucidating the long-term effects of cannabis use on different neuronal networks of the brain.
The hippocampal region plays a crucial role in forming new associations or episodic memories, including memories for faces (Sperling et al, 2001; Crane and Milner, 2002; Zeineh et al, 2003). Using a face-name task, Zeineh and colleagues showed that learning associations between faces and names most prominently activates the anterior CA 2 and 3 fields and the DG of the hippocampus. Based on the extant literature concerning behavioural and functional activity differences in cannabis users related to learning and memory (Grant et al, 2003; Pope et al, 2001; Pope, 2002; Rodgers et al, 2001; Solowij et al, 2002, Block et al, 2002; Jager et al, 2007), the current study reports two separate experiments. In experiment 1, high functioning regular users of cannabis and demographically matched controls were compared behaviourally, using a face-name task previously shown to engage the hippocampal formation (Zeineh et al, 2003). Arising from the observation of performance differences in experiment 1, experiment 2 compared brain activity, under fMRI conditions, between cannabis users and drug-naive controls using a modified version of the face-name task. Given the effect of chronic cannabis use on this structure, and the potentially taxing effects of face-memory learning on hippocampal functioning, we hypothesised the following. In experiment 1, cannabis users would show inferior learning and memory performance for face-name associations, with performance related to life-time cannabis use; and in experiment 2, using a modified version of the face-name task, cannabis users would show dysfunctional hippocampal and prefrontal-dependent activity during learning, with altered activity related to life-time cannabis consumption.
Experiment 1

Material and Methods

Participants

35 current users of cannabis and 38 controls were recruited from the general public and academic institutions around Dublin city. All participants underwent a comprehensive telephone screening, during which detailed information concerning past and present psychiatric, neurological and substance use was taken. Information pertaining to any form of treatment (counselling, psychological, psychiatric), past or present, was carefully detailed, with any potential participant describing any major life-time psychiatric event or head injury (e.g., head trauma resulting in a loss of consciousness, seizure or stroke) considered ineligible for the study. Cannabis and control participants also completed inventories for mood (BDI II) and drug use (questionnaire taken from the Addiction Severity index Lite-CF) (see questionnaires section below) prior to testing, to screen for depression and past or concurrent abuse of other substances. Therefore, cannabis and controls participants were additionally considered ineligible if they reported concurrent or past dependence on other drugs (e.g., alcohol, amphetamines, benzodiazepines, cocaine, MDMA, hallucinogens and opiates) at the practice session prior to testing. No cannabis or control participants reported the current or past use of any other psychoactive substances (e.g., nicotine, nutraceuticals). All information concerning drug use in each participant was indexed in years (life-time) and recent (last 30 days) and fully recorded prior to testing.

Cannabis participants were required to have regularly consumed cannabis (5-7 days/week) for the previous 2 years in order to be eligible as cannabis users for experiment 1. All cannabis users provided a positive urine sample for Δ⁹-
tetrahydrocannabinol ($\Delta^8$THC) prior to behavioural testing, with additional screening for methadone, benzodiazepines, cocaine, amphetamine, opiates, barbiturates and tricyclic antidepressants (Cozart® RapiScan, UK) taking place. While the identification and quantification of cannabis metabolites in urine may have proved advantageous as a potential predictor of cognition and brain functioning, past studies have reliably shown that estimates of recent use, life-time use and age of onset of use, are reliable predictors of behavioural impairments and BOLD activity in cannabis users (Block & Ghoneim, 1993; Bolla et al, 2002; Pope & Yurgelun-Todd, 1996; Solowij et al, 2002; Block & Ghoneim, 1993; Solowij, 1995; Pope et al, 2003; Bolla et al, 2005; Chang et al, 2006). Control participants were also tested for $\Delta^8$THC and the above adulterants. Nine control participants reported past infrequent use of cannabis (<10 times lifetime use). None of the controls used cannabis in the 30 days preceding study participation (see Table 1). The sample of cannabis users reported a mean lifetime consumption of 5.7 years (range = 1.5-17), a mean 23 days (range = 7-30) of use in the 30 days preceding study participation and had been abstinent from cannabis, on average, for 15 hours (range = 2-45) prior to testing. All research participants provided informed consent and were financially compensated.

**Learning and Memory Face-Name Pairs Task**

The Face-name learning task was adapted from a paradigm in Zeineh et al (2003), and modified to provide a serial-learning format similar to that of the Rey Auditory Verbal Learning Test (RAVLT) (Rey, 1941, 1964). The task structure included learning, distraction and recall phases, with the learning phase requiring participants to study eight serially presented pairs of faces and names (each presented for 3.5 seconds). A distracter task was inserted between each learning and recall phase to prevent rote
rehearsal of the face-name associations. The distracter task required participants to press a button (the "1" key on the key pad) each time a central visual display (an empty circle) contained a black star (See Figure 1 below). Eight distracter trials, separated by intervals of 2 to 5 seconds were presented prior to the beginning of the cued recall phase. During each recall trial participants were presented (in random order) with one of the eight ‘learning phase’ faces (for 3.5 seconds), and required to verbally respond with the correct name association. The learning, distraction, and recall procedure was repeated five times for the original set of faces, following which the procedure was conducted with a new set of unfamiliar faces, which acted as a “diversion memory set.” Immediately following the recall phase of the diversion set, participants were once again presented with the original set of faces (in random order) and asked to correctly identify their names with a verbal response. This constituted the “short delay” component of the face-name cued recall task. Approximately 25 minutes later, participants were again presented with the original set of faces (in random order), and asked to correctly identify the name (with a verbal response) associated with each face. This constituted the “long delay” component of the face-name cued recall task. Finally, participants completed the recognition component of the task, during which they were presented with 24 faces (each presented for 3.5 seconds in a randomised order), eight of which were part of the original set and 16 of which were from either the diversion set or previously unseen. Here, participants were required to press a button (i.e., the “1” key on the key pad) only when they were presented with a face from the original set of faces.
A series of dependent measures were derived from this task: learning curve (trials 1-5), learning performance (sum of trials 1-5), short and long delay recall, percentage recall consistency \( \left( \frac{100}{\text{sum of trials 1-4}} \times \text{sum of conjoint recalls of faces between trials 1, 2; 2, 3; 3, 4; 4, 5} \right) \), which is a measure of both working memory and organization of memory (based on Delis et al, 1987) and the percentage recognition score, which constituted the number of original faces correctly identified. The task was programmed and run using *E-Prime version 1.1* (Psychology Software Tools, Pittsburgh, USA).

**Figure 1 Experiment 1.** Face-name cued recall task in which participants were required to learn and recall face-name pairs over a total of 7 trials.
**Questionnaires**

The National Adult Reading Test (NART) (Nelson & O'Connell, 1978) and the Beck Depression Inventory-II (Beck et al, 1996) were administered to all participants during the testing session. Information concerning recent and lifetime alcohol and drug use (see Table 1) was obtained from all participants using a questionnaire taken from the Addiction Severity index *Lite-CF* (McLellan et al, 1992).

**Results**

*Demographics and drug use*

Demographic data for the two groups are shown in Table 1. Overall, the groups did not differ significantly on any variable except lifetime alcohol use (*p*<0.05). Despite this group difference, there were no associations between reported alcohol use and face-name memory performance. 45% of the cannabis group reported past MDMA use, with 4 cannabis users reporting minor usage of MDMA in the last month, despite urinalyses indicating an absence of MDMA in these participants. A comparison of cannabis users with no history of MDMA use (*n*=19) and those with a history of use (*n*=16), on all components of the face-name cued recall task revealed no significant differences, nor were there any significant correlations between MDMA use and behavioural performance in those subjects with a history of MDMA use. Exploration of MDMA use in the cannabis group also showed that both lifetime and recent use were significantly positively skewed. These data were, therefore, appropriately log transformed and used, together with lifetime alcohol use, as co-variates in all analyses of behavioural data.
Table 1 Experiment 1. Mean and SEM for control and cannabis groups on demographic and drug use history (*p<0.05; **p<0.01, ***p<0.001 versus control group).

<table>
<thead>
<tr>
<th></th>
<th>Control (n=38)</th>
<th>Cannabis (n=35)</th>
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<tbody>
<tr>
<td>Age</td>
<td>22.0 ± 0.4</td>
<td>22.3 ± 0.5</td>
</tr>
<tr>
<td>Years of Education</td>
<td>16.0 ± 0.3</td>
<td>16.0 ± 0.3</td>
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<tr>
<td>Verbal Intelligence Score (NART)</td>
<td>121.8 ± 0.6</td>
<td>120.1 ± 0.8</td>
</tr>
<tr>
<td>Beck Depression Inventory II Score</td>
<td>5.2 ± 0.7</td>
<td>4.7 ± 0.6</td>
</tr>
<tr>
<td>Males/Females</td>
<td>29/9</td>
<td>32/3</td>
</tr>
<tr>
<td>Years of Alcohol Use</td>
<td>5.2 ± 0.4</td>
<td>6.9 ± 0.5*</td>
</tr>
<tr>
<td>Alcohol Use in the Last Month (no. days)</td>
<td>6.9 ± 0.9</td>
<td>9.2 ± 1.0</td>
</tr>
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<td>Alcohol Use Age of Onset (Years)</td>
<td>16.7 ± 0.3</td>
<td>15.7 ± 0.4</td>
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<tr>
<td>Amphetamine Use (Years)</td>
<td>1.0 ± 1.0</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>Amphetamine Use in the Last Month (no. days)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Cocaine Use (Years)</td>
<td>2.0 ± 0.7</td>
<td>1.3 ± 0.3</td>
</tr>
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<td>Cocaine Use in the Last Month (no. days)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>MDMA Use (Years)</td>
<td>0.0 ± 0.0</td>
<td>0.9 ± 0.2</td>
</tr>
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<td>MDMA Use in the Last Month (no. days)</td>
<td>0.0 ± 0.0</td>
<td>0.2 ± 0.1</td>
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<tr>
<td>Hallucinogenic Use (Years)</td>
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<td>0.2 ± 1.0</td>
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<td>Hallucinogenic Use in the Last Month (no. days)</td>
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<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Cannabis Use (Years)</td>
<td>0.2 ± 0.1</td>
<td>5.7 ± 0.6***</td>
</tr>
<tr>
<td>Cannabis Use in the Last Month (no. days)</td>
<td>0.0 ± 0.0</td>
<td>23.1 ± 1.0***</td>
</tr>
<tr>
<td>Cannabis Age of Onset (Years)</td>
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<td>16.5 ± 0.4**</td>
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<td>Years of Nicotine Use</td>
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<td>Nicotine Use in the Last Month (no. days)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

Memory Performance

Memory performance for the two groups is shown in Figure 2. A two (group) by five (trial) repeated measures analysis of variance (ANOVA) found a significant main effect of trial (F=106.7, df=4,68, p<0.001), reflecting an increase in memory performance over the first five trials. There was also a significant group effect (F=12.7, df=1,71, p<0.01) with mean scores indicating cannabis users had lower
overall levels of recall performance when compared to controls. These group differences still remained when co-varying for lifetime alcohol (F=8.3, df=1,70, p<0.01), lifetime MDMA (F=4.4, df=1,70, p<0.05) and recent MDMA use (F=13.0, df=1,70, p<0.01). Analyses revealed no trial by group interaction (F=1.3, df=4,68, p=0.3), suggesting there was no difference between groups in the gradient of the learning curves. Independent t-tests showed that the two groups significantly differed on the short (p<0.01) and long (p<0.01) delay recall components of the task. Univariate analyses showed that inferior short delay performance remained when controlling for lifetime alcohol (F_{1,70}=6.0, p<0.05), lifetime MDMA (F_{1,70}=5.0, p<0.05) and recent MDMA use (F_{1,70}=8.7, p<0.01), as did long delay performance when controlling for lifetime alcohol (F_{1,70}=5.2, p<0.05), lifetime MDMA (F_{1,70}=3.7, p<0.05) and recent MDMA use (F_{1,70}=7.8, p<0.01).

![Figure 2 Experiment 1](image)

**Figure 2 Experiment 1.** Mean performance on the first five trials, trial 6 (short delay) and trial 7 (long delay) of the face-name cued recall task in controls and cannabis users (means and standard error means). Learning curves (trials 1-5) were analyzed using a repeated measures design. Performances on trials 6 and 7 were analyzed using independent tests (**p<0.01; ***/p<0.001).
Figures 3 and 4 below show significant differences between the two groups on learning performance ($p<0.001$) and percentage recall consistency ($p<0.01$). There was no significant group difference on percentage recognition ($p=0.2$). The inferior learning performance of cannabis users remained when controlling for lifetime alcohol ($F_{1,70}=8.9$, $p<0.01$), lifetime MDMA ($F_{1,70}=7.7$, $p<0.01$) and recent MDMA use ($F_{1,70}=13.5$, $p<0.001$) as did recall consistency when controlling for lifetime alcohol ($F_{1,70}=6.6$, $p<0.05$), lifetime MDMA ($F_{1,70}=6.1$, $p<0.05$) and recent MDMA use ($F_{1,70}=10.7$, $p<0.01$).

*Drug use correlations*

There were no correlations between cannabis abstinence or self-reported use of cannabis (e.g., years of use, days of use in last month and age of onset of use) and behavioural performance on the face-name cued recall task in the cannabis-using group.

Finally, correlations between supraspan (recall performance on the first trial), and recall consistency were observed in the cannabis-using group ($r = .56$, $p<0.001$), in the control group ($r = .54$, $p<0.001$) and when the samples were combined ($r = .67$, $p<0.001$).
Figure 3 Experiment 1. Mean learning performance score (LPS) in the control and cannabis groups on the face-name cued recall task (**p<0.001).

![Figure 3: Mean Learning Performance Score](image)

Figure 4 Experiment 1. Mean percentage recall consistency in the control and cannabis groups on the face-name cued recall task (**p<0.01).

![Figure 4: Mean Recall Consistency](image)
Discussion

The results indicate that high functioning chronic cannabis users and non-drug-using controls differed significantly on a task previously shown to actively engage hippocampal functioning. Despite the reported life-time alcohol and MDMA use (which were not found to relate to task performance), the control and cannabis groups were well matched for education, verbal IQ, and mood, thereby avoiding some of the common confounds of previous studies (a cohort effect), which may contribute to group differences. The group differences in this study appear to be consistent with recent findings indicating deficits in learning and memory (Grant et al, 2003; Pope et al, 2001; Pope, 2002; Rodgers et al, 2001; Solowij et al, 2002), supporting the notion that cannabis may impair learning, short and long-term memory processing.

The present results do not appear to be influenced by cannabis intoxication at the time of testing the cannabis-using group. While there is evidence indicating that significant cognitive impairment accompanies cannabis use up to 24 hours after smoking (Robbe & O’Hanlon, 1993), we found no significant associations between hours of abstinence and task performance in our sample of cannabis users. Unlike previous studies (Block & Ghoneim, 1993; Bolla et al, 2002; Pope & Yurgelun-Todd, 1996; Solowij et al, 2002), we found no relationship between the frequency (days) of use in the preceding month and memory performance, nor did we observe any association between lifetime cannabis use or age of cannabis use onset and performance, like previous studies (Block & Ghoneim, 1993; Solowij, 1995; Pope et al, 2003).
The face-name cued recall task has previously been shown to selectively engage the hippocampal formation (Zeineh et al, 2003). The behavioural effects of cannabis on learning and memory appear to be mediated by CB₁ receptors, which, as noted, are expressed at especially high densities in the hippocampus proper and dentate gyrus regions (Herkenham et al, 1991; Tsou et al, 1998). Our high functioning cannabis-using group also showed inferior recall consistency on the face-name task, which may have been due to working memory dysfunction (Alexander et al, 2003) or a reduced organization of memory during learning (Waters and Waters, 1976; Sternberg and Tulving, 1977). In support of deficits in working memory, correlation analyses showed a significant positive relationship between supraspan (recall performance on the first trial), an indirect measure of working memory capacity, and recall consistency in this study. In addition, the learning curves for the two groups were mostly parallel (supported by the lack of a trial x group interaction), suggesting that there was no deficit in learning over-and-above that observed on the first trial, which may support prefrontal/working memory impairment. Studies suggest the memory-impairing effects of cannabinoids are the result of prefrontal dysfunction (Diana et al, 1998; Jentsch et al, 1997; 1998; Verrico et al, 2003), with imaging studies confirming prefrontal hypoactivity in cannabis users (Amen and Waugh, 1998; Yurgelun-Todd et al, 1999; Lundqvist et al, 2001; Block et al, 2002; Eldreth et al, 2004; Bolla et al, 2005; Gruber and Yurgelun-Todd, 2005; Chang et al, 2006; Jager et al, 2007).

The hippocampal region plays an important role in forming new associations or episodic memories, including memories for faces (Sperling et al, 2001; Crane and Milner, 2002; Zeineh et al, 2003), with observations that learned associations between faces and names most prominently activate the anterior CA 2 and 3 fields and the DG.
of the hippocampus (Zeineh et al, 2003). The results here suggest that learning and cued recall for face-name associations are significantly compromised in high functioning regular cannabis users, possibly due to cannabis use and its effects on hippocampal and/or prefrontal neuronal functioning. Based on the extant literature concerning both behavioural and functional activity differences in cannabis users related to learning and memory (Grant et al, 2003; Pope et al, 2001; Pope, 2002; Rodgers et al, 2001; Solowij et al, 2002, Block et al, 2002; Jager et al, 2007), and the results of experiment 1, experiment 2 used functional magnetic resonance imaging (fMRI) to further explore the effects of cannabis on both hippocampal and prefrontal functioning within the context of learning and memory processing for faces.
Experiment 2

Material and Methods

Participants

14 cannabis users and 14 control subjects meeting the same criteria as in experiment 1 were recruited from the general public and academic institutions around Dublin. None of these individuals participated in experiment 1. Twelve controls reported past infrequent use of cannabis (no more than 10 times). None of the controls used cannabis in the 30 days preceding study participation (see Table 2). Cannabis users in experiment 2, as in experiment 1, were required to have consumed cannabis 5-7 days/week for the past two years. All participants in the cannabis group were additionally required to have smoked a minimum of 500 joints in their life-time. This additional criterion was introduced to maximize the examination of possible "dose-response" relationships between this index of cannabis consumption and BOLD activity, as addressed in previous studies (Bolla et al, 2005; Chang et al, 2006). Information concerning alcohol, nicotine and cannabis use in each participant was indexed in years (life-time) and recent (last 30 days). Other drug use information in each participant was indexed by the total number of separate occasions (life-time), total number of recent separate occasions (last 30 days) and the length of time (days or months) since a substance was used. Cannabis and control participants did not report the concurrent or past misuse of any other psychoactive substances (e.g., nicotine, neutraceuticals), which may have altered BOLD activity. Cannabis users tested positive for $\Delta^9$THC. The cannabis group reported, on average, 7.2 years (range = 2-16) of lifetime cannabis use, consumption of 7925 joints (range = 988-33,653), an average of 19 days of use in the last 30 days (range = 1-30) and a mean abstinence of 80.8 hours (range = 3-686). All participants were right-handed as confirmed by the
Edinburgh Handedness Inventory (Oldfield, 1971) during the telephone screening process. Control and cannabis participants completing the study were neurologically normal (as confirmed by a registered radiographer who examined each structural MRI). All research participants provided informed consent and were financially compensated.

**Learning and Memory for Faces Task**

The task used for the imaging procedure was adapted from that used by Zeineh et al (2003) and modified\(^1\) from experiment 1, into a face-number associative learning paradigm. The modified task structure involved button-press responses during recall so as to avoid possible head motion associated with verbal responses. It consisted of two runs of three blocks, with each block containing rest, learning, distraction and recall phases. The beginning of each block involved participants resting for 30 seconds. During the learning phase, participants were required to learn eight serially presented face-number pairs (presented for 3 seconds each). The numbers paired with each face were randomly selected from a set including 11, 12, 13, 14, 21, 22, 23 and 24. During recall, these numbers were selected using two handheld keypads (left keypad contained the 1 and 2 keys, right keypad contained the 3 and 4 keys). Following the presentation of each face-number pair, a fixation crosshair was presented for a variable period of 1 to 7 seconds. The face-number association remained consistent throughout each learning phase, with each learning phase lasting 60 seconds. Eight distracter trials were presented between each learning and recall phase, lasting a total of 22 seconds. The distracter task required participants to press a

\(^1\) An initial pilot study comparing the face-name and face-number versions of the task found identical learning and recall performance.
key (the 3 key on the right hand-held keypad) each time the central visual display, an empty circle, contained a black star (see Figure 5). The eight distracter trials were separated by delay intervals of 3 to 5 seconds. During each recall trial, participants were presented (in random order) with one of the eight ‘learning phase’ faces (for 3 seconds), and required to respond with the correct double digit number for each face. A fixation crosshair of variable duration (1 to 7 sec) followed the presentation of each face. Each recall phase was 60 seconds in duration, with a different presentation order administered during each of the six recall blocks. Each run of the face-memory task lasted 570 seconds. Dependent measures for the behavioural task were the learning curve (percentage correct recall on each of the six recall trials) and percentage learning performance (Mean percentage of trials 1-6). The task was programmed using *E-Prime version 1.1* (Psychology Software Tools, Pittsburgh, USA).

**Figure 5 Experiment 2.** Face memory task administered during fMRI data collection. Participants were required to learn and recall face-number pairs over a series of 6 blocks.
Questionnaires

In addition to the National Adult Reading Test (NART) (Nelson & O'Connell, 1978), Beck Depression Inventory-II (Beck et al, 1996) and a drug and alcohol use questionnaire (McLellan et al, 1992) used in experiment 1, cannabis users also provided information concerning withdrawal and cannabis craving prior to scanning. The Marijuana Craving Questionnaire (Heishman et al, 2001) is made up of 12 statements, which the participant has to rate according to a seven-point Likert-type scale from “strongly disagree” to “strongly agree”. Responses to the questionnaire are then divided into four specific constructs made up of compulsivity, emotionality, expectancy and purposefulness related to cannabis use. Information regarding withdrawal, modified from a cocaine withdrawal checklist (Brower et al, 1988) was obtained using a thirty two-item checklist, where participants were required to rate, on a scale of 0 (none) to 3 (severe), symptoms they had experienced in the previous 24 hours.

fMRI acquisition

All scanning was conducted on a Philips Intera Achieva 3.0 Tesla MR system (Best, The Netherlands) equipped with a mirror that reflected the visual display, which was projected onto a panel placed behind the participants’ head outside the magnet. The mirror was mounted on the head coil in the participants’ line of vision.

Each scanning sequence began with a reference scan to resolve sensitivity variations. A parallel Sensitivity Encoding (SENSE) approach (Pruessmann et al, 1999) with a reduction factor of 2 was utilised for all T1-weighted image acquisitions. 180 high-resolution T1-weighted anatomic MPRAGE axial images (FOV 230 mm, thickness
0.9 mm, voxel size 0.9 x 0.9 x 0.9) were then acquired (total duration 325 seconds), to allow subsequent activation localization and spatial normalization.

Functional data were collected using a T2* weighted echo-planar imaging sequence that acquired 32 non-contiguous (10% gap) 3.5 mm axial slices covering the entire brain (TE = 35 ms, TR = 2000 ms, FOV 224 mm, 64 x 64 mm matrix size in Fourier space). The functional scans had a total duration of 570 seconds per run.

**Data processing and analyses**

All analyses were conducted using AFNI software (Cox, 1996). Following image reconstruction, the two 3-D time series (runs 1 and 2) were concatenated and motion-corrected using 3-D volume registration (least-squares alignment of three translational and three rotational parameters). Activation outside the brain was also removed using edge detection techniques.

A block analysis was performed to estimate the activation for the learning and recall periods separately. These ON-OFF block regressors were convolved with a standard haemodynamic response to accommodate the lag time of the blood oxygen level-dependent (BOLD) response. Multiple regression analyses were then used to determine the average level of block activation as a percentage change relative to the distraction period (baseline). The baseline activation was derived from averaging the distraction periods in each block over both runs of the task.
The percentage change map (block activation) voxels were re-sampled at 1 mm³ resolution, then warped into standard Talairach space and spatially blurred with a 3-mm isotropic rms Gaussian kernel. Group activation maps for each condition of the task (learning and recall) were determined with one-sample $t$-tests against the null hypothesis of zero activation change (i.e., no change relative to the distraction-period baseline). Significant voxels passed a voxelwise statistical threshold ($t = 3.4$, $p<0.005$) and were required to be part of a larger 278μl cluster of contiguous significant voxels. Thresholding was determined through Monte Carlo simulations and resulted in a 5% probability of a cluster surviving due to chance.

To compare activations between the control and cannabis groups, thresholded group $t$-test maps for each condition in both groups were combined to form OR maps. For example, the selection OR map includes the significant voxels from either group. This process was performed independently for the learning and recall periods. The mean activation for clusters in the OR map was calculated for the purposes of a whole brain analysis, and these data were used for group independent $t$-tests.

We also performed a small-volume correction region of interest (ROI) analysis, given a priori interest in hippocampal involvement in this task. A second volume threshold was applied for voxels that fell within an anatomically defined medial temporal lobe region that included the hippocampus and parahippocampal gyrus. Significant voxels passed the same voxelwise statistical threshold ($t = 3.4$, $p<0.005$) and were required to be part of a 114μl cluster of contiguous significant voxels. All between-groups analyses of mean activation clusters were conducted using independent $t$-tests in SPSS (SPSS Inc).
Results

Demographics and drug use

Table 2 shows the group demographic and drug use history for both samples. The groups did not significantly differ on age, years of education, pre-morbid intelligence or alcohol and other drug use.

Memory Performance

Figure 6 below shows the learning curves for the two groups over the 6 blocks of recall trials. A two (group) by six (trial) repeated measures analysis revealed an effect of trial (F=32.9, df=5.22, p<0.001), but no effect of group (F=0.78, df=1.26, p=0.39) and no trial by group interaction (F=0.4, df=5.22, p=0.85).

![Figure 6 Experiment 2. Mean percentage performance on trials 1-6 of the fMRI face memory task in controls and cannabis users (means and standard error means).](image-url)
Table 2 Experiment 2. Mean and SEM for control and cannabis groups on demographic and drug use history.

<table>
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<tr>
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<th>Control (n=14)</th>
<th>Cannabis (n=14)</th>
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<td>Age</td>
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<tr>
<td>Years of Education</td>
<td>16.6 ± 0.4</td>
<td>16.0 ± 0.5</td>
</tr>
<tr>
<td>Verbal Intelligence Score (NART)</td>
<td>122.6 ± 0.7</td>
<td>122.9 ± 0.9</td>
</tr>
<tr>
<td>Beck Depression Inventory II Score</td>
<td>3.6 ± 0.8</td>
<td>6.1 ± 1.3</td>
</tr>
<tr>
<td>Females/Males</td>
<td>2/12</td>
<td>2/12</td>
</tr>
<tr>
<td>Years of Alcohol Use</td>
<td>7.7 ± 1.3</td>
<td>9.0 ± 1.2</td>
</tr>
<tr>
<td>Alcohol Use in the Last Month (no. days)</td>
<td>2.2 ± 0.2</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>Alcohol Use Age Onset (Years)</td>
<td>16.1 ± 0.6</td>
<td>15.4 ± 0.5</td>
</tr>
<tr>
<td>Last Alcohol Use (hrs)</td>
<td>118.3 ± 33.5</td>
<td>197.6 ± 153.4</td>
</tr>
<tr>
<td>Years of Nicotine Use</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Nicotine Use in the Lat Month (no. days)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
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<tr>
<td>Amphetamine Use (no. times)</td>
<td>2.9 ± 2.9</td>
<td>2.6 ± 1.0</td>
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<tr>
<td>Amphetamine Use in the Last Month (no. times)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Last Amphetamine Use (mths)</td>
<td>36.0 ± 36.0</td>
<td>42.7 ± 13.7</td>
</tr>
<tr>
<td>Cocaine Use (no. times)</td>
<td>2.2 ± 2.1</td>
<td>6.0 ± 2.9</td>
</tr>
<tr>
<td>Cocaine Use in the Last Month (no. times)</td>
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<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Last Cocaine Use (mths)</td>
<td>40.5 ± 31.5</td>
<td>13.6 ± 6.7</td>
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<tr>
<td>MDMA Use (no. times)</td>
<td>3.3 ± 2.8</td>
<td>9.4 ± 3.7</td>
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<td>MDMA Use in the Last Month (no. times)</td>
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<td>0.0 ± 0.0</td>
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<td>Last MDMA Use (mths)</td>
<td>84.0 ± 45.4</td>
<td>32.0 ± 10.6</td>
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<tr>
<td>Hallucinogenic Use (no. times)</td>
<td>20.0 ± 0.0</td>
<td>10.4 ± 3.4</td>
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<tr>
<td>Hallucinogenic Use in the Last Month (no. times)</td>
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<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Last Hallucinogenic Use (mths)</td>
<td>12.0 ± 0.0</td>
<td>15.0 ± 3.8</td>
</tr>
<tr>
<td>Cannabis Use (Years)</td>
<td>0.0 ± 0.0</td>
<td>7.2 ± 1.1</td>
</tr>
<tr>
<td>Lifetime Joints (number)</td>
<td>5.5 ± 1.5</td>
<td>7925.9 ± 2253.7</td>
</tr>
<tr>
<td>Days of use in last month (number)</td>
<td>0.0 ± 0.0</td>
<td>19.1 ± 2.7</td>
</tr>
<tr>
<td>Joints in last month (number)</td>
<td>0.0 ± 0.0</td>
<td>82.8 ± 19.4</td>
</tr>
<tr>
<td>Cannabis Age of Onset (Years)</td>
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<td>17.0 ± 0.9</td>
</tr>
<tr>
<td>Cannabis Abstinence (Hours)</td>
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<td>10.5 ± 1.8</td>
</tr>
<tr>
<td>Cannabis Withdrawal Score (out of 32)</td>
<td></td>
<td>10.5 ± 1.8</td>
</tr>
<tr>
<td>Cannabis Craving Scores (each item out of 21)</td>
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<td></td>
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<tr>
<td>Compulsivity</td>
<td>6.7 ± 1.2</td>
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</tr>
<tr>
<td>Emotionality</td>
<td>8.4 ± 1</td>
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<tr>
<td>Expectancy</td>
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</tr>
<tr>
<td>Purposefulness</td>
<td>11.6 ± 1.2</td>
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</tr>
</tbody>
</table>

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Whole brain analyses

Table 3 lists the areas of significant activity during the learning and recall phases of the face-memory task. Figure 7 also demonstrates the general patterns of activation in both the cannabis and controls groups during the learning phase of the face-memory task. Five regions were found to have passed the voxel and cluster-size threshold for the learning phase and included the right superior temporal gyrus (RSTG, BA 39), right superior frontal gyrus (RSFG, BA 6 and BA 9), left superior frontal gyrus (LSFG, BA 8), and right middle frontal gyrus (RMFG, BA 8).

Figure 7 Experiment 2. Activation ttest maps (p=0.005) showing horizontal sections during the learning phase of the face-memory task across the whole brain (left is left and right is right).
Table 3 Experiment 2. Regions of activation during the learning and recall phases of the face memory task. Shown are the regions for whole brain and small volume correction ROI analyses. Positive values for $x$, $y$ and $z$ Talairach coordinates denote, respectively, locations that are left, posterior and inferior relative to the anterior commissure. Table abbreviations indicate: BA=Brodmann area; HS=hemisphere; Vol=activity cluster volume in microliters, Ctrl=control group, THC=cannabis group; FG=frontal gyrus; TG=temporal gyrus; PHG=parahippocampal gyrus (*$p<0.05$, **$p<0.01$, ***$p<0.001$ Independent t-test). Only positive activations are reported.

<table>
<thead>
<tr>
<th>Structure</th>
<th>BA</th>
<th>HS</th>
<th>Vol (μl)</th>
<th>Talairach co-ordinates</th>
<th>$P$</th>
<th>Direction of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Learning</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$x$</td>
<td>$y$</td>
<td>$z$</td>
</tr>
<tr>
<td>Learning</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>(Whole brain)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior TG</td>
<td>39</td>
<td>R</td>
<td>2063</td>
<td>50</td>
<td>57</td>
<td>21</td>
</tr>
<tr>
<td>Superior FG</td>
<td>6</td>
<td>R</td>
<td>1561</td>
<td>14</td>
<td>-26</td>
<td>55</td>
</tr>
<tr>
<td>Superior FG</td>
<td>9</td>
<td>R</td>
<td>1226</td>
<td>16</td>
<td>-45</td>
<td>38</td>
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<tr>
<td>Middle FG</td>
<td>8</td>
<td>R</td>
<td>590</td>
<td>33</td>
<td>-25</td>
<td>44</td>
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<tr>
<td>Superior FG</td>
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<td>479</td>
<td>-12</td>
<td>-39</td>
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<tr>
<td>Recall</td>
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<td>(Whole brain)</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Middle TG</td>
<td>39</td>
<td>R</td>
<td>1920</td>
<td>47</td>
<td>63</td>
<td>22</td>
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<tr>
<td>Superior TG</td>
<td>39</td>
<td>L</td>
<td>581</td>
<td>-51</td>
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<td>(ROI analysis)</td>
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<td></td>
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<tr>
<td>PHG</td>
<td>27</td>
<td>R</td>
<td>832</td>
<td>20</td>
<td>30</td>
<td>-6</td>
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<tr>
<td>PHG</td>
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<td>L</td>
<td>741</td>
<td>-22</td>
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<tr>
<td>PHG</td>
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<td>L</td>
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<tr>
<td>PHG</td>
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<tr>
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<td>R</td>
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<td>25</td>
<td>7</td>
<td>-23</td>
<td>*</td>
</tr>
<tr>
<td>PHG</td>
<td>R</td>
<td>138</td>
<td>19</td>
<td>54</td>
<td>-4</td>
<td>ns</td>
</tr>
</tbody>
</table>

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The cannabis group showed significantly lower levels of BOLD activity in the RSTG, BA 39 ($p<0.01$), RSFG, BA 6 ($p<0.001$), RSFG, BA 9 ($p<0.01$), RMFG, BA 8 ($p<0.001$) and LSFG, BA 8 ($p<0.05$) (See Figures 8a and b below). Areas significantly active across the control and cannabis groups for the recall phase were the right middle temporal gyrus (RMTG, BA 39) and left superior temporal gyrus (LSTG, BA 39). There were no group differences in BOLD activity during recall.

Figure 8 Experiment 2. BOLD activity (mean % change from baseline) in a) the right superior frontal gyrus [BA9] and b) left superior frontal gyrus [BA8] in the cannabis and control groups during the learning phase of the face-memory task. (*$p<0.05$; **$p<0.01$).
Region of interest (ROI) analyses

Table 3 also demonstrates the results of a small volume correction region of interest (ROI) analysis during the learning phase, which included the hippocampal and parahippocampal regions. Five areas were found to be significantly active, consisting of the right parahippocampal gyrus (RPHG, BA 27); left PHG (LPHG, BA 27); LPHG, BA 19; LPHG, BA 30 and the RPHG. A between-groups comparison indicated that the cannabis group had significantly greater activity in the right parahippocampal gyrus (x=25, y=7, z=-23) during the learning phase (See Figure 9 below), when compared to control participants. There were no significant clusters of activation during recall.

Figure 9 Experiment 2. BOLD activity (mean % change from baseline) in the right parahippocampal gyrus ROI in the cannabis and control groups during the learning phase of the face-memory task (*p<0.05).
Performance and BOLD Correlations

There were no correlations between BOLD activity (whole brain and ROI results) and task performance scores in either the control or cannabis groups.

Drug-use Correlations

There were no correlations between cannabis abstinence or self-reported use of cannabis (e.g., years of use, lifetime joints, days of use in last month, joints in last month and age of use onset) and behavioural performance on the face-memory task in the cannabis-using group. Nor were there any associations between these measures and BOLD activations in the cannabis group.

Drug Craving and Withdrawal Correlations

Figure 10 below shows two significant correlations. Cannabis withdrawal scores were positively associated with activity in LPHG, BA 19 during learning ($r = .53$, $p=0.05$) and negatively associated with BOLD activity in the LSTG, BA 39 during recall ($r = -0.59$, $p<0.05$). Finally, we found no significant correlations between reported craving (compulsivity, emotionality, expectancy and purposefulness) and behavioural performance or BOLD activity.
Figure 10 Experiment 2. Correlations between a) withdrawal score and LSTG, BA 39 BOLD activity during recall ($r = -0.59$, $p<0.05$) and b) LPHG, BA 19 BOLD activity during learning ($r = 0.53$, $p=0.05$).
Discussion

Experiment 2 investigated the effects of chronic cannabis use on learning and recall-related brain activity. In a sample of high functioning cannabis users, demographically well matched to a comparison control group, we found evidence of hypoactivity in the right superior temporal gyrus, right superior frontal gyrus, right middle frontal gyrus and left superior frontal gyrus during associative learning. These were observed in the absence of group differences in recall-related activity or recall performance. The lack of a performance effect may be due to the smaller sample size and lower statistical power of experiment 2: The absence of performance effects can be advantageous, however, enabling us to discount performance-related effects (e.g., error-related activity, frustration) from confounding the group comparison (Murphy and Garavan, 2004). Cortical hypoactivation in cannabis users has previously been argued to reflect the sub-acute effects of cannabis at the time of testing (Block et al, 2002). The present differences in BOLD activity do not appear to be influenced by cannabis intoxication at the time of testing the cannabis-using group. We also failed to observe any significant association between self-reported measures of use, such as the frequency (days) of use or number of joints in the month prior to testing, and either task performance or BOLD activity.

Animal studies suggest some memory-impairing effects of cannabinoids are the result of a dysfunction in the prefrontal cortex (Diana et al, 1998; Jentsch et al, 1997; 1998; Verrico et al, 2003), with human imaging studies also demonstrating prefrontal hypoactivity in cannabis users (Amen and Waugh, 1998; Yurgelun-Todd et al, 1999; Lundqvist et al, 2001; Block et al, 2002; Eldreth et al, 2004; Bolla et al, 2005; Pillay et al, 2004; Gruber and Yurgelun-Todd, 2005; Chang et al, 2006; Jager et al, 2007).
Prefrontal areas are critical to working memory (WM) function (D’Esposito et al, 1995; 1999; Petrides, 2000; Blumenfeld and Ranganath, 2006), with the suggestion that the organization of learning is highly contingent upon WM processes (Tulving and Pearlstone, 1966; Alexander et al, 2003). Patients with LSFG lesions exhibit WM deficits when compared with control groups (du Boisgueheneuc et al, 2006), and there is evidence of prefrontal hypoactivity in cannabis users during verbal and associative learning (Block et al, 2002; Jager et al, 2007). Recently, Hermann et al (2007) showed that cannabis users have diminished N-acetylaspartate/total creatine (NAA/tCr) in the dorsolateral prefrontal cortex (DLPFC), suggestive of reduced cortical neuronal and axonal integrity. Our results showing reduced bilateral SFG activity during facial associative learning may, therefore, indicate a dysfunction related to WM processing, associated with reduced neuronal functioning in this area. These differences, however, may well have preceded cannabis use, however.

Cannabis users also demonstrated increased right parahippocampal gyrus activity during the learning phase, when compared to control participants. The face-memory associative learning task has previously been shown to selectively involve the hippocampal formation (Zeineh et al, 2003). The parahippocampal gyrus includes the entorhinal cortex, an area known to have extensive connections with the hippocampus and DG, and is thought to be involved in the translation of temporary hippocampal information storage during learning (Rolls, 2000). The behavioural effects of cannabis appear to be mediated by CB₁ receptors, which are expressed at especially high densities in the hippocampus proper and DG regions (Herkenham et al, 1991; Tsou et al, 1998). Our findings of parahippocampal hyperactivity are contrary to a recent study conducted by Jager and colleagues (2007), which showed that cannabis users
demonstrated parahippocampal hypoactivity during an associative learning task. This inconsistency may reflect the differing recall demands of the two tasks; in the present study participants were required to recall the digits associated with a face, whereas Jager et al. required recognition of previous picture pairs.

Previous studies have found that greater activity in the parahippocampal region during learning predicts subsequent recall, suggesting a strong association between parahippocampal neuronal activity and memory encoding (Fernandez et al, 1998; Brewer et al, 1998; Wagner et al, 1998). Jansma et al (2004) have suggested that hyperactivity may represent a greater ‘neurophysiological’ effort in order to maintain normal behavioural performance, which in this case, may account for the absence of significant recall deficits in the cannabis user group due to relative parahippocampal hyperactivity during learning. Previous research studies using other cognitive paradigms have also reported increased hippocampal and parahippocampal activity in cannabis users (Eldreth et al, 2004; Bolla et al, 2005). The frontal and temporal cortical hypoactivity and parahippocampal hyperactivity observed in the present study may, therefore, suggest a neural compensatory mechanism, whereby the latter is compensating for the cannabis-related lack of prefrontal-mediated involvement in memory formation. Interestingly, different patterns of activity in other drug-using groups, such as cocaine users, have also been observed during fMRI paradigms, which are assumed to reflect a reliance on sub-optimal circuits (Hester and Garavan, 2004). This conclusion may be consistent with the behavioural results of experiment 1, during which a prefrontal deficit was identified as the likely source of poorer memory performance, and where the reliance upon compensatory neural circuits would prove behaviourally sub-optimal in a much larger sample of cannabis users.
Adolescent and adult users seeking treatment for cannabis dependence (Crowley et al, 1998; Budney, Novy and Hughes, 1999; Budney et al, 2003; Dawes et al, 2006) and frequent users not seeking treatment (Wiesbeck et al, 1996) have both been shown to experience withdrawal symptoms. In the present study, we assessed cannabis withdrawal prior to scanning using a questionnaire designed to elucidate potential physical, affective and behavioural withdrawal symptoms (Brower et al, 1988). The cannabis-using group in our study had a mean score of 10.5 (range= 0-21) of reported withdrawal, which suggests that they were experiencing a low-moderate level of withdrawal during the testing session. Rating scores were also found to be positively correlated with LPHG activity during learning and negatively correlated with LSTG activity during recall. These results might suggest that the cannabis-using group was experiencing a level of withdrawal that affected memory-related brain activity, although there were no associations observed between withdrawal scores and cortical areas where group differences were present.

Experiment 1 of the current study demonstrated learning and memory deficits in a group of high functioning cannabis users on a task previously shown to engage activity within the hippocampal region. These behavioural findings taken from a sample of moderate cannabis users concur with the extant literature, which has shown deficits related to learning and memory, which may result from prefrontal and/or hippocampal impairment. Using a modified version of this task, sensitive to memory impairments, experiment 2 has demonstrated learning-related functional brain alterations in a cohort of equally high functioning cannabis-users with heavier use than those of experiment 1. Hypoactivity in frontal and temporal cortices, and relative hyperactivity in the parahippocampus during learning, may suggest discordant
compensatory and adaptive functioning to overcome diminished activity in normal neural networks. One of the major limitations of experiment 2, however, was the use of a block design. For example, this experiment was unable to model neural responses when groups encoded and recalled information successfully and unsuccessfully; therefore, unable to elaborate on the current literature regarding neural differences between cannabis and non-cannabis users with respect to behavioural performance. Notwithstanding this limitation, the results may still help reconcile learning and memory impairments in cannabis users, challenging the strongly held view that such deficits are (para)hippocampal in origin, with evidence to suggest that deficits in associative learning are also related to prefrontal dysfunction.
References


Chapter 3 - Cannabis Use and Non-Drug Reward Processing
Abstract

Despite an increased understanding of the pharmacology and long-term cognitive effects of cannabis in humans, there has been no research to date examining its chronic effects upon reward processing in the brain. Motivational theories regarding long-term drug use posit contrasting predictions with respect to how drug users are likely to process non-drug incentives. The reward deficiency syndrome (RDS) of addiction, for example, posits that there are deficits in dopamine (DA) motivational circuitry for non-drug rewards, such that only drugs of abuse are capable of normalizing DA in the ventral striatum (VS). Alternatively, the opponent process theory (OPT) holds that in individuals prone to drug use, there exists some form of mesolimbic hyperactivity, in which there is a bias towards reward-centred behaviour concomitant with impulsivity. Using fMRI, the current study examined VS responses in 14 recreational cannabis users and 14 drug-naïve controls, using a monetary incentive delay (MID) task. Despite no significant behavioural differences between the two groups, cannabis users demonstrated significantly more right VS BOLD activity during the anticipation of non-drug rewards. Correlation analyses also demonstrated that this right VS response was significantly correlated with life-time years of cannabis use and the number of reported life-time cannabis joints consumed. These results appear to argue against a reward deficiency syndrome in the VS circuitry of recreational cannabis users during instrumental response anticipation for non-drug rewards.
Introduction

The long-term recreational use of cannabis has been linked to deficits in learning, memory, and executive functioning in humans (Nestor et al, 2008; Grant et al, 2003; Solowij et al, 2002; Bolla et al, 2002). Critically, it has been proposed that compromised cognitive processing in chronic cannabis users may promote the continuation of drug consumption, which may be exacerbated following acute withdrawal (Goldstein et al, 2002; Volkow et al, 2002). Subjective reports concerning the effects of cannabis (See Iversen, 2000, 2003) suggest that its initial reinforcing properties, like other drugs of abuse, are related to its effects on the brain’s “reward circuitry”. Despite an increased understanding concerning the cognitive pharmacological effects of cannabis (Iversen, 2000, 2003) little is known regarding its chronic effects upon reward processing in the human brain.

Despite significant evidence of VS responses to drug-associated stimuli in substance dependence (London et al, 1999; Garavan, 2000; Grusser et al, 2004; Sinha and Li, 2007), there is little evidence that drug users are abnormal in their responses to stimuli which predict non-drug rewards. Motivational theories regarding drug use posit contrasting predictions with respect to how drug users may differentially recruit the VS in response to cues which signal non-drug incentives. The reward deficiency syndrome (RDS) (Blum et al, 2000) and the allostatic hypotheses (AH) (Koob et al, 2004), for example, view addiction as a deficit in DA motivational circuitry for non-drug rewards, such that only drugs of abuse are able to normalize DA at the VS. The incentive salience hypothesis (ISH) (Robinson and Berridge 2001) attributes compulsive drug-use to alterations in striatal functioning, in which drug-cues reputedly acquire increased incentive-motivational value. In support of this, and the
RDS described above, are findings that detoxified alcoholics demonstrate reductions in VS responses to non-drug reward cues, while eliciting increased VS activity to alcohol associated stimuli (Wrase et al, 2007).

Alternatively, the opponent process theory (OPT) (Solomon and Corbit, 1973) holds that in individuals prone to drug use, there exists some combination of both mesolimbic reward hyperactivity and hypoactive frontocortical punishment-avoidance circuitry (Bechara, 2005; Bickel et al, 2007). Indeed, substance dependent patients have been shown to exhibit both impulsive (Bickel and Marsch, 2001) and reward centred (Bechara et al, 2001) choice behaviour under laboratory conditions, with cocaine (Heil et al, 2006) and alcohol (Bjork et al, 2004) dependence associated with an increased preference for small immediate over larger delayed rewards. Therefore, drugs such as cannabis, capable of engaging the VS, may facilitate further drug consumption by affecting DA reward circuitry.

Given evidence that the anticipation of non-drug incentives reliably activates the VS for goal-objects (Schultz et al, 1997; Knutson et al, 2001; O’Doherty, 2004) the current study sought to examine VS reward functioning in recreational cannabis users and drug-naïve controls. The current investigation set out to 1) test whether chronic recreational cannabis use is associated with either VS reward deficiency or hyperactivity and 2) investigate whether this VS reward activity is associated with reported life-time cannabis consumption.
Material and Methods

Participants

14 current users of cannabis and 14 controls were recruited from the general public and academic institutions around Dublin city. All participants underwent a comprehensive telephone screening, during which detailed information concerning past and present psychiatric, neurological and substance use was taken. Information pertaining to any form of treatment (counselling, psychological, psychiatric), past or present, was carefully detailed, with any potential participant describing any major life-time psychiatric event or head injury (e.g., head trauma resulting in a loss of consciousness, seizure or stroke) considered ineligible for the study. Cannabis and control participants also completed inventories for mood (BDI II) and drug use (questionnaire taken from the Addiction Severity index Lite-CF; see questionnaires section below) to screen for depression and past or concurrent abuse of other substances. Therefore, cannabis and control participants were additionally considered ineligible if they reported concurrent or past dependence on other drugs (e.g., alcohol, amphetamines, benzodiazepines, cocaine, MDMA, hallucinogens and opiates) at the practice session prior to scanning. Information concerning alcohol, nicotine and cannabis use in each participant was indexed in years (life-time) and recent (last 30 days). Other drug use information on each participant was indexed by the total number of separate occasions (life-time) and the total number of recent separate occasions (last 30 days).

Cannabis participants were required to have regularly consumed cannabis (5-7 days/week) for the previous 2 years in order to be eligible for the study. Participants in the cannabis group were additionally required to have smoked a minimum of 500
joints in their life-time, in order to eliminate potential participants with only negligible cannabis use. All cannabis users provided a positive urine sample for $\Delta^9$-tetrahydrocannabinol ($\Delta^9$THC) prior to scanning, with additional screening for methadone, benzodiazepines, cocaine, amphetamines, opiates, barbiturates and tricyclic antidepressants (Cozart® RapiScan, UK) taking place. Control participants were also tested for $\Delta^9$THC and the above adulterants. While the identification and quantification of cannabis metabolites in urine may have proved advantageous as a potential predictor of brain functioning, past studies have shown that estimates of recent use, life-time use and age of onset of use, are reliable predictors of behavioural performance and BOLD activity in cannabis users (Block & Ghoneim, 1993; Bolla et al, 2002; Bolla et al, 2005; Pope et al, 2003; Pope & Yurgelun-Todd, 1996; Solowij et al, 1995, 2002; Chang et al, 2006). Therefore, urinalyses were conducted merely to verify the presence of $\Delta^9$THC in cannabis participants and the absence of all other drug metabolites in both control and cannabis participants.

The cannabis group reported, on average, 6.1 years (range = 2.5-17) of life-time cannabis use, the consumption of 7258 life-time cannabis joints (range = 700-34,403), 4.5 years of heavy\(^2\) life-time cannabis use (range = 1-15) an average of 20 days use in the last 30 days (range = 6-30), an average of 64 (range = 15-140) cannabis joints consumed in the last 30 days, and had been abstinent from cannabis, on average, 108 hours (range = 12-504) prior to testing. All participants were right-handed as confirmed by the Edinburgh Handedness Inventory (Oldfield, 1971) during the telephone screening process. Control and cannabis participants completing the study

\(^2\) Heavy use here is defined by the number of years in which the user reports being intoxicated from cannabis on a daily or near daily basis.
were neurologically normal (as confirmed by a registered radiologist who examined each structural MRI). All research participants provided informed consent and were financially compensated.

Monetary Incentive Delay Task (MID)

We used a "monetary incentive delay task" (MID), which was based on that originally employed by Knutson et al (2001). The version used in the current study, however, differed from that originally developed on two levels. First, we did not use differential magnitudes of financial reward and loss in our version of the task. Second, in the original version of the task, the anticipatory cue and trial outcome periods were time invariantly yoked. To overcome any possible cross contamination between anticipatory cue and outcome maps, we used extended temporal jittering between cue periods and target responses, and between outcome notifications and the commencement of the next trial. In doing this, we were able to separate the BOLD signal related to instrumental response anticipation and outcome deliveries using deconvolution analyses. This was confirmed during the initial designing of the MID task, during which canonical regressors for all the anticipation and outcome periods were run through the fMRI software programme (see below), demonstrating that there was no multicollinearity between the regressors of interest.

During the practice session, prior to scanning, participants were informed that they would receive the total amount of money won during the imaging experiment. While being scanned, participants performed the MID task, during which they anticipated potential monetary gain, loss or no potential monetary outcome. During each trial, participants saw one of three coloured squares ("cue"; 2-8 sec) which indicated they
could win fifty cent, lose fifty cent or experience no financial outcome upon their response to an upcoming visual target. The cue signalling potential financial gain (+0.50c) was a green square; the cue signalling potential financial loss (-0.50c) was a red square and the cue signalling no potential financial outcome (0.00) was a blue square (See Fig 1 below). Each cue was presented for a variable duration (2-8 sec), after which participants made a button press response upon the presentation of a visual target (star located within a circle) (See Fig 1). Following their response to the visual target, participants received feedback (1500 ms), after which there was an end fixation period (2-8 sec) before the commencement of the next trial (See Fig 1). All responses to the visual target falling within (“hits”) or outside (“misses”) a four hundred millisecond response deadline received feedback appropriate for that particular trial (See Fig 1). Therefore, participants had four hundred milliseconds to respond to the visual target in order to be successful on a win, loss or no-outcome trial. There were a total of twenty seven trials in each run (nine win, nine loss and nine no-outcome), with each trial lasting between six and eighteen seconds. The MID was composed of three runs, with each run lasting 340 seconds. The order of cue presentation within each run was different across the three runs. Dependent measures were percentage accuracy and reaction time for win, loss and no-outcome conditions. The task was programmed and run using E-Prime version 1.1 (Psychology Software Tools, Pittsburgh, USA).
Figure 1. Monetary Incentive Delay (MID) task structure. Participants were cued (2-8 sec) regarding potential financial reward, loss or no financial outcome using one of three coloured squares. Participants were then required to respond to a target stimulus following cue presentation. The target stimulus was presented for 400ms, during which participants were required to respond, as quickly as possible, with a button press on a hand-held key pad. Following this, participants received feedback regarding their response (1500ms), before the presentation of an end fixation period (2-8 sec), during which they saw a centrally located crosshair.
Questionnaires

The National Adult Reading Test (NART) (Nelson & O’Connell, 1978) and the Beck Depression Inventory-II (Beck et al, 1996) were administered to all participants prior to scanning. Information concerning alcohol and drug use (See Table 1) was obtained from all participants using a questionnaire taken from the Addiction Severity index Lite-CF (McLellan et al, 1992). Prior to scanning, cannabis users also provided information concerning withdrawal and cannabis craving. The Marijuana Craving Questionnaire (Heishman et al, 2001) is made up of 12 statements, which the participant has to rate according to a seven-point Likert-type scale from “strongly disagree” to “strongly agree”. Responses to the questionnaire are then divided into four specific constructs made up of compulsivity, emotionality, expectancy and purposefulness, related to cannabis use. Information regarding withdrawal, modified from a cocaine withdrawal checklist (Brower et al, 1988) was obtained using a thirty two-item checklist, where participants were required to rate, on a scale of 0 (none) to 3 (severe), symptoms they had experienced in the previous 24 hours.

fMRI acquisition

All scanning was conducted on a Philips Intera Achieva 3.0 Tesla MR system (Best, The Netherlands). Please refer to pages 82-83 in chapter 2 (Learning and memory in cannabis users) for a full description of imaging acquisitions.
Data processing and analyses

All analyses were conducted using AFNI software (Cox, 1996). Following image reconstruction, the three 3-D time series (runs 1, 2 and 3) were concatenated and motion-corrected using 3-D volume registration (least-squares alignment of three translational and three rotational parameters). Activation outside the brain was also removed using edge detection techniques.

i) Cue analyses

An estimate of activation for the win, loss and no-outcome cue periods was conducted. These three regressors were convolved with a standard haemodynamic response to accommodate the lag time of the blood oxygen level-dependent (BOLD) response. Multiple regression analyses calculated activation as a percentage change relative to the baseline, which consisted of the fixation period at the end of each trial.

The percentage change activation maps were re-sampled to 1 mm³ resolution, warped into standard Talairach space (Talairach and Tournoux, 1988) and spatially blurred with a 3-mm isotropic rms Gaussian kernel. Group activation maps for each condition of the task (win, loss and no-outcome cue) were determined with one-sample t-tests against the null hypothesis of zero activation change (i.e. no change relative to the between trial fixation periods). Significant voxels passed a voxelwise statistical threshold ($t = 3.4, p<0.005$) and were required to be part of a larger 276ml cluster of contiguous significant voxels. Thresholding was determined through Monte Carlo simulations and resulted in a 5% probability of a cluster surviving due to chance.
Initially, the thresholded group *t*-test map for the win, loss and no-outcome cue periods were combined within each group to form group OR maps. Following this, groups OR maps were combined to form an overall OR map. The overall OR map included those significant voxels from either the win, loss and no-outcome cue periods in both the control and cannabis groups. This final map yielded functionally-defined regions of interest for comparisons between groups and between conditions.

ii) *Outcome analyses*

To examine neural activations in response to reward and loss outcomes in the control and cannabis groups, an event-related analysis was performed that estimated activations for the six MID outcomes (+0.50 hit; +0.50 miss; -0.50 hit; -0.50 miss; 0.00 hit and 0.00 miss) separately. Using a nonlinear regression programme, we determined the best-fitting gamma-variate function for these six impulse-response functions (Cohen, 1997) as described previously (Murphy and Garavan, 2005) the area under the curve of the gamma-variate function was expressed as a percentage of the area under the baseline. The baseline for the six outcome measures was the same as that used for the block analyses described above.

The percentage area (event-related activation) voxels were re-sampled to 1 mm³ resolution, before being warped into standard Talairach space and spatially blurred with a 3-mm isotropic rms Gaussian kernel. Group activation maps for each MID outcome condition were also determined with one-sample *t*-tests against the null hypothesis of zero activation change (i.e. no change relative to the between trial fixation periods). As above, significant voxels passed the same voxelwise statistical threshold (*t* = 3.4, *p* < 0.005) and were also required to be part of a 276μl cluster of
contiguous significant voxels. Thresholded group $t$-test maps for each outcome period were combined within each group to form group OR maps. Following this, group OR maps were combined to form an overall outcome OR map. The overall outcome OR map included those significant voxels from each outcome period in both the control and cannabis groups.

We observed significant activations from baseline in a number of areas during the outcome periods, which included the anterior cingulate (ACC), insula [BA13], dorsolateral prefrontal cortex (dIPFC) and nucleus accumbens (NAcc). Due to the volume of thresholded outcome activation maps observed in these areas, we decided to perform a series of anatomically-defined region of interest (ROI) analyses in both left and right hemispheres, given an *a priori* interest in areas previously associated with reward and loss outcomes (Knutson et al, 2003; Rogers et al, 2004). Using the same whole brain thresholding described above, each left and right ROI was combined with the overall outcome OR map to form left and right AND maps for each ROI. The AND map included those significant voxels from the overall outcome OR map and each ROI in both control and cannabis groups. The ROI areas were the ACC, insula [BA13], dIPFC and NAcc. By choosing areas typically associated with the receipt of reward (as cited above), we were also able to minimize BOLD activity occurring during response execution, such as that likely to occur in the primary motor cortex (BA4).
All between-groups analyses of mean activation clusters for the cue and outcome periods were initially conducted using univariate analyses of variance. The observation of either a group effect or group x condition interaction justified post hoc analyses using between group independent t-tests on each of the cue and outcome conditions. All between group analyses were conducted in SPSS (SPSS Inc).

Results

Demographics and drug use

Table 1 shows the group demographic and drug use history for both samples. The groups did not significantly differ on age, years of education, verbal intelligence, BDI or alcohol, nicotine and other drug use. Cannabis use data (no. life-time joints) was found to be significantly skewed in the cannabis sample, and was therefore, log transformed (log10) to be used in further analyses.
Table 1. Mean and SEM for control and cannabis groups on demographic and drug use history.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=14)</th>
<th>Cannabis (n=14)</th>
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<tbody>
<tr>
<td>Age</td>
<td>23.1 ± 1.2</td>
<td>22.1 ± 1.2</td>
</tr>
<tr>
<td>Years of Education</td>
<td>16.1 ± 0.4</td>
<td>17.1 ± 0.6</td>
</tr>
<tr>
<td>Verbal Intelligence Score (NART)</td>
<td>123.0 ± 0.8</td>
<td>123.9 ± 0.8</td>
</tr>
<tr>
<td>Beck Depression Inventory II Score</td>
<td>7.2 ± 2.1</td>
<td>8.1 ± 2.1</td>
</tr>
<tr>
<td>Females/Males</td>
<td>3/11</td>
<td>2/12</td>
</tr>
<tr>
<td>Years of Alcohol Use</td>
<td>6.9 ± 1.2</td>
<td>6.1 ± 1.2</td>
</tr>
<tr>
<td>Alcohol Use in Last Month (no. days)</td>
<td>7.8 ± 1.4</td>
<td>8.3 ± 1.8</td>
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<td>Alcohol Use Age Onset (Years)</td>
<td>16.2 ± 0.5</td>
<td>15.6 ± 0.5</td>
</tr>
<tr>
<td>Years of Nicotine Use</td>
<td>7.9 ± 1.7</td>
<td>5.2 ± 1.3</td>
</tr>
<tr>
<td>Pack-Years</td>
<td>4.6 ± 1.8</td>
<td>4.1 ± 1.3</td>
</tr>
<tr>
<td>Cigarettes/Day</td>
<td>10.0 ± 2.4</td>
<td>10.0 ± 2.8</td>
</tr>
<tr>
<td>Nicotine Use in Last Month (no. days)</td>
<td>15.0 ± 4.2</td>
<td>12.9 ± 4.1</td>
</tr>
<tr>
<td>Number of Packs in Last Month</td>
<td>11.8 ± 3.6</td>
<td>12.0 ± 3.8</td>
</tr>
<tr>
<td>Nicotine Use Age Onset (Years)</td>
<td>16.20 ± 0.6</td>
<td>16.4 ± 1.0</td>
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<tr>
<td>Amphetamine Use (no. times)</td>
<td>3.3 ± 1.2</td>
<td>3.0 ± 1.8</td>
</tr>
<tr>
<td>Amphetamine Use in Last Month (no. times)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Cocaine Use (no. times)</td>
<td>4.8 ± 0.3</td>
<td>6.1 ± 1.2</td>
</tr>
<tr>
<td>Cocaine Use in Last Month (no. times)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>MDMA Use (no. times)</td>
<td>3.6 ± 0.9</td>
<td>4.5 ± 0.6</td>
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<tr>
<td>MDMA Use in Last Month (no. times)</td>
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<td>0.0 ± 0.0</td>
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<tr>
<td>Hallucinogenic Use (no. times)</td>
<td>2.0 ± 0.4</td>
<td>3.2 ± 0.4</td>
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<td>Hallucinogenic Use in Last Month (no. times)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
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<tr>
<td>Cannabis Use (Years)</td>
<td>0.0 ± 0.0</td>
<td>6.1 ± 1.1</td>
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<tr>
<td>Life-time Joints (number)</td>
<td>3.0 ± 0.6</td>
<td>7258.6 ± 2512.8</td>
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<td>Heavy Cannabis Use (Years)</td>
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<td>4.5 ± 1.1</td>
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<td>Days of Use in Last Month (number)</td>
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<td>20.1 ± 2.5</td>
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<tr>
<td>Joints in Last Month (number)</td>
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<td>64.8 ± 10.7</td>
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<tr>
<td>Cannabis Use Age Onset (years)</td>
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<td>16.1 ± 0.4</td>
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<tr>
<td>Cannabis Abstinence (hours)</td>
<td>108.0 ± 39.7</td>
<td>11.6 ± 2.1</td>
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<tr>
<td>Cannabis Withdrawal Score (out of 32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cannabis Craving Scores (each item out of 21)</td>
<td></td>
<td></td>
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<tr>
<td>Compulsivity</td>
<td>5.9 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Emotionality</td>
<td>7.1 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Expectancy</td>
<td>10.6 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Purposefulness</td>
<td>12.1 ± 1.5</td>
<td></td>
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</table>
MID Performance

Figure 2a shows the MID accuracy (% “hits”) for the three conditions in the control and cannabis groups. A two (group) by three (condition) univariate analysis of variance showed that there was a significant effect of cue (F=6.5, df=2, 78, p<0.01); no effect of group (F=0.02, df=1, 78, p=0.9) and no cue x group interaction (F=0.5, df=2, 78, p=0.6). Pairwise comparisons confirmed that there was a significant accuracy difference between the no-outcome and loss (p<0.05) and between the no-outcome and win (p<0.01), but not between the loss and win conditions (p=1.0).

Figure 2b demonstrates MID reaction time (milliseconds) on “hit” trials on the three conditions for both groups. Here, there was a significant effect of cue (F=5.8, df=2, 78, p<0.01); no effect of group (F=3.1, df=1, 78, p=0.08) and no cue x group interaction (F=0.2, df=2, 78 p=0.8). Pairwise comparisons indicated a significant difference in reaction time between the no-outcome and win (p<0.01), but not between the no-outcome and loss (p=0.06) conditions.

Figure 2. a) Mean percentage accuracy and b) mean reaction time (milliseconds) for no-outcome, loss and win trials in controls and cannabis users (means and standard error means). Data were analyzed using two (group) x three (cue) univariate analyses (*p<0.05 loss versus no-outcome, **p<0.01 win versus no-outcome Pairwise comparisons).
fMRI

Cue analyses

Table 2 lists the areas of significant activity during the win, loss and no-outcome cue presentation periods in both the control and cannabis groups. Figure 3 below additionally demonstrates the general patterns of activation in both the cannabis and control groups during the cue periods of the MID task. All data across conditions and between groups in each brain region were found to be normally distributed.

**Figure 3.** Activation $t$-test maps for the cannabis and control groups ($p=0.005$) showing coronal sections during win cue anticipation across the whole brain.
Table 2. Brain activations elicited by win, loss and no-outcome cue periods. Positive values for x, y and z Talairach coordinates denote, respectively, locations that are left, posterior and inferior relative to the anterior commissure. Table abbreviations indicate: L=left; R=right; Ctrl=controls; THC=cannabis; W=win; L=loss NO=no outcome; FG=frontal gyrus. *P indicates differences between the two groups in BOLD activity following post hoc two-sample t-tests (*p<0.05).

<table>
<thead>
<tr>
<th>Structure</th>
<th>BA</th>
<th>HS</th>
<th>Vol (μl)</th>
<th>Talairach co-ordinates</th>
<th>Direction of significance</th>
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<td>Medial FG</td>
<td>6</td>
<td>L</td>
<td>668</td>
<td>-5 -13 62</td>
<td>Ctrl&gt;THC</td>
</tr>
<tr>
<td>Medial FG</td>
<td>6</td>
<td>R</td>
<td>394</td>
<td>10 -9 57</td>
<td>Ctrl&gt;THC</td>
</tr>
<tr>
<td>Fusiform gyrus</td>
<td>37</td>
<td>L</td>
<td>468</td>
<td>-35 -46 -10</td>
<td>Ctrl&gt;THC* [W]</td>
</tr>
<tr>
<td>Cingulate gyrus</td>
<td>24</td>
<td>L</td>
<td>2220</td>
<td>-2 4 43</td>
<td>Ctrl&gt;THC* [W]</td>
</tr>
<tr>
<td>Cuneus</td>
<td>18</td>
<td>R</td>
<td>276</td>
<td>2 -88 14</td>
<td>THC&gt;Ctrl</td>
</tr>
<tr>
<td>Ventral striatum</td>
<td></td>
<td>R</td>
<td>2008</td>
<td>20 8 -4</td>
<td>THC&gt;Ctrl* [W]</td>
</tr>
<tr>
<td>Declive of vermis</td>
<td>18</td>
<td>R</td>
<td>16868</td>
<td>1 -77 -19</td>
<td>THC&gt;Ctrl* [L] &amp; [W]</td>
</tr>
</tbody>
</table>

Activated areas for both groups were observed in both left and right hemispheres, and included the cerebellum, cingulate gyrus, ventral striatum, medial frontal gyrus, fusiform gyrus and cuneus. Two (group) x three (cue period) univariate analyses showed that there were significant BOLD activity differences between the two groups in a number of areas. The right cerebellar (declive of vermis) BOLD response showed no effect of cue ($F=0.5$, $df=2$, $78$, $p=0.6$); a significant effect of group ($F=5.7$, $df=1$, $78$, $p<0.05$) wherein the cannabis group had significantly more BOLD activity than controls, and a significant cue x group interaction ($F=3.9$, $df=2$, $78$, $p<0.05$). The interaction was driven by significantly greater BOLD activity during the loss ($p<0.05$) and win ($p<0.05$) cue periods in the cannabis group relative to the controls (See Figs 4a & b).
We additionally observed a significant positive correlation between BOLD activity in this region during the “win” cue period and the number of reported life-time cannabis joints consumed \( r=0.8, p<0.001 \) (See Fig 4c).

**Figure 4.** a) Mean brain activity in the right declive of vermis \( (x = 1, y = -77, z = -19) \), b) graph showing cannabis and control groups significantly differed in right declive of vermis BOLD activity during loss and win cue presentation \(* p<0.05 \) Independent \( t \)-tests) and c) correlation between right devlive of vermis BOLD activity during win cue presentation and the number of reported life-time cannabis joints smoked \( r=0.7, p<0.01 \)
Figure 5. a) Mean brain activity in the right VS (x = 20, y = 8, z = -4), b) graph showing cannabis and control groups significantly differed in right VS BOLD activity during win cue presentation (*p<0.05 Independent t-tests) and c) correlation between right VS BOLD activity during win cue presentation and the number of reported lifetime cannabis joints smoked r=.6, p<0.05).

In the right VS there was no effect of cue ($F=2.3, df=2, 78, p=0.1$), a significant effect of group ($F=6.0, df=1, 78, p<0.05$) in which the cannabis group had significantly more activity than controls, and a significant cue x group interaction ($F=4.5, df=2, 78, p<0.05$). Post hoc tests indicated greater BOLD activity in the cannabis group during the win cue ($p<0.05$) period only (See Figs 5a & b below). We also observed a significant positive correlation between BOLD activity in this region during the win
cue period and the number of reported life-time cannabis joints consumed (r=0.6, p<0.05) (See Fig 5c).

BOLD activity in the right medial frontal gyrus [BA6] showed no effect of cue (F=0.4, df=2, 78, p=0.7); a significant effect of group (F=6.2, df=1, 78, p<0.05; Control > Cannabis) but no significant cue x group interaction (F=0.8, df=2, 78, p=0.5). In the left fusiform gyrus [BA37], there was a significant effect of cue (F=4.4, df=2, 78, p<0.05); a significant effect of group (F=6.8, df=1, 78, p<0.05; Control > Cannabis) and a significant cue x group interaction (F=3.9, df=2, 78, p<0.05).

Pairwise comparisons for cue showed that there was greater BOLD activity during win compared to the loss (p<0.05) and no-outcome (p<0.05) periods. Further post hoc tests of the interaction revealed that it was only the control group that had significantly more BOLD activity during the win cue compared to the loss (p<0.05) and no-outcome (p<0.05) cue periods. Moreover, the control group also had significantly more activity than cannabis users (p<0.05) during the win cue period.

Finally, for the right cuneus [BA18], there was no effect of cue (F=1.0, df=2, 78, p=0.4); a significant effect of group (F=4.4, df=1, 78, p<0.05; Cannabis > Control) but no cue x group interaction (F=2.1, df=2, 78, p=0.1).

Due to the large size of the observed right VS cluster (2008 µl, encompassing the caudate, lentiform nucleus and putamen), we additionally used a higher voxelwise threshold (t = 4.2, p≤0.001) to determine the exact signal location for the observed group BOLD difference during the win cue period. This produced two smaller clusters. The first cluster (114 µl) was located in the right ventral putamen (x= 22, y =3, z= -5) and showed a significant effect of cue (F=3.9, df=2, 78, p<0.05); a
significant effect of group \( (F=4.6, df=1, 78, p<0.05; \text{Cannabis} > \text{Control}) \) but no cue x group interaction \( (F=0.4, df=2, 78, p=0.7). \)

Figure 6. a) Mean brain activity in the right ventral putamen \((x=22, y=3, z=-5)\), b) graph showing cannabis users had a greater BOLD response to loss and win cues compared with no-outcome cues \((p<0.05 \text{ Paired } t\text{-tests})\) in the right ventral putamen and that cannabis users had a greater BOLD response compared to controls in the right ventral putamen during win cue presentation \((p<0.05 \text{ Independent } t\text{-tests})\), c) graph showing cannabis users had a greater BOLD response in the right putamen \((x=19, y=10, z=-1)\) compared to controls during win cue presentation \((p<0.05 \text{ Independent } t\text{-tests})\) and d) correlation between right putamen BOLD activity during win cue presentation and the number of reported life-time cannabis joints smoked \((r=.7, p<0.01)\).
Pairwise comparisons for cue indicated that there was significantly greater BOLD activity during the loss cue compared to the no-outcome period \((p<0.05)\). Within group post hoc testing showed that in the cannabis group there was significantly more BOLD activity during the loss \((p<0.05)\) and win \((p<0.05)\) cue periods compared to the no-outcome cue period (See Fig 6a & b). The second cluster \((66\mu l)\), located in the right putamen \((x=19, y=10, z=-1)\), showed no effect of cue \((F=0.4, df=2, 78, p=0.7)\); no effect of group \((F=1.2, df=1, 78, p=0.3)\) but a significant cue x group interaction \((F=3.6, df=2, 78, p<0.05)\). Between group post hoc tests showed that the cannabis group had significantly greater BOLD activity than the control group \((p<0.05)\) during the win cue period (See Fig 6a & c below). We additionally observed a significant positive correlation between BOLD activity in this putamen cluster during the win cue period and the number of reported life-time cannabis joints consumed \((r=0.7, p<0.01)\) (See Fig 6d).

*MID outcome analyses*

Due to the large volume of activation observed during the outcome periods, we performed a series of region of interest (ROI) analyses in both left and right hemispheres given an *a priori* interest in areas previously associated with reward and loss outcomes (Knutson et al, 2003; Rogers et al, 2004) (See Table 3 below). Exploration of the outcome data in all ROIs, across all conditions and across both groups demonstrated that the data were significantly skewed. Therefore, all data were log (log 10) transformed to eliminate skew and establish normality prior to analyses. Each ROI was tested using two (group) x two (hemisphere) x six (outcome) univariate ANOVAs. For all areas in table 3, there were no group effects of interest or higher order interactions involving group.
Table 3. Brain activations elicited by the MID outcome periods. Positive values for x, y and z Talairach coordinates denote, respectively, locations that are left, posterior and inferior relative to the anterior commissure. Table abbreviations indicate: L=left; R=right; Ctrl=controls; THC=cannabis; NAcc=nucleus accumbens; dlPFC=dorsolateral prefrontal cortex; dACC=dorsal anterior cingulate; rACC=rostral anterior cingulate.

<table>
<thead>
<tr>
<th>Structure</th>
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<th>HS</th>
<th>Vol (µl)</th>
<th>Talairach co-ordinates</th>
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<td></td>
<td></td>
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<tr>
<td>Insula</td>
<td>13</td>
<td>R</td>
<td>30072</td>
<td>41</td>
</tr>
<tr>
<td>Subcallosal Gyrus</td>
<td>13</td>
<td>L</td>
<td>453</td>
<td>-17</td>
</tr>
<tr>
<td>Subcallosal Gyrus</td>
<td>13</td>
<td>R</td>
<td>296</td>
<td>17</td>
</tr>
<tr>
<td>dlPFC</td>
<td>9/46</td>
<td>L</td>
<td>44636</td>
<td>-31</td>
</tr>
<tr>
<td>dlPFC</td>
<td>9/46</td>
<td>R</td>
<td>45876</td>
<td>38</td>
</tr>
<tr>
<td>dACC</td>
<td>24/32</td>
<td>L</td>
<td>6803</td>
<td>-6</td>
</tr>
<tr>
<td>dACC</td>
<td>24/32</td>
<td>R</td>
<td>6641</td>
<td>8</td>
</tr>
<tr>
<td>rACC</td>
<td>24/32</td>
<td>L</td>
<td>3893</td>
<td>-6</td>
</tr>
<tr>
<td>rACC</td>
<td>24/32</td>
<td>R</td>
<td>3935</td>
<td>7</td>
</tr>
</tbody>
</table>

We did observe significant effects of hemisphere for the dorsal anterior cingulate gyrus ($F=10.3$, $df=1$, 312, $p<0.01$) and the dorsolateral prefrontal cortex ($F=18.6$, $df=1$, 312, $p<0.001$). We additionally observed a significant effect of outcome for the subcallosal gyrus ($F=3.0$, $df=5$, 312, $p<0.05$), with pairwise comparisons indicating that there was more BOLD activity during successful no-outcome trials compared to loss trials ($p<0.05$).
Drug-use correlations

Table 4 above indicates that there were a number of significant positive correlations between cannabis use demographics and BOLD activity during win cue presentation of the MID task. Particularly interesting were the correlations between the number of life-time cannabis joints smoked and right declive of vermis BOLD ($r=.8, p<0.001$) (See Fig 4c above); right ventral striatal BOLD ($r=.6, p<0.05$) (see Fig 5c above) and right putamen BOLD ($r=.7, p<0.01$) (See Fig 6d above). Also, cannabis withdrawal scores were negatively correlated with left fusiform [BA37] BOLD activity during win cue presentation ($r=-.7, p<0.01$).

There were no other correlations between self-reported use of cannabis and BOLD activity during either the loss or no-outcome cues periods. Nor were there any correlations between cue BOLD activity and MID behavioural performance (i.e. accuracy and reaction time) in either the cannabis or control groups. There were no correlations between BOLD activity during any outcome period, and either cannabis use or MID behavioural performance. There were no correlations between cannabis withdrawal measures and MID behavioural performance. Finally, there were no correlations between cannabis craving and MID behavioural performance or BOLD activity.
Table 4. Correlations between BOLD activity during win cue presentation in the cannabis group and cannabis use and withdrawal demographics. MFG=medial frontal gyrus.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Use (Years)</th>
<th>Heavy Use (Years)</th>
<th>Life-Time Joints</th>
<th>Withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left MFG/BA6</td>
<td>r=.6, p&lt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right MFG/BA6</td>
<td>r=.5, p&lt;0.05</td>
<td>r=.7, p&lt;0.01</td>
<td>r=.7, p&lt;0.01</td>
<td>r=-7, p&lt;0.01</td>
</tr>
<tr>
<td>Fusiform gyrus/BA37</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Cingulate gyrus</td>
<td>r=.6, p&lt;0.05</td>
<td>r=.7, p&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right cuneus/BA18</td>
<td>r=.6, p&lt;0.05</td>
<td>r=.8, p&lt;0.001</td>
<td>r=.7, p&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Right ventral striatum</td>
<td>r=.6, p&lt;0.05</td>
<td></td>
<td>r=.6, p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Right putamen</td>
<td>r=.5, p&lt;0.05</td>
<td>r=.8, p&lt;0.01</td>
<td>r=.7, p&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Right declive of vermis</td>
<td>r=.7, p&lt;0.01</td>
<td>r=.8, p&lt;0.001</td>
<td>r=.8, p&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

The current investigation, using a monetary incentive delay (MID) task, examined neural activity in cannabis users during the processing of cues predicting non-drug rewards and non-drug losses. In a cohort of cannabis users, demographically matched to a control group, the current study specifically demonstrated an increased BOLD response in the right ventral striatum (VS) for cues predicting non-drug rewards. The current study also observed an increased BOLD response in the right declive of vermis of cannabis users during both loss and win cue periods. The different BOLD responses observed during reward and loss cues occurred in the absence of any behavioural group effect on the MID or any group differences in outcome-related activity, enabling us to discount performance-related neural effects from confounding these group comparisons. The observed elevated VS response to cues predictive of non-drug rewards is in contrast to that previously observed in alcoholism and dual alcohol and cocaine dependence (Wrase et al, 2007; Bjork et al, 2008), suggesting that the relationship between cannabis use and VS activity may be qualitatively different from that of other drugs.

Animal research suggests that cues for primary reinforcers can activate DA neurons in the ventral tegmental area (VTA) and elicit DA release in the VS (Schultz et al, 1997). Human imaging studies have also demonstrated that cues for non-drug incentives reliably activate the VS BOLD response for goal-objects (Knutson et al, 2001; Knutson and Copper, 2005; McClure et al, 2004; O'Doherty, 2004), possibly through endogenous DA release (Dixon et al, 2005; Choi et al, 2006). DA D2 receptor down-regulation and reductions in pre-synaptic DA release in some drug-using groups (Heinz et al, 2004; Martinez et al, 2005; Volkow et al 1997), it is argued, may increase
the threshold required for non-drug reinforcers to activate the VS (Martin-Solch et al, 2001), thereby inducing a reward deficiency syndrome (Blum et al, 2000). The availability of striatal DA D2 receptors, however, has not been shown to significantly differ between cannabis users and drug-naïve controls (Sevy et al, 2008), potentially ruling out a dopaminergic reward deficiency hypothesis. Our finding of increased VS activity during cues for non-drug rewards, may instead provide support for an opponent process view (Solomon and Corbit, 1973), in which the VS neuronal response of cannabis users may be indicative of a hypersensitivity to reward (Bechara et al, 2001, 2002). Given that the VTA contains a moderately high density of cannabinoid (CB1) receptors (Herkenham et al, 1991; Tsou et al, 1998), and evidence that cannabinoids increase midbrain DA neuron activity (French 1997; Chen et al, 1990), this may be a mechanism by which chronic cannabis use alters reward processing in humans.

This account is also consistent with the associations observed between the VS response and cannabis use history. Past research has shown that estimates of life-time cannabis use and life-time “dose” (i.e. cannabis joints) are reliable predictors of behavioural performance and BOLD activity in cannabis users (Block & Ghoneim, 1993; Bolla et al, 2002; Pope & Yurgelun-Todd, 1996; Solowij et al, 1995, 2002; Pope et al, 2003; Bolla et al, 2005; Chang et al, 2006). Correlation analyses revealed that a participant’s reported years of cannabis use and the number of life-time cannabis joints smoked independently predicted their VS BOLD response during cues for non-drug rewards. Importantly, this VS correlation was observed in the absence of any correlations between craving indices and BOLD activity in this area, potentially ruling out this factor as a contributor to the VS response.
We do not believe that the present results were influenced by cannabis intoxication at the time of testing the cannabis-using group, as we found no significant associations between hours of abstinence and task performance or BOLD responses. Cannabis users did demonstrate a reduced BOLD response in the left fusiform gyrus [BA 37], which was significantly negatively correlated with reported cannabis withdrawal. We do not believe, however, that our VS findings were in any way indicative of cannabis withdrawal as there were no associations between withdrawal scores and BOLD activity in the VS. Furthermore, animal research suggests that there is a decline in mesolimbic DA activity during cannabis withdrawal (Diana et al, 1998), which might predict reductions in the VS BOLD response during cues for non-drug rewards in humans, rather than increases, as observed herein. These findings, therefore, may suggest that in current, chronic users of cannabis, there exists some form of mesolimbic reward hyperactivity, which is significantly influenced by life-time cannabis “dosage”.

As already stated, the incentive-sensitization theory of addiction proposes that sensitized neural circuits function to attribute incentive salience to reward-related stimuli, allowing reward cues to trigger excessive “wanting” for the reward (Berridge and Robinson, 1998). In drug addiction, however, the focus of sensitized “wanting” is believed to be primarily towards drug cues and drug rewards, rather than natural rewards (Robinson and Berridge, 1993, 2000). Despite this assertion, sensitization has been shown to enhance the pursuit of natural rewards in animals, where pre-treatment with amphetamine, cocaine and morphine have been observed to significantly increase cue elicited approach behaviour for food, water and sexual contact (Mitchell and Stewart, 1990; Fiorino and Phillips, 1999; Harmer and Phillips, 1999; Taylor and
Horger, 1999; Wyvell and Berridge, 2001). This may suggest that chronic pre-exposure to cannabis in humans might sensitize mesolimbic neural circuits, an effect which is manifested by cue-triggered VS responses, indicating the pursuit for non-drug rewards such as money. Therefore, if these results in chronic cannabis users are indicative of sensitization within the VS during cues predictive of non-drug rewards, they may also have significant implications for the future use and misuse of other drugs. For example, there is evidence that Δ⁹THC exposure in animals affects the developmental plasticity of the reward system (Singh et al, 2006), and that the consumption of cannabis can predict a significantly higher risk for the subsequent use of other more dangerous illicit substances in humans (Fergusson and Horwood, 2000; Lessem et al, 2006). Therefore, one hypothesis, arising from the current findings is that chronic cannabis use in humans may induce a VS hypersensitivity to general rewards, thus increasing the likelihood of future reward seeking, which may come in the form pursuing more dangerous and illicit drugs of abuse.

The current study also demonstrated that cannabis users had a significantly greater BOLD response than controls in the right declive of vermis during both loss and win cue periods. There is evidence that the cerebellum plays a role in cognitive processes required for executing goal-directed behaviours (Paradiso et al, 1999) and conditioned response learning (Logan and Grafton, 1995). Moreover, there is evidence for cerebellar vermis connections to DA cell body regions in the VTA (Snider et al, 1976), with the VTA shown to project to the cerebellum (Ikai et al, 1992). Vermis activity has been shown to occur during the provision of non-drug rewards or their anticipation (Rogers et al, 1999; Kunig et al, 2000; Martin-Solch et al, 2001; Knutson et al, 2001), which may explain activation patterns observed herein. Furthermore, the
vermis has been shown to respond to drug-related stimuli in cocaine (Volkow et al, 2003) and alcohol (Schneider et al, 2001) dependence, with increased cerebellar activity observed during cognitive tasks in alcoholics (Desmond et al, 2003) and cocaine addicts (Hester and Garavan, 2004). The present study also demonstrated that in the cannabis-using group, there were significant relationships between BOLD activity in the declive of vermis (during the win cue period) and cannabis use history (years of use and life-time joints). This group difference in vermis BOLD activity, together with the observed association with cannabis use, may suggest that chronic cannabis use exaggerates cerebellar goal-directed activity in response to cues predictive of non-drug rewards.

The findings of the current study suggest that in chronic cannabis users, there is an increased VS BOLD response to stimuli which predict potential non-drug rewards. Furthermore, the observed VS hyperactivity during reward anticipation was associated with the duration (in years) of cannabis use and the estimated number of life-time cannabis joints consumed. Notwithstanding the distinct possibility that these VS differences may have preceded cannabis use, these findings may suggest a "dose-response" sensitization effect on DA incentive processing within mesolimbic circuitry. Future studies will be needed to examine factors which determine whether it is drug or non-drug rewards that become excessively "wanted" in chronic cannabis users, and indeed other drug-using populations.
References


Ikai, Y., Takada, M., Shinonaga, Y., & Mizuno, N. (1992). Dopaminergic and non-dopaminergic neurons in the ventral tegmental area of the rat project, respectively, to the cerebellar cortex and deep cerebellar nuclei. *Neuroscience, 51*(3), 719-728.


Chapter 4 - Nicotine Use and Non-Drug Reward Processing
Abstract

Investigating differences in brain functioning between current and long-term abstinent drug users represents a significant area within drug addiction research. Importantly, exploring such differences may be informative with respect to the functioning of underlying neural substrates which necessitate successful abstinence and protect against drug relapse. Laboratory studies in animals and human brain imaging research have provided evidence for deficits in ventral striatal (VS) reward-related circuitry following long-term nicotine exposure. This may suggest that drugs of abuse such as nicotine are capable of raising the required threshold for natural reinforcers to activate DA at the VS, an effect which may serve to facilitate continued nicotine consumption and provoke relapse. To investigate the long-term effects of nicotine exposure and abstinence on reward processing, the current study examined VS functioning in 13 controls, 10 ex-smokers and 13 smokers using a monetary incentive delay (MID) task. While results demonstrated significant BOLD activity in response to cues predictive of non-drug rewards and losses in the ventral/dorsal striatum and cingulate gyrus of all groups, there was no evidence of group differences in these areas. With respect to feedback-related neural activity, cigarette-smokers demonstrated BOLD reductions in the dorsolateral prefrontal cortex (dLPFC) compared to both controls and ex-smokers, and in the dorsal anterior cingulate (dACC) compared to controls. Both ex-smokers and smokers showed a diminished BOLD response in the insula compared to controls, with ex-smokers eliciting a reduced BOLD response in the nucleus accumbens (NAcc) compared to both controls and smokers. Reductions in smokers and ex-smokers may, therefore, be indicative of long-term alterations in neural functioning as a consequence of chronic nicotine use, effects which may fail to subside following long-term abstinence.
**Introduction**

Nicotine and other drugs of abuse are conceived to commandeer some of the same neural substrates which have evolved to support beneficial forms of synaptic plasticity, such as learning and memory (Gerdeman et al, 2003). Laboratory studies in animals and humans have consistently demonstrated that dopamine (DA) release within the ventral striatum (VS), underlies the reinforcing properties of nicotine (Koob, 1992; Leshner and Koob, 1999; Benwell and Balfour, 1992; Corrigall et al, 1992; Brody et al, 2002; Dewey et al, 1999; Marenco et al, 2000; Tsukada et al, 2002; Nakamura et al 2000; Stein et al 1998). The VS has additionally been shown to represent a critical neuroanatomical substrate for the processing of non-drug rewards in humans (Knutson et al, 2001; O'Doherty et al, 2004) and has also been established as a key structure underlying incentive salience for goal-objects (Knutson and Copper, 2005; McClure et al, 2004; O'Doherty, 2004). Therefore, drugs such as nicotine which are capable of engaging VS “reward circuitry” may potentially alter VS processing for non-drug rewards, an effect which may significantly contribute to relapse and further nicotine use.

While substantial evidence exists to confirm a VS response to drug-associated stimuli in substance users (Braus et al, 2001; Grusser et al, 2004; London et al, 1999; Sinha and Li, 2007; Wilson et al, 2004), there is little evidence demonstrating that substance-using populations are abnormal in their responses to stimuli predictive of non-drug rewards. Motivational theories regarding drug use make varying predictions with respect to how drug users may respond to cues which signal non-drug incentives. According to the reward deficiency syndrome (RDS) (Blum et al, 2000), addiction represents a deficit in DA motivational circuitry for non-drug rewards, such that only
drugs of abuse are able to normalize DA at the VS. The allostatic hypothesis (AH) (Koob et al, 2004) further proposes that chronic drug use renders the mesolimbic incentive circuitry hyporesponsive to stimuli which signal the potential availability of non-drug rewards (Koob and Le Moal, 2005). These two proposals, relating the effects of drug use to a hypodopaminergic state are particularly relevant, given the existing evidence of long-lasting reductions in DA D2 receptors in drug users (Volkow et al, 1990, 1993, 1996; Heinz et al, 2004), including chronic cigarette smokers (Fehr et al, 2008). These findings may further invoke the notion that as drug associated experiences gain in significance, the threshold required for natural reinforcers to activate DA at the VS, increases (Martin-Solch et al, 2001).

The incentive salience hypothesis (ISH) established by Robinson and Berridge (2001) attributes alterations in striatal circuitry to compulsive drug-taking behaviour. Drug-associated cues supposedly acquire increased incentive-motivational salience as a result of drug consumption, irrespective of changes in hedonic experience. In support of this hypothesis, and the RDS described above, are findings that detoxified alcoholics demonstrate a reduction in VS activation in response to cues which predict non-drug rewards, while eliciting increased VS responses to alcohol associated stimuli (Wrase et al, 2007). There is also evidence for increased VS activity in nicotine addicts in response to cigarette cues (Due et al, 2002; David et al, 2005), with one previous study demonstrating mesolimbic hypoactivity in smokers in response to monetary reinforcement (Martin-Solch et al, 2001). This may suggest that nicotine addiction, like alcoholism, is associated with biased VS reward processing for drug-related stimuli, together with a reduced dopaminergic response for non-drug rewards.
Conversely, substance dependent patients have also been shown to exhibit both impulsive (Bickel and Marsch, 2001) and reward centred (Bechara et al, 2001) choice behaviour under laboratory conditions, with cocaine (Heil et al, 2006) and alcohol (Bjork et al, 2004) dependence associated with an increased preference for small immediate over larger delayed rewards. Importantly, research in cigarette smokers has shown that nicotine administration enhances the incentive value of monetary rewards following an acute period of abstinence (Dawkins et al, 2006) suggesting that smokers may show an increased sensitivity to non-drug inducements. Furthermore, a recent study, using a delayed discounting task, showed that smokers demonstrated a steeper devaluation of delayed cigarette and food rewards compared to money (Odum and Baumann, 2008). These observed effects appear to conform to an opponent process view (OPT) (Solomon and Corbit, 1973) of addiction, which holds that in individuals prone to substance use there is trait impulsivity due to some combination of both mesolimbic reward hyperactivity and deficient frontocortical punishment-avoidance circuitry (Bechara, 2005; Bickel et al, 2007; Newman and Wallace, 1993). Therefore, alterations in nicotine users with respect to the processing of rewards, may equally demonstrate hypersensitivity for non-drug incentives, an effect which may be confirmed by elevations in VS functioning.

Few studies to date have specifically explored the effects of long-term nicotine abstinence on behaviour or brain functioning, particularly with a view to relating functional and behavioural differences in reward processing to the ability to remain abstinent. Bickel et al (1999) investigated delayed discounting in smokers, ex-smokers and controls and revealed that while current cigarette smokers discounted the value of delayed financial rewards, ex-smokers responded in a similar fashion to
controls. Munafo et al (2003) assessed behavioural responses to nicotine-related cues in smokers, ex-smokers and drug naïve controls during a modified Stroop task, demonstrating that while current smokers revealed an attentional bias towards nicotine-related stimuli, ex-smokers showed similar patterns of performance to the control group. These two studies are particularly relevant to the current investigation, given that they do no appear to conform to the RDS model of addiction (Blum et al, 2000) in former cigarette smokers; where if there was a residual effect on VS reward-related circuitry as a consequence of previous nicotine exposure, ex-smokers would be expected to elicit behavioural patterns not that dissimilar from current smokers. Therefore, the results of these two previous studies warrant further attention with respect to the neural substrates of nicotine abstinence on reward processing.

Given the known pharmacological effects of nicotine within the mesolimbic system (Koob, 1992; Leshner and Koob, 1999; Benwell and Balfour, 1992; Corrigall et al, 1992; Brody et al, 2002; Dewey et al, 1999; Marenco et al, 2000; Tsukada et al, 2002; Nakamura et al 2000; Stein et al 1998) and evidence that the anticipation of non-drug incentives reliably activates the VS for goal-objects (Schultz et al, 1997; Knutson et al, 2001; Knutson and Copper, 2005; McLure et al, 2004; O’Doherty, 2004) the current study examined VS reward functioning in demographically matched smokers, ex-smokers and controls using a monetary incentive delay (MID) task. Long-term changes in reward-related brain functioning for non-drug incentives as a consequence of chronic nicotine use may elucidate mechanisms by which adaptations in neural circuits contribute to its continued consumption. Furthermore, examining the effects of chronic nicotine exposure during a period of extended abstinence, may help establish whether neural mechanisms responsible for non-drug reward processing are
significantly different from current nicotine users, the results of which my explicate changes in reward processing which are necessary for drug abstinence.

**Material and Methods**

**Participants**

13 current cigarette smokers, 10 ex-smokers and 13 controls completed the current investigation. All participants underwent a comprehensive telephone screening, during which detailed information concerning past and present psychiatric, neurological and substance use was taken. Information pertaining to any form of treatment (counselling, psychological, psychiatric), past or present, was carefully detailed, with any potential participant describing any major life-time psychiatric event or head injury (e.g., head trauma resulting in a loss of consciousness, seizure or stroke) considered ineligible for the study. Prior to scanning, all participants completed an inventory for drug use (questionnaire taken from the Addiction Severity index Lite-CF; see questionnaires section below) to screen for past or the concurrent abuse of substances. Therefore, participants were additionally considered ineligible if they reported concurrent or past dependence on other drugs (e.g., alcohol, amphetamines, benzodiazepines, cocaine, MDMA, hallucinogens and opiates) at the practice session prior to scanning. Ex-smokers were additionally considered ineligible if they reported past or current use of products to facilitate nicotine abstinence (e.g., gum, patches, lozenges, nasal spray and inhalators). Information concerning alcohol and nicotine use in each participant was indexed in years (life-time) and recent (last 30 days). Other drug use information in each participant was indexed by the total number of separate occasions (life-time) and the total number of recent separate occasions (last 30 days).
Cigarette smokers were required to have regularly consumed nicotine (10-15 cigarettes/day) for the previous 2 years in order to be eligible, with ex-smokers having also met this requirement prior to abstinence (See Table 1 below). Ex-smokers must also have been abstinent from nicotine for a minimum of twelve months prior to enrolment in the study. Control participants must never have smoked cigarettes. All participants were required to provide a negative urine sample for various drugs of abuse prior to scanning, specifically screening for the presence of methadone, benzodiazepines, cocaine, amphetamine, opiates, barbiturates and tricyclic antidepressants (Cozart® RapiScan, UK). Cigarettes smokers each consumed one cigarette 15 minutes prior to scanning in order to avoid withdrawal and craving during the imaging procedure.

All participants were right-handed as confirmed by the Edinburgh Handedness Inventory (Oldfield, 1971) during the telephone screening process. Smoking abstinence in controls and ex-smokers was confirmed by expired carbon monoxide (CO) in parts per million (ppm) prior to scanning. All participants completing the study were neurologically normal (as confirmed by a registered radiologist who examined each structural MRI). All research participants provided informed consent and were financially compensated.
Questionnaires

The National Adult Reading Test (NART) (Nelson & O’Connell, 1978) was administered to all participants prior to scanning to assess verbal intelligence. Information concerning alcohol and drug use (See Table 1 below) was obtained from all participants using a questionnaire taken from the Addiction Severity Index Lite-CF (McLellan et al, 1992). Prior to scanning, the Fagerström test of nicotine dependence (FTND) was administered to participants in the smoking group. The FTND (Heatherton et al, 1991) is a 6-item questionnaire that measures the degree of nicotine dependence in an individual smoker. The Shiffman-Jarvik smoking withdrawal questionnaire (SJWQ) and the urge to smoke (UTS) scale were administered to both the smoker and ex-smoker groups prior to the scanning procedure. The 25-item SJWQ (Shiffman and Jarvik, 1976) asks individuals to respond to questions using a 7-point Likert-type scale from “very definitely” (7) to “very definitely not” (1), with respect to how they feel at that moment regarding potential, separate withdrawal symptoms. These withdrawal symptoms are comprised of craving, physical, psychological, sedation and appetite constructs. Each construct is given a mean score, with the mean for each construct added together to provide an overall withdrawal score in an individual. The 10-item UTS scale (Jarvik et al, 2000) assesses responses to craving-related questions, using a 7-point Likert-type scale from “very definitely” (7) to “very definitely not” (1).
Monetary Incentive Delay Task (MID)

Please refer to pages 110-112 in chapter 3 for a full description of this task.

fMRI acquisition

All scanning was conducted on a Philips Intera Achieva 3.0 Tesla MR system (Best, The Netherlands). Please refer to pages 82-83 in chapter 2 for a full description of imaging acquisitions.

Data processing and analyses

All analyses were conducted using AFNI software (Cox, 1996). Following image reconstruction, the three 3-D time series (runs 1, 2 and 3) were concatenated and motion-corrected using 3-D volume registration (least-squares alignment of three translational and three rotational parameters). Activation outside the brain was also removed using edge detection techniques.

i) Cue analyses

An estimate of activation for the win, loss and no-outcome cue periods was conducted. These three regressors were convolved with a standard haemodynamic response to accommodate the lag time of the blood oxygen level-dependent (BOLD) response. Multiple regression analyses calculated activation as a percentage change relative to the baseline, which consisted of the fixation period at the end of each trial.

The percentage change activation maps were re-sampled to 1 mm³ resolution, warped into standard Talairach space (Talairach and Tournoux, 1988) and spatially blurred with a 3-mm isotropic rms Gaussian kernel. Group activation maps for each condition of the task (win, loss and no-outcome cue) were determined with one-sample $t$-tests
against the null hypothesis of zero activation change (i.e. no change relative to the between trial fixation periods). Significant voxels passed a voxelwise statistical threshold \( t = 3.4, p < 0.005 \) and were required to be part of a larger 274μl cluster of contiguous significant voxels. Thresholding was determined through Monte Carlo simulations and resulted in a 5% probability of a cluster surviving due to chance.

Initially, the thresholded group \( t \)-test map for the win, loss and no-outcome cue periods were combined within each group to form group OR maps. Following this, groups OR maps were combined to form an overall OR map. The overall OR map included those significant voxels from either the win, loss and no-outcome cue periods in both the control and cannabis groups. This final map yielded functionally-defined regions of interest for comparisons between groups and between conditions.

ii) Outcome analyses

To examine neural activations in response to reward and loss outcomes in the control and cannabis groups, an event-related analysis was performed that estimated activations for the six MID outcomes (+0.50 hit; +0.50 miss; -0.50 hit; -0.50 miss; 0.00 hit and 0.00 miss) separately. Using a nonlinear regression programme, we determined the best-fitting gamma-variate function for these six impulse-response functions (Cohen, 1997) as described previously (Murphy and Garavan, 2005) the area under the curve of the gamma-variate function was expressed as a percentage of the area under the baseline. The baseline for the six outcome measures was the same as that used for the block analyses described above.
The percentage area (event-related activation) voxels were re-sampled to 1 mm\(^3\) resolution, before being warped into standard Talairach space and spatially blurred with a 3-mm isotropic rms Gaussian kernel. Group activation maps for each MID outcome condition were also determined with one-sample \(t\)-tests against the null hypothesis of zero activation change (i.e. no change relative to the between trial fixation periods). As above, significant voxels passed the same voxelwise statistical threshold \((t = 3.4, p < 0.005)\) and were also required to be part of a 274\(\mu\)l cluster of contiguous significant voxels. Thresholded group \(t\)-test maps for each outcome period were combined within each group to form group OR maps. Following this, group OR maps were combined to form an overall outcome OR map. The overall outcome OR map included those significant voxels from each outcome period in both the control and cannabis groups.

We observed significant activations from baseline in a number of areas during the outcome periods, which included the anterior cingulate (ACC), insula [BA13], dorsolateral prefrontal cortex (dLPFC) and nucleus accumbens (NAcc). Due to the volume of thresholded outcome activation maps observed in these areas, we decided to perform a series of anatomically-defined region of interest (ROI) analyses in both left and right hemispheres, given an \(a\ priori\) interest in areas previously associated with reward and loss outcomes (Knutson et al, 2003; Rogers et al, 2004). Using the same whole brain thresholding described above, each left and right ROI was combined with the overall outcome OR map to form left and right AND maps for each ROI. The AND map included those significant voxels from the overall outcome OR map and each ROI in both control and cannabis groups. The ROI areas were the ACC, insula [BA13], dLPFC and NAcc.
Between-groups two-way univariate analyses (condition x group) examined the difference between control, ex-smoker and smoker groups on mean activation clusters for the cue conditions. For outcome condition comparisons, three-way univariate analyses (hemisphere x condition x group) were conducted. The observation of either a group effect or group x condition interaction was necessary for post hoc testing using Bonferonni-corrected pairwise comparisons. All between group analyses were conducted in SPSS (SPSS Inc).

Results

Demographics and drug use

Table 1 shows the demographic, nicotine and drug use history for the control, ex-smoker and smoker groups. The groups did not significantly differ on age, years of education, verbal intelligence or alcohol and other drug use. Furthermore, there were no differences between smokers and ex-smokers with respect to nicotine use demographics, such as years of use, pack-years and the number of cigarettes smoked per day. Expired CO levels were significantly lower in controls and ex-smokers compared to smokers, confirming nicotine abstinence in both these groups prior to testing.
Table 1. Mean and SEM for the control, ex-smoker and smoker groups on demographic, nicotine use and drug use history (denotes usage prior to abstinence; *p<0.001 versus control and ex-smoker).

<table>
<thead>
<tr>
<th></th>
<th>Control (n=13)</th>
<th>Ex-smoker (n=10)</th>
<th>Smoker (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>23.6 ± 1.3</td>
<td>25.4 ± 1.6</td>
<td>24.3 ± 1.2</td>
</tr>
<tr>
<td>Years of Education</td>
<td>17.3 ± 0.8</td>
<td>17.9 ± 0.9</td>
<td>16.8 ± 0.6</td>
</tr>
<tr>
<td>Verbal Intelligence Score (NART)</td>
<td>122.9 ± 1.2</td>
<td>123.2 ± 1.0</td>
<td>121.0 ± 1.0</td>
</tr>
<tr>
<td>Females/Males</td>
<td>8/5</td>
<td>7/3</td>
<td>6/7</td>
</tr>
<tr>
<td>Years of Alcohol Use</td>
<td>6.3 ± 1.3</td>
<td>9.0 ± 1.5</td>
<td>8.0 ± 1.2</td>
</tr>
<tr>
<td>Alcohol Use in the Last Month (no. days)</td>
<td>6.4 ± 1.2</td>
<td>6.6 ± 1.6</td>
<td>8.8 ± 1.3</td>
</tr>
<tr>
<td>Alcohol Use Age Onset (Years)</td>
<td>16.4 ± 0.4</td>
<td>16.4 ± 0.6</td>
<td>16.3 ± 0.5</td>
</tr>
<tr>
<td>Years of Nicotine Use</td>
<td>0.0 ± 0.0</td>
<td>7.1 ± 1.7</td>
<td>7.7 ± 1.4</td>
</tr>
<tr>
<td>Pack-Years</td>
<td>0.0 ± 0.0</td>
<td>5.9 ± 1.5</td>
<td>6.3 ± 1.7</td>
</tr>
<tr>
<td>Number of Cigarettes/Day</td>
<td>0.0 ± 0.0</td>
<td>16.0 ± 2.5</td>
<td>15.0 ± 1.3</td>
</tr>
<tr>
<td>Nicotine Use in the Last Month (no. days)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>29.6 ± 0.4</td>
</tr>
<tr>
<td>Number of Packs in the Last Month</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>21.3 ± 2.3</td>
</tr>
<tr>
<td>Nicotine Abstinence (wks)</td>
<td></td>
<td>84.8 ± 13.6</td>
<td></td>
</tr>
<tr>
<td>Amphetamine Use (no. times)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Amphetamine Use in the Last Month (no. times)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Cocaine Use (no. times)</td>
<td>0.0 ± 0.0</td>
<td>0.4 ± 0.3</td>
<td>0.5 ± 0.4</td>
</tr>
<tr>
<td>Cocaine Use in the Last Month (no. times)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>MDMA Use (no. times)</td>
<td>0.1 ± 0.1</td>
<td>1.0 ± 0.7</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>MDMA Use in the Last Month (no. times)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Hallucinogenic Use (no. times)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Hallucinogenic Use in the Last Month (no. times)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Cannabis Use (no. times)</td>
<td>4.7 ± 2.5</td>
<td>9.8 ± 3.1</td>
<td>10.6 ± 2.0</td>
</tr>
<tr>
<td>Cannabis Use in the Last Month (no. times)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Expired Carbon Monoxide (ppm)</td>
<td>3.0 ± 0.0</td>
<td>3.0 ± 0.0</td>
<td>15.2 ± 0.9*</td>
</tr>
</tbody>
</table>
Table 2. Mean and SEM for withdrawal, dependence and craving measures in the ex-smoker and smoker groups.

<table>
<thead>
<tr>
<th>Subscales of Shiffman/Javik Withdrawal Scale</th>
<th>Ex-smoker (n=10)</th>
<th>Smoker (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Craving</td>
<td>3.4 ± 0.2</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>Physical Symptoms</td>
<td>2.0 ± 0.3</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>Psychological Symptoms</td>
<td>3.4 ± 0.1</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>Sedation</td>
<td>3.8 ± 0.2</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>Appetite</td>
<td>2.0 ± 0.3</td>
<td>3.4 ± 0.4**</td>
</tr>
<tr>
<td>Total Score</td>
<td>14.4 ± 0.7</td>
<td>14.5 ± 0.7</td>
</tr>
<tr>
<td>Fagerström Score</td>
<td></td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>Urge to Smoke Scale Score</td>
<td>12.3 ± 1.1</td>
<td>33.9 ± 5.2**</td>
</tr>
</tbody>
</table>

Table 2 above shows that on withdrawal and craving measures, only the appetite construct of the SJWQ significantly differed between the smoker and ex-smoker groups. Here, ex-smokers appeared to have less appetite compared to smokers. Smokers demonstrated a significantly greater UTS score at the testing session prior to scanning.

**MID Performance**

For MID accuracy (% “hits”), there was a significant effect of condition (F=5.6, df=2, 99, p<0.01); no effect of group (F=0.3, df=2, 99, p=0.8) and no condition x group interaction (F=0.1, df=4, 99, p=1.0). *Post hoc* analyses indicated accuracy differences between the no-outcome and loss (p<0.01) and no-outcome and win (p<0.01) conditions (See Fig 1 below). For MID reaction time on hits only (See Fig 2 below), there was no effect of condition (F=0.4, df=2, 99, p=0.7); no effect of group (F=1.3, df=2, 99, p=0.2) and no condition x group interaction (F=0.03, df=4, 99, p=1.0).
Figure 1. Mean percentage accuracy scores for the control, ex-smoker and smoker groups on the no-outcome, loss and win conditions of the MID task (**p<0.01 win and loss condition versus no-outcome condition).

Figure 2. Mean reaction time scores (milliseconds) for the control, ex-smoker and smoker groups on the no-outcome, loss and win conditions of the MID task.
**fMRI**

**Cue analyses**

**Table 3.** Brain activations elicited by win, loss and no-outcome cue anticipation periods during the MID task in the control, ex-smoker and smoker groups. Positive values for $x$, $y$ and $z$ Talairach coordinates denote, respectively, locations that are left, posterior and inferior relative to the anterior commissure. Table abbreviations indicate: L=left; R=right; Ctrl=controls; Ex=ex-smokers; Smk=smokers; FG=frontal gyrus.

<table>
<thead>
<tr>
<th>Structure</th>
<th>BA</th>
<th>HS</th>
<th>Vol (μl)</th>
<th>Talairach co-ordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Middle FG</td>
<td>L</td>
<td>580</td>
<td>-38 47</td>
<td>21</td>
</tr>
<tr>
<td>Medial FG</td>
<td>L</td>
<td>567</td>
<td>0 34 43</td>
<td></td>
</tr>
<tr>
<td>Medial FG</td>
<td>6</td>
<td>R</td>
<td>4025</td>
<td>6 -8 60</td>
</tr>
<tr>
<td>Cingulate</td>
<td>24</td>
<td>L</td>
<td>1760</td>
<td>-9 -1 46</td>
</tr>
<tr>
<td>Cingulate</td>
<td>32</td>
<td>L</td>
<td>387</td>
<td>-2 12 35</td>
</tr>
<tr>
<td>Cingulate</td>
<td>32</td>
<td>R</td>
<td>682</td>
<td>12 11 40</td>
</tr>
<tr>
<td>Cuneus</td>
<td>19</td>
<td>L</td>
<td>299</td>
<td>-1 -86 29</td>
</tr>
<tr>
<td>Caudate</td>
<td>R</td>
<td>1385</td>
<td>12 -3 16</td>
<td></td>
</tr>
<tr>
<td>Ventral Striatum</td>
<td>R</td>
<td>3261</td>
<td>24 3 1</td>
<td></td>
</tr>
<tr>
<td>Putamen</td>
<td>L</td>
<td>346</td>
<td>-22 2 5</td>
<td></td>
</tr>
<tr>
<td>Insula/Claustrum</td>
<td>13</td>
<td>L</td>
<td>1380</td>
<td>-35 5 1</td>
</tr>
<tr>
<td>Declive</td>
<td>L</td>
<td>8578</td>
<td>-3 -84 -16</td>
<td></td>
</tr>
<tr>
<td>Declive</td>
<td>R</td>
<td>355</td>
<td>-25 -66 -22</td>
<td></td>
</tr>
<tr>
<td>Cerebellar Tonsil</td>
<td>R</td>
<td>302</td>
<td>31 -55 -34</td>
<td></td>
</tr>
<tr>
<td>Cerebellar Culmen</td>
<td>L</td>
<td>277</td>
<td>-36 -48 -30</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 above shows that a number of left and right hemisphere activations were observed during the MID cue periods. Areas of activation included bilateral medial frontal, cingulate and cerebellar regions, as well as unilateral occipital and striatal structures. There were no significant between group differences in BOLD activity in any area during MID cue presentation. Within group analyses further revealed that there were no significant differences between conditions in either the control, ex-smoker or smoker groups.
Table 4. Brain activations elicited by MID outcome periods in the control, ex-smoker and smoker groups. Shown are the regions of interest (ROI). Table abbreviations indicate: L=left; R=right; Ctrl=controls; Ex=ex-smokers; Smk=smokers; NAcc=nucleus accumbens; dLPFC=dorsolateral prefrontal cortex; dACC=dorsal anterior cingulate; rACC=rostral anterior cingulate (*p<0.05; **p<0.01; ***p<0.001 Bonferroni Pairwise comparisons for group).

<table>
<thead>
<tr>
<th>Structure</th>
<th>BA</th>
<th>HS</th>
<th>Vol (μl)</th>
<th>Talairach co-ordinates</th>
<th>Group differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x</td>
<td>y</td>
<td>z</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAcc L</td>
<td>L</td>
<td>130</td>
<td>-12</td>
<td>8</td>
<td>-8</td>
</tr>
<tr>
<td>NAcc R</td>
<td>R</td>
<td>157</td>
<td>12</td>
<td>8</td>
<td>-8</td>
</tr>
<tr>
<td>Insula L</td>
<td>13</td>
<td>L</td>
<td>31468</td>
<td>-40</td>
<td>-8</td>
</tr>
<tr>
<td>Insula R</td>
<td>13</td>
<td>R</td>
<td>29524</td>
<td>41</td>
<td>-6</td>
</tr>
<tr>
<td>Subcallosal Gyrus L</td>
<td>13</td>
<td>L</td>
<td>453</td>
<td>-17</td>
<td>14</td>
</tr>
<tr>
<td>Subcallosal Gyrus R</td>
<td>13</td>
<td>R</td>
<td>296</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>dLPFC L</td>
<td>9/46</td>
<td>L</td>
<td>35636</td>
<td>-31</td>
<td>32</td>
</tr>
<tr>
<td>dLPFC R</td>
<td>9/46</td>
<td>R</td>
<td>61111</td>
<td>38</td>
<td>34</td>
</tr>
<tr>
<td>dACC L</td>
<td>24/32</td>
<td>L</td>
<td>6803</td>
<td>-6</td>
<td>6</td>
</tr>
<tr>
<td>dACC R</td>
<td>24/32</td>
<td>R</td>
<td>6641</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>rACC L</td>
<td>24/32</td>
<td>L</td>
<td>3893</td>
<td>-6</td>
<td>37</td>
</tr>
<tr>
<td>rACC R</td>
<td>24/32</td>
<td>R</td>
<td>3935</td>
<td>7</td>
<td>37</td>
</tr>
</tbody>
</table>

Table 4 above shows that there were a number of significant BOLD group differences during the outcome conditions of the MID in the predefined regions of interest (ROI). Two (hemisphere) x three (condition) x three (group) univariate analyses for NAcc BOLD, showed that there was only a significant effect of group (F=6.6, df=2, 396, p<0.01). Pairwise comparisons at group level revealed that ex-smokers showed significantly less BOLD than the control (p<0.01) and smoker (p<0.01) groups (See Figs 3 and 4 below).
Figure 3. BOLD response (mean % change from baseline) in the left nucleus accumbens (NAcc) of the control, ex-smoker and smoker groups during the 6 outcome periods of the MID task, where ex-smokers showed significantly less activity compared to the control and smoker groups ($p<0.01$ - Bonferroni Pairwise comparisons).

![Graph showing BOLD response in the left NAcc](image)

Figure 4. BOLD response (mean % change from baseline) in the right nucleus accumbens (NAcc) of the control, ex-smoker and smoker groups during the 6 outcome periods of the MID task, where ex-smokers showed significantly less activity compared to the control and smoker groups ($p<0.01$ - Bonferroni Pairwise comparisons).

![Graph showing BOLD response in the right NAcc](image)
BOLD activity in the insula [BA13] ROI also showed that there was a significant effect of group (F=4.4, df=2, 396, p<0.05), but no other main effects or interactions. Pairwise comparisons at group level showed that controls had significantly greater BOLD activity compared to both the ex-smoker (p<0.05) and smoker (p<0.05) groups (See Figures 5 and 6 below). Within the insula [BA13] ROI, there was a second, much smaller cluster (x= -17, y= 14, z= -12), which fell within the subcallosal gyrus. This cluster showed a significant effect of group (F=3.1, df=2, 396, p<0.05) in the absence of any other significant main effects or interactions. Pairwise comparisons showed that ex-smokers had less BOLD activity than controls (p<0.05). The dLPFC showed a significant effect of condition (F=2.4, df=2, 396, p<0.05), whereby pairwise comparisons demonstrated that there was a greater BOLD response during the missed outcome (+0.50 miss) compared to the neutral win (+0.00 hit) outcome (p<0.05). There was also a group effect in this region (F=12.4, df=2, 396, p<0.001) in the absence of any interactions involving group. Pairwise comparisons showed that the smoker group had significantly less BOLD activity (p<0.001) compared to both the control and ex-smoker groups (See Figures 7 and 8 below). Finally, in the dorsal anterior cingulate (dACC), there was a significant effect of group (F=3.3, df=2, 396, p<0.05). Pairwise comparisons showed that smokers were significantly lower compared to controls (p<0.05) with respect to BOLD activity in this group.
**Figure 5.** BOLD response (mean % change from baseline) in the left insula [BA13] of the control, ex-smoker and smoker groups during the 6 outcome periods of the MID task, where controls showed significantly greater activity compared to the ex-smoker and smoker groups ($p<0.05$ - Bonferroni Pairwise comparisons).

**Figure 6.** BOLD response (mean % change from baseline) in the right insula [BA13] of the control, ex-smoker and smoker groups during the 6 outcome periods of the MID task, where controls showed significantly greater activity compared to the ex-smoker and smoker groups ($p<0.05$ - Bonferroni Pairwise comparisons).
Figure 7. BOLD response (mean % change from baseline) in the left dLPFC [BA9/46] of the control, ex-smoker and smoker groups during the 6 outcome periods of the MID task, where smokers showed significantly less activity compared to the control and ex-smoker groups (p<0.001 - Bonferroni Pairwise comparisons).

Figure 8. BOLD response (mean % change from baseline) in the right dLPFC [BA9/46] of the control, ex-smoker and smoker groups during the 6 outcome periods of the MID task, where smokers showed significantly less activity compared to the control and ex-smoker groups (p<0.001 - Bonferroni Pairwise comparisons).
Nicotine-use behaviour BOLD correlations

In the ex-smoker group, BOLD activity in the right NAcc during the saved monetary outcome condition (-0.50 hit) was negatively correlated with the reported number of cigarettes smoked per day (r = -0.9, p < 0.001); with BOLD activity in the right NAcc during the lose monetary outcome condition (-0.50 miss) positively correlated with nicotine use age onset (r = 0.8, p < 0.001). In the smoker group, BOLD activity in the left NAcc in response to the monetary win outcome condition (+0.50 hit) was negatively correlated with the number of cigarettes smoked per day (r = -0.6, p < 0.05) (See Fig 9 below). The BOLD response in the right NAcc during the monetary save outcome condition (-0.50c hit) was positively correlated with years of nicotine use (r = 0.8, p < 0.01) and the pack-year history (r = 0.9, p < 0.01). BOLD activity in the right NAcc during the monetary win outcome condition (+0.50 hit) was negatively correlated with nicotine use age onset (r = -0.6, p < 0.05). BOLD activity in the left dlPFC during the saved outcome condition (-0.50c hit) was negatively correlated with urge to smoke score (r = -0.8, p < 0.005) (See Fig 10 below). BOLD activity in the right dlPFC during the lose outcome condition (-0.50c miss) was also negatively correlated with urge to smoke score (r = -0.6, p < 0.05), with activity in this region during the saved outcome condition (-0.50c hit) additionally correlated with urge to smoke score (r = -0.7, p < 0.01). There were no other correlations observed.
Figure 9. Correlation in the smoker group between left NAcc BOLD during the win outcome condition (+0.50c hit) and the number of reported cigarettes smoked per day ($r = -.6$, $p<0.05$).

Figure 10. Correlation in the smoker group between left dLPFC BOLD during the saved outcome condition (-0.50c hit) and urge to smoke score ($r = -.8$, $p<0.005$).
Discussion

The current study examined and compared neural responses in current cigarette smokers, ex-smokers and controls, using a monetary incentive delay (MID) task. Despite recruiting regular current smokers, who on average had consumed 15 cigarettes a day for the last 7.5 years, there were no significant group differences in the ventral striatum (VS), or other brain areas, in response to cues predictive of non-drug rewards and losses. We did observe significant BOLD changes from baseline, in all three groups, in the VS (ventral putamen), dorsal striatum (caudate), cingulate gyrus and cerebellum; areas which have consistently been shown to respond to reward and loss predictive cues during this task (Knutson et al, 2001, 2003, 2005; Schlagenhauf et al, 2008; Strohle et al, 2008). Furthermore, we observed behavioural differences in all three groups with respect to MID performance, in which there were significantly greater response accuracies for loss and win trials, demonstrating the incentive value of our MID paradigm. Therefore, our findings are consistent with the extant literature regarding behavioural and neural activity during the MID task, effects which did not appear, however, to be significantly different between demographically well matched cohorts of cigarette smokers, ex-smokers and controls.

The current investigation also observed robust, significant changes from baseline in all three groups during MID outcomes. We used specific regions of interest (ROIs) to examine differences between groups in brain areas previously shown to respond to rewarding and aversive outcomes (Knutson et al, 2003; Rogers et al, 2004; Berns et al, 2001; Elliott et al, 2003; Taylor et al, 2006). Results demonstrated that ex-smokers, compared to both the control and current smoker groups, had an overall significantly reduced BOLD response during MID outcome deliveries in the NAcc. The NAcc is
traditionally associated with the receipt of rewards (Berns et al, 2001; Kim et al, 2006; Elliot et al, 2003), suggesting that ex-smokers may have reductions in VS functioning as a consequence previous, chronic exposure to nicotine. Indeed, previous research in drug users has demonstrated long-lasting reductions in DA D$_2$ receptors (Volkow et al, 1990, 1993, 1996; Heinz et al, 2004), including chronic cigarette smokers (Fehr et al, 2008), which may indicate the residual effects of nicotine on NAcc functioning. Furthermore, we also observed that the number of cigarettes smoked per day during nicotine usage in ex-smokers, negatively predicted the NAcc BOLD response when money was saved (loss avoidance) in the MID. The fact that smokers also showed significantly more BOLD activity in the NAcc, may speak to the acute effects of nicotine at this region in smokers who are nicotine satiated (David et al, 2007).

Following MID outcome notifications on the MID, both ex-smokers and current smokers showed a reduced BOLD response in the insula [BA13]. Error-related activity in the insula, for example, has previously been demonstrated in healthy controls (Garavan et al, 2002; Hester et al, 2005; Klein et al, 2007), with Klein and colleagues highlighting that insula activity reflects interoceptive (i.e. bodily) awareness (Critchley et al, 2004). Furthermore, recent work suggests that the insula and interoceptive awareness are critical to drug craving and dependence (Paulus, 2007; Naqvi et al, 2007; Gray and Critchley, 2007), whereby the insula monitors interoceptive "urges" for rewarding stimuli such as drugs. Our finding of diminished neural activity in the ex-smoker and smoker groups in response to MID feedback may therefore, imply that chronic nicotine exposure is associated with reductions in neurophysiological responses, which represent interoceptive awareness.
We also observed that ex-smokers had significantly reduced BOLD activity, compared to controls, in the subcallosal gyrus during reward and loss feedback. The subcallosal gyrus is part of the ventromedial prefrontal cortex, is known to participate in awareness and emotional processing (Adolphs et al, 2002) and is an area associated with reward and dopaminergic functioning (Breiter et al, 1997). This BOLD reduction in response to reward and punishment feedback may represent a conditioned “dampening” down of neural activity in circuits associated with arousal, emotional processing and reward; a response which may serve to protect against symptoms which lead to drug relapse. The present study observed significant reductions in the dIPFC of smokers compared to controls and ex-smokers during outcome feedback. The dIPFC is known to be involved in performance monitoring (Bush et al, 1998; Carter et al, 1998; Kiehl et al, 2000), with previous research in smokers demonstrating deficits in this region during cognitive processing (Xu et al, 2006a, 2006b; Musso et al, 2007; Loughead et al, 2008). Importantly, long-term nicotine use has been associated with a reduced grey matter volume in this region (Brody et al, 2004; Gazdzinski et al, 2005; Gallinat et al, 2006), potentially implicating changes in dIPFC volume integrity with respect to “top-down” cognitive control mechanisms; an effect, which functionally, may abate following long-term nicotine abstinence.

Anatomical investigations have revealed divisions along several distinct axes of the ACC (Devinsky et al, 1995; Vogt et al, 1995). The dorsal area, with its marked projections to the dIPFC and motor systems, is referred to as the “cognitive” division of the ACC (Bush et al, 2000; Whalen et al, 1998). Error-related activity has been observed within the dACC (Kiehl et al, 2000; Braver et al, 2001; Ullsperger and von Cramon, 2001, 2004; Garavan et al, 2002) during cognitive tasks, with suggestions
that the involvement of the dACC in emotional processing, may reflect its general role in the modulation of autonomic arousal and the evaluation of responses related to adaptive behavioural control (Gehring and Knight, 2002; Critchley et al, 2003). The current study revealed that current smokers had significantly lower neural activity in the dACC compared to controls. ACC dysfunction has been implicated in the progress and maintenance of addictive behaviour (Goldstein and Volkow, 2002; Peoples et al, 2002; Volkow et al, 2002), with cigarette smokers (Neuhaus et al, 2006; Musso et al, 2007), cannabis (Gruber and Yurgelun-Todd, 2005), cocaine (Hester et al, 2004; Kaufman et al) and methamphetamine users (London et al, 2005) manifesting diminished activity in this region. Therefore, chronic nicotine use (and drug use in general) may have significant implications for reductions in ACC “top-down”cognitive control; with ACC deficits potentially remitting, following a protracted period of abstinence in former nicotine users.

The release of DA within the VS has been shown to underlie the reinforcing properties of nicotine (Koob, 1992; Leshner and Koob, 1999; Benwell and Balfour, 1992; Corrigall et al, 1992; Brody et al, 2002; Dewey et al, 1999; Marenco et al, 2000; Tsukada et al, 2002; Nakamura et al 2000; Stein et al 1998). Research into the long-term effects of nicotine has also provided evidence for reductions in striatal DA D2 receptors (Fehr et al, 2008) and mesolimbic hypoactivity in response to non-drug reinforcement (Martin-Solch et al, 2001) following chronic nicotine exposure in humans. Using a task, previously shown to activate striatal and cortical structures (Knutson et al, 2001, 2003, 2005; Schlagenauf et al, 2008; Strohle et al, 2008), the current study found no evidence for altered meso-cortico-limbic reward processing in response to cues predictive of non-drug rewards in current cigarette smokers. Nor did
we observe any evidence of possible residual effects on striatal reward processing in ex-smokers in response to reward predictive stimuli. The current study did, however, reveal evidence for feedback-related neural hypoactivity in the NAcc of former cigarette smokers; an effect which may indicate, potentially, the residual effects of nicotine on striatal dopaminergic functioning in response to secondary reinforcers. Neural activity in response to feedback was also found to be significantly reduced in current cigarette smokers in regions associated with cognitive control, such as the ACC and dlPFC; an affect which was absent in ex-smokers. These findings may, therefore, suggest that executive, “top-down” processes recover following prolonged nicotine abstinence, at least within the context of processing reward and punishment outcomes; an effect which may serve to protect against symptoms which provoke drug relapse.
References


Chapter 5

Nicotine Use and Attentional Bias for Drug-Predictive Stimuli
Abstract

Studies using stimulus-response selection paradigms have provided strong evidence for an attentional bias towards drug-related stimuli in various substance-using populations, such as cigarette smokers. There is also evidence that nicotine and conditioned cues can maintain cigarette use and trigger smoking relapse. An attentional bias towards smoking-related stimuli, it is suggested, may be due to an increased “incentive salience” for nicotine cues, which mediate drug “wanting”. The current study utilized a paradigm to investigate neural responses during the processing of drug-related stimuli in demographically matched smokers, ex-smokers and controls. Here the study focused on exploring differential neural responses to drug-predictive cues as a result of both long-term current nicotine use and as a function of chronic nicotine abstinence. Behaviourally, no significant group differences were observed with respect to the processing of drug-related stimuli. Consistent with previous research concerning neural responses to smoking cues, however, significant changes in BOLD activity were observed in the nucleus accumbens (NAcc), amygdala, anterior cingulate (ACC), insula and thalamus. Current cigarette smokers demonstrated, overall, significantly greater BOLD activity in the left amygdala compared to controls; with greater right NAcc activity compared to ex-smokers, specifically in response to smoking cues. By and large, ex-smokers demonstrated greater BOLD activity in the rostral ACC and insula compared to controls and current smokers; while in the posterior cingulate, smokers showed general hypoactivity compared to controls and smokers. Taken together, these differences appear to reflect both the effects of chronic nicotine use upon striatal and amygdaloid functioning in current nicotine addiction, while also revealing the functional consequences of successfully abstaining from nicotine, in prefrontal and subcortical circuits.
Introduction

Research has demonstrated that dopamine (DA) release at the level of the ventral striatum (VS) and prefrontal cortex (PFC) underlies the reinforcing properties of drugs of abuse (Koob, 1992; Leshner and Koob, 1999; Pierce et al, 1997). These areas, while clearly implicated in the acute pharmacological effects of drugs, may also be activated by cues which predict drug availability. A major contributing factor to continued drug use is the development of craving or "wanting" upon the presentation of environmental stimuli associated with drug consumption (Niaura et al, 1988, 1998; Rohsenow et al, 1990). Studies using stimulus-response selection paradigms, for example, have provided strong evidence for an attentional bias towards drug-related stimuli in different populations (Field et al, 2004; Field, 2005; Hester et al, 2006; Vadhan et al, 2007; Franken et al, 2000, 2004; Lubman et al, 2000; Townshend and Duka, 2001; Bradley et al, 2008; Mogg et al, 2003, 2005; Waters et al, 2003; Drobos et al, 2006; Munafo et al, 2003), suggesting the development of conditioned cue-induced behavioural arousal upon the presentation of drug-predictive cues. This attentional bias and its accompanying neural response may have significant implications for continued drug use and drug relapse, as well as treatment strategies which attempt to facilitate abstinence.

Cue-induced craving (CIC) is a clinically relevant phenomenon given that it is a major contributing factor within the cycle of drug relapse during drug addiction (O'Brien et al, 1998). While DA is strongly implicated in the development of addiction, it is also known to be involved in reward and reward prediction (Schultz et al, 1997; Wise and Rompre, 1989), and appears to play a significant role in cue elicited drug craving. An attentional bias towards drug-related stimuli involving a
dopaminergic mechanism may be supported by animal studies which have demonstrated both drug-seeking behaviour and DA release at the ventral and dorsal striatum in response to stimuli which have previously been paired with drug administration (Di Ciano and Everitt, 2004; Kiyatkin and Stein, 1996; Phillips et al, 2003; Vanderschuren et al, 2005; Weiss et al, 2000; Duvauchelle et al, 2000; Ito et al, 2002). Furthermore, drug users have been shown to exhibit increased DA release in the ventral and dorsal striatum (caudate and putamen) upon the presentation of drug-related stimuli, an effect found to be associated with elevated levels of drug craving (Volkow et al, 2006; Wong et al, 2006; Zijlstra et al, 2008). fMRI studies have additionally demonstrated that cues responsible for evoking drug-craving activate an integrated neural network involved in the appetitive, emotional and motivational processes of addiction, specifically in the VS, amygdala and anterior cingulate (ACC) (Grusser et al, 2004; Tapert et al, 2003, 2004; Maas et al, 1998; Garavan et al, 2000; Goldstein et al, 2007; Due et al, 2002; David et al, 2005; Smolka et al, 2006; McClernon et al, 2007), all of which are heavily influenced by DA (Koob and Swerdlow, 1988; Wise, 1996).

Cigarette use is a chronic form of compulsive/addictive behaviour, likely leading to cellular adaptations in the brain which may be responsible for CIC and attentional bias towards nicotine-related stimuli. The α4β2 nicotinic acetylcholine receptor (nAChR) appears to be responsible for mediating the reinforcing properties of nicotine and is distributed throughout the mesolimbic DA pathway, particularly on the cell bodies of the ventral tegmental area (VTA) and nerve terminals within the nucleus accumbens (NAcc) of the VS (Le Novere et al, 1996; Quik et al, 2000; Klink et al, 2001; Champtiaux et al, 2003; Wooltorton et al, 2003). α4β2 nAChRs have been
found to be increased in the brains of rodents (Marks et al, 1983; Schwartz and Kellar, 1983), monkeys (McCallum et al, 2006) and humans (Benwell et al, 1988) following chronic nicotine treatment, with evidence for increased α4β2 nAChR-evoked DA release within the NAcc (McCallum et al, 2006) following chronic nicotine exposure. There is also existing evidence for long-lasting reductions in striatal DA D2 receptors in chronic cigarette smokers (Fehr et al, 2008), which may lend further support to the disrupting effects of long-term nicotine within the mesolimbic DA system.

Both nicotine and conditioned cues have been shown to maintain cigarette smoking and trigger relapse (Henningfield and Goldberg, 1983; Rose, 2006). There have been proposals that the release of DA at the NAcc attributes “incentive salience” to drug-associated stimuli, likely to increase the motivational value and attentional processing of drug cues, which mediates drug “wanting” rather than drug “liking” (Robinson and Berridge, 1993). Therefore, sustained cue-induced changes in VS DA discharge may code possible reward availability and elicit a selective form of attentional bias or arousal (Fiorillo et al, 2003). Furthermore, it has been proposed that the low availability of D2 receptors in the VS may mediate an excessive attribution of incentive salience to drug-associated cues, responsible for the pathological “wanting” to consume drugs such as nicotine (Robinson and Berridge, 1993). As already stated, chronic cigarettes smokers demonstrate reductions in striatal DA D2 receptors (Fehr et al, 2008), which may provide a mechanism by which “trait” reductions in dopaminergic functioning interfere with error-detection signals which indicate the availability of drug reward (Schultz et al, 1997).
Furthermore, individuals who have successfully embarked upon a period of nicotine abstinence may show exaggerated functional reductions in brain reward circuitry, associated with the attribution of incentive salience. Moreover, these functional reductions may be concomitant with an increase in functional responses associated with conflict resolution, error detection and avoidance. For example, the “affective” rostral ACC (rACC) has previously been implicated in the assessment of salience and in the regulation of emotional responses (Bush et al, 2000), where the suppression of task-irrelevant emotional information is required (Whalen et al, 1998). Also, the “cognitive” dorsal ACC (dACC) has been implicated in demanding tasks involving stimulus-response selection, such as Stroop paradigms, where there are competing streams of information (Bush et al, 2000), particularly involving conflict resolution (Carter et al, 1998), which encompass emotionally valence (Davis et al., 2005).

Therefore, increased activity in these areas as a product of nicotine abstinence may point to neural substrates which adapt during the presentation of smoking-related cues, which serve to assess the salience of environmental stimuli, regulate emotional processes and resolve conflict. These responses, therefore, may facilitate in maintaining nicotine abstinence and protecting against relapse.

Using an attentional bias paradigm, in which individuals are required to make stimulus-response selections in the presence of neutral, evocative and drug-related stimuli, the current research sought to investigate neuronal functioning in controls, ex-smokers and smokers, with a view to exploring the effects of both current nicotine use and protracted nicotine abstinence. We hypothesized that upon the presentation of drug-related cues for nicotine, current cigarette smokers would demonstrate greater BOLD responses in the VS (NAcc), amygdala and medial PFC, while ex-smokers
would show increased activity in areas implicated in error/risk detection and avoidance behaviour, such as the rostral anterior cingulate cortex (rACC) and insula.

**Material and Methods**

**Participants**

13 current cigarette smokers, 10 ex-smokers and 13 controls completed the current investigation. For a full description of participant screening, questionnaires and other particulars, please refer to the material and methods section in chapter 4, pages 148-156.

**Attentional Bias Paradigm**

Participants in the control, ex-smoker and smoker groups performed an attentional bias paradigm during fMRI scanning. Participants viewed pictures, made up of three different types of stimulus category. Stimulus categories were made up of coloured neutral (musical instruments), evocative (photographs taken from the International Affective Picture System (Lang et al, 1999) and drug (smoking-related) pictures. Each picture within each stimulus category was contained within one of four different coloured borders (i.e. blue, yellow, green and red). During the presentation of each stimulus, participants were simply required to respond to the colour of each border with a button press, using one of four coloured buttons on two separate hand-held key pads.
Figure 1. Attentional bias paradigm where participants were required to make a response to the colour of a border surrounding neutral, evocative and drug-related (smoking) stimuli. Participants were required to respond to the colour (blue, yellow, green and red) of the border surrounding each picture with a button press using one of four different coloured keys (blue, yellow, green and red).

The four different coloured buttons on two separate hand-held key pads (i.e. blue and yellow on the left hand-held, green and red on the right hand-held key pad) corresponded to the four coloured borders used to contain each picture within each stimulus category. The beginning of each block involved participants resting for 20 seconds. Following this rest period, participants performed one of the three stimulus category blocks (neutral, evocative or drug). Each picture within each stimulus category block was presented for 1.5 seconds, followed by a pseudo-randomized inter-stimulus interval of 1, 1.5 or 2 seconds, such that each block was exactly 30 seconds in duration (See Fig 1 above). The task structure consisted of two blocks of
each stimulus category (neutral, evocative and drug). Each stimulus category block consisted of 10 pictures, with a different set of pictures presented in each block. Each block of the task was pseudo-randomized within and between runs. There were a total of two runs, each of which lasted 340 seconds. Dependent measures for the task were the mean percentage correct response and the mean reaction time for each stimulus category. The task was programmed using E-Prime version 1.1 (Psychology Software Tools, Pittsburgh, USA).

fMRI acquisition

All scanning was conducted on a Philips Intera Achieva 3.0 Tesla MR system (Best, The Netherlands). Please refer to pages 82-83 in chapter 2 (Learning and memory in cannabis users) for a full description of imaging acquisitions.

Data processing and analyses

All analyses were conducted using AFNI software (Cox, 1996). Following image reconstruction, the 3-D time series (runs 1 and 2) were concatenated and motion-corrected using 3-D volume registration (least-squares alignment of three translational and three rotational parameters). Activation outside the brain was also removed using edge detection techniques.

A block analysis was performed to estimate the activation for each separate stimulus category. These ON-OFF block regressors were convolved with a standard haemodynamic response to accommodate the lag time of the blood oxygen level-dependent (BOLD) response. Multiple regression analyses were then used to determine the average level of block activation as a percentage change relative to the
rest period (baseline). The baseline activation was derived from averaging the rest periods in each block over both runs of the task.

The percentage change map (block activation) voxels were re-sampled to 1 mm$^3$ resolution, then warped into standard Talairach space (Talairach and Tournoux, 1988) and spatially blurred with a 3-mm isotropic rms Gaussian kernel. Group activation maps for each condition of the task (neutral, evocative and drug) were determined with one-sample $t$-tests against the null hypothesis of zero activation change (i.e., no change relative to the rest period baseline). Due to the very robust BOLD response observed across all groups during the task, we opted for a whole-brain corrected threshold of $p \leq 0.01$. Here significant voxels which passed a voxelwise statistical threshold ($t = 3.6, p=0.001$) were required to be part of a 328μl cluster of contiguous significant voxels. This cluster size criterion was determined through Monte Carlo simulations ($p=0.01$ corrected) resulting in a 1% probability of a cluster surviving due to chance.

To compare activations between the control, ex-smoker and smoker groups, thresholded group $t$-test maps for each condition (neutral, evocative, drug) in all groups were combined to form OR maps. This within group OR map includes the significant voxels from each condition. Following this, each group OR map was combined to form an overall OR map. This between group overall OR map included significant voxels from each condition from each group. The mean activation for clusters in the group overall OR map was calculated for the purpose of a cluster level functionally defined region of interest analysis.
We also performed a small-volume correction region of interest (ROI) analysis for the amygdala, given *a priori* interest in BOLD responses in this region, due to its collection of separate nuclei and previous research implicating it in response to drug and evocative stimuli (Britton et al, 2006; Hariri et al, 2002; Rasia-Filho et al, 2000; Paradiso et al, 1999; Garavan et al, 2000; Due et al, 2002; Wang et al, 2007; Franklin et al, 2007). For the amygdale, a second volume threshold was applied for voxels that fell within anatomically defined left and right amygdale. Here significant voxels passed a voxelwise statistical threshold \((t = 3.4, p < 0.005)\) and were required to be part of a \(26\mu l\) cluster of contiguous significant voxels. We also performed a ROI analysis on the nucleus accumbens (NAcc). Due to its very small size, we, simply averaged over the voxels in this area to calculate the mean BOLD percentage change score during neutral, evocative and drug conditions in both the left and right NAcc.

Within group mean activation cluster analyses examined differences between the neutral, evocative and drug conditions. Between groups examined differences between smokers, ex-smokers and controls on these conditions. All analyses were conducted using SPSS (SPSS Inc).
Results

Demographics and drug use

Tables 1 and 2 on pages 154-156 in chapter 4 show the group demographic, nicotine and drug use history for all samples, as these same participants completed both the nicotine MID and the current attentional bias paradigm reported here.

Attentional Bias Paradigm Performance

Three (group) by three (condition) univariate analyses showed that for percentage accuracy (See Fig 2 below), there was an effect of condition (F=3.7, df=2, 99, p<0.05); an effect of group (F=7.2, df=2, 99, p<0.001) but not condition by group interaction (F=0.4, df=4, 99, p=0.8). Post hoc testing showed that at a condition level, accuracy during the drug condition was significantly higher (p<0.05) compared to the neutral condition. At a group level, analyses showed that ex-smokers had significantly poorer accuracy than smokers (p<0.001), while differences between controls and smokers only approached significance (p=0.08). For reaction time (See Fig 3 below), there was no effect of condition (F=0.8, df=2, 99, p=0.5); no effect of group (F=0.4, df=2, 99, p=0.7) and no condition by group interaction (F=0.2, df=4, 99, p=1.0).
Figure 2. Mean percentage accuracy during the neutral, evocative and drug conditions for the control, ex-smoker and smoker groups on the attentional bias paradigm (***p<0.001 smoker versus ex-smoker).

Figure 3. Mean reaction time during the neutral, evocative and drug conditions for the control, ex-smoker and smoker groups on the attentional bias paradigm.
### Table 3. Regions of activation during the neutral, evocative and drug conditions of the attentional bias paradigm in all three groups. Shown are the regions for whole brain and small volume ROI analyses. Positive values for x, y and z Talairach coordinates denote, respectively, locations that are left, posterior and inferior relative to the anterior commissure. Table abbreviations indicate: BA=Brodman area; HS=hemisphere; Vol=activity cluster volume in microliters; Ctrl=controls; Ex=ex-smokers, Smk=smokers; Neutral=neutral condition; Drug=drug condition; FG=frontal gyrus; ACC=anterior cingulate; PCC=posterior cingulate; PHG=parahippocampal gyrus; NAcc=nucleus accumbens.

<table>
<thead>
<tr>
<th>Structure</th>
<th>BA</th>
<th>HS</th>
<th>Vol (µl)</th>
<th>Talairach co-ordinates</th>
<th>Group differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lingual Gyrus</td>
<td>18/19</td>
<td>R</td>
<td>153371</td>
<td>x:2, y:-83, z:-5</td>
<td>Ex&gt;Ctr*; Ex&gt;Smk**</td>
</tr>
<tr>
<td>Inferior FG/Insula</td>
<td>45</td>
<td>L</td>
<td>967</td>
<td>x:-32, y:24, z:4</td>
<td></td>
</tr>
<tr>
<td>Inferior FG/Insula</td>
<td>13</td>
<td>R</td>
<td>7730</td>
<td>x:36, y:18, z:8</td>
<td>Ex&gt;Ctr &amp; Smk**</td>
</tr>
<tr>
<td>ACC</td>
<td>32</td>
<td>R</td>
<td>4432</td>
<td>x:5, y:40, z:-5</td>
<td></td>
</tr>
<tr>
<td>Cingulate</td>
<td></td>
<td></td>
<td></td>
<td>x:1, y:4, z:47</td>
<td></td>
</tr>
<tr>
<td>PCC</td>
<td>30</td>
<td>L</td>
<td>1531</td>
<td>x:-18, y:-51, z:8</td>
<td>Ctr&gt;Smk*; Ex&gt;Smk**</td>
</tr>
<tr>
<td>PCC</td>
<td></td>
<td>R</td>
<td>896</td>
<td>x:16, y:47, z:6</td>
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</tr>
<tr>
<td>Precentral Gyrus</td>
<td>6</td>
<td>L</td>
<td>2920</td>
<td>x:-49, y:2, z:30</td>
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<tr>
<td>Precentral Gyrus</td>
<td>4</td>
<td>R</td>
<td>1971</td>
<td>x:34, y:-14, z:51</td>
<td>Ctr&gt;Smk**</td>
</tr>
<tr>
<td>Precentral Gyrus</td>
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<td>R</td>
<td>6806</td>
<td>x:43, y:1, z:29</td>
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<tr>
<td>Paracentral Lobule</td>
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<td>R</td>
<td>1359</td>
<td>x:2, y:-30, z:56</td>
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</tr>
<tr>
<td>PHG</td>
<td></td>
<td>L</td>
<td>426</td>
<td>x:-21, y:-27, z:-7</td>
<td></td>
</tr>
<tr>
<td>Fusiform Gyrus</td>
<td>19</td>
<td>L</td>
<td>386</td>
<td>x:-51, y:-67, z:-14</td>
<td></td>
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<tr>
<td>Insula</td>
<td>13</td>
<td>R</td>
<td>478</td>
<td>x:36, y:-20, z:15</td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td></td>
<td>L</td>
<td>880</td>
<td>x:-13, y:-16, z:4</td>
<td>Drug&gt;Neutral* - Smk</td>
</tr>
<tr>
<td>Thalamus</td>
<td></td>
<td>R</td>
<td>2090</td>
<td>x:12, y:-13, z:3</td>
<td></td>
</tr>
</tbody>
</table>

**ROI analysis**

<table>
<thead>
<tr>
<th>Structure</th>
<th>BA</th>
<th>HS</th>
<th>Vol (µl)</th>
<th>Talairach co-ordinates</th>
<th>Group differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdala</td>
<td>L</td>
<td>267</td>
<td>-20</td>
<td>x:-20, y:-4, z:-15</td>
<td>Smk&gt;Ctr**</td>
</tr>
<tr>
<td>Amygdala</td>
<td>R</td>
<td>112</td>
<td>26</td>
<td>x:26, y:-5, z:-18</td>
<td>Smk&gt;Ctr**</td>
</tr>
<tr>
<td>NAcc</td>
<td>L</td>
<td>130</td>
<td>-12</td>
<td>x:-12, y:8, z:-8</td>
<td>Smk&gt;Ex* - Drug</td>
</tr>
<tr>
<td>NAcc</td>
<td>R</td>
<td>157</td>
<td>12</td>
<td>x:12, y:8, z:-8</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 above lists the brain regions significantly activated during the neutral, evocative and drug conditions across the three groups. All data across conditions and between groups in each brain region were found to be normally distributed.
Figure 4. Sagittal sections show horizontal regions activated during neutral, evocative and drug conditions for the attentional bias paradigm. Shown are $t$-test maps ($p=0.001$) for the control, ex-smoker and smoker groups.
In the right thalamus, there was a significant effect of condition (F=3.4, df=2, 99, p<0.05); no effect of group (F=2.0, df=2, 99, p=0.1) and no condition x group interaction (F=0.7, df=4, 99, p=0.6). Pairwise comparisons at condition level showed that the drug condition elicited a greater BOLD response than the neutral condition (p<0.05). To explore this further, a within group one-way ANOVA revealed a significant condition effect only in the smoker group (F(2,36) =4.8, p<0.05), with post hoc tests confirming greater activity during the drug condition compared to the neutral condition (p<0.05). The right precentral gyrus showed no effect of condition (F=0.2, df=2, 99, p=0.8); a significant effect of group (F=7.6, df=2, 99, p<0.01) but no condition x group interaction (F=0.3, df=4, 99, p=0.9). Pairwise comparisons at a group level showed that smokers had significantly less BOLD than controls (p<0.01). Right ACC [BA32] BOLD showed no effect of condition (F=0.2, df=2, 99, p=0.8); a significant effect of group (F=9.2, df=2, 99, p<0.001) but no condition x group interaction (F=0.2, df=4, 99, p=0.9). Pairwise comparisons revealed that ex-smokers had significantly greater BOLD compared to both controls and smokers (p<0.01) (See Fig 5 below). For a cluster located in the posterior portion of the left cingulate gyrus (PCC), there was no effect of condition (F=0.2, df=2, 99, p=0.8); a significant effect of group (F=5.7, df=2, 99, p<0.01) but no condition x group interaction (F=0.3, df=4, 99, p=1.0). Pairwise comparisons showed that smokers had significantly lower BOLD activity compared to both controls (p<0.05) and ex-smokers (p<0.01). In the right inferior frontal gyrus/insula [BA13], there was no effect of condition (F=0.7, df=2, 99, p=0.5); a significant effect of group (F=5.8, df=2, 99, p<0.01) but no condition x group interaction (F=0.5, df=4, 99, p=0.7). Group level pairwise comparisons indicated that ex-smokers had greater BOLD activity compared to both controls (p=0.05) and smokers (p<0.01) (See Fig 6 below).
**Figure 5.** BOLD response (mean % change from baseline) in the right rostral anterior cingulate (rACC) [BA32] during the attentional bias paradigm. Shown is a line graph of the BOLD response in each condition for the three groups, where ex-smokers showed significantly greater activity compared to the control and smoker groups ($p<0.01$ - Bonferroni Pairwise comparisons).

**Figure 6.** BOLD response (mean % change from baseline) in the right insula [BA13] during the attentional bias paradigm. Shown is a line graph of the BOLD response in each condition for the three groups, where ex-smokers showed significantly greater activity compared to the control ($p=0.05$) and smoker groups ($p<0.01$ - Bonferroni Pairwise comparisons).
Small volume correction analyses - Amygdala

Table 3 above additionally shows the results for the left and right amygdala. Using a two (hemisphere) by three (condition) by three (group) univariate analysis, there was a significant effect of condition (F=6.5, df=2, 198, p<0.01) and a significant effect of group (F=6.9, df=2, 198, p<0.01). There were no other main effects or interactions. Pairwise comparisons at the condition level indicated that the evocative condition elicited a greater BOLD response compared to the neutral (p<0.01) and drug conditions (p<0.01). Pairwise comparisons at the group level showed that smokers had significantly more BOLD activity compared to controls (p<0.01) (See Fig 7 below).

ROI analyses - NAcc

Table 3 also shows the results for the left and right NAcc. Using the same analyses as described above for the amygdala, we observed a significant condition by group interaction (F=6.9, df=4, 198, p<0.05). No other effects were observed. Between group one-way ANOVA analyses exploring on which condition and in which hemisphere the groups differed, showed a significant difference during the drug condition in the right NAcc (F(2.33)=3.8, p<0.05). Post hoc analyses demonstrated that smokers had significantly more BOLD activity (p<0.05) compared to ex-smokers (See Fig 8 below).
Figure 7. BOLD response (mean % change from baseline) in a) the left amygdala and b) the right amygdala during the neutral, evocative and drug conditions in the control, ex-smoker and smoker groups. Shown are line graphs of the BOLD response in each condition for the three groups, where smokers showed significantly overall greater activity compared to the control group (p<0.01 - Bonferroni Pairwise comparisons).
Figure 8. BOLD response (mean % change from baseline) in the right NAcc in the control, ex-smoker and smoker groups. Shown are a) line graph of BOLD responses during the neutral, evocative and drug conditions and b) scatter plot showing activation scores for individuals in each group on the drug condition of the attentional bias paradigm (*p<0.05 smoker versus ex-smoker group).
Correlations

There were no correlations between nicotine use demographics and behavioural performance and BOLD activity in the ex-smoker group. In the smoker group, a significant correlation was observed between the left NAcc BOLD response during the drug condition and total score on the Shiffman-Jarvik smoking withdrawal questionnaire (SJWQ) ($r=0.7, p<0.05$) (See Fig 9 below).

Figure 9. Correlation between the left NAcc BOLD response during the presentation of drug-related stimuli in smokers and the total score on the Shiffman-Jarvik smoking withdrawal questionnaire (SJWQ) ($r=0.7, p<0.05$).
Discussion

The current study investigated an attentional bias towards drug-related stimuli in current cigarette smokers, ex-smokers and controls. Behaviourally, we did not observe any performance differences in response accuracy or response latency between the three groups. Previous research studies, utilizing different paradigms, have demonstrated an attentional bias towards nicotine-related stimuli (Bradley et al, 2004; Mogg et al, 2003, 2005; Waters et al, 2003; Drobes et al, 2006; Munafo et al, 2003), which appear to support the idea of conditioned behavioural responses to drug predictive cues in cigarette smokers. Furthermore, there is growing evidence from both animal (Caggiula et al, 2001) and human (Henningfield and Goldberg, 1983; Rose et al, 2006) studies that conditioned sensory stimuli play an important role in the maintenance of drug use behaviour, including nicotine use. We recruited regular current smokers, who on average had consumed 15 cigarettes a day for the last 7.5 years, with an average pack-year history of just under 6.5 years. Prior to scanning, we additionally observed significant differences between the smoker and ex-smoker groups with respect urge to smoke (UTS) scores, indicative of a greater desire to consume nicotine. Nicotine users exhibited greater performance accuracy on drug condition compared to ex-smokers, but no group differed significantly with respect to reaction time.

The current investigation did reveal a number of significant within and between group differences with respect to BOLD responses during the attentional bias paradigm. All three groups demonstrated significant changes from baseline in the anterior cingulate (ACC), posterior cingulate (PCC), insula, thalamus and parahippocampal gyrus; all of which have previously been shown to respond to evocative and drug-related stimuli.
(Britton et al, 2006; Grusser et al, 2004; Tapert et al, 2003, 2004; Maas et al, 1998; Garavan et al, 2000; Goldstein et al, 2007; Due et al, 2002; David et al, 2005; Smolka et al, 2006; McClernon et al, 2007). In the smoker group, there was significantly greater BOLD activity in the right ventral lateral nucleus of the thalamus during the processing of drug-related compared to neutral stimuli. The thalamus has been shown to contain a high number of α4β2 nAChRs (Adem et al, 1988; Brody et al, 2006) which are known to stimulate dopamine (DA) release, with previous research observing a thalamic response to nicotine-related cues during fMRI in cigarette smokers (Due et al, 2002; Franklin et al, 2007; McBride et al, 2006). The thalamus is responsible for the relaying of information to the cortex (Steriade et al, 1997), with differential thalamic nuclei thought to functionally gate the amount of information reaching cortical areas during cognitive processing (Van Der Werf et al, 2001). While we failed to observe a significant group difference in the thalamus, smokers did demonstrate a greater BOLD response compared to the control and ex-smoker groups during the drug condition, perhaps suggesting a greater bias towards smoking cues in this region as a consequence of nicotine addiction.

In the right precentral gyrus [BA4] smokers showed, by and large, significantly less BOLD activity compared to controls. The paradigm used in the current study required individuals to respond to the colour of each border, using one of four separate keys, surrounding neutral, evocative and drug-related stimuli. These responses required the use of both hands, therefore, likely to activate both the left and right primary motor cortices. The current finding that smokers showed overall hypoactivity in the right motor cortex while responding, may suggest a differential functional engagement of both left and right motor areas. Nicotinic receptors are particularly abundant in the
primary motor cortex (Perry et al, 1992; Sihver et al, 1998) with a recent study demonstrating that smokers show reduced motor cortex excitability during transcranial magnetic stimulation (Lang et al, 2007). These results have prompted Lang and colleagues to suggest that chronic nicotine intake, while strengthening cholinergic inhibitory circuits, may also be associated with a reduced integrity in specific neuronal circuits of the motor cortex. Conversely, research has shown that the severity of nicotine dependence and craving intensity are independently associated with increased activation in the primary motor cortex during the presentation of smoking cues (Smolka et al, 2006), effects which we failed to observe in the current study.

We observed, overall, significantly greater BOLD activity in the right ACC [BA32] of ex-smokers compared to controls and smokers during the attentional bias paradigm. The ACC has been divided into “cognitive” dorsal and “affective”/rostral subdivisions (Bush et al, 2000; Devinsky et al, 1995). The activation cluster observed here was located in the rostral portion of the ACC (rACC). The rACC, which occupies BA32, projects directly to the amygdala, NAcc, hippocampus and orbitofrontal cortex (Devinsky et al, 1995). There is considerable evidence implicating the rACC in the assessment of emotional information as well as in the regulation of emotional processing (Bush et al, 2000; Devinsky et al, 1995). Greater overall BOLD activity in ex-smokers in this region may suggest differences in attentional/emotional processing as a consequence of nicotine abstinence. The rACC has previously been implicated in the assessment of salience and in the regulation of emotional responses (Bush et al, 2000), where the suppression of task-irrelevant emotional information is required (Whalen et al, 1998). Cognitive control is equally important when task-irrelevant
emotional stimuli interfere with task relevant processing. The current paradigm required individuals to make stimulus response selections in the presence of competing irrelevant stimuli. Increased rACC BOLD activity has also previously been observed in neuroimaging studies during the presentation of fearful faces and negatively valenced words (Bishop et al., 2004; Mohanty et al., 2005; Vuilleumier et al., 2001; Whalen et al., 1998). Therefore, taken together, the observation of greater rACC activity may suggest that, in ex-smokers, there is an overall greater implementation of ACC-dependent cognitive control during task-relevant processing.

We additionally observed that ex-smokers had significantly greater BOLD activity than both controls and smokers in the right insula. The anterior, agranular regions of the insula are known to have reciprocal connections with the ACC, ventromedial prefrontal cortex, amygdala and ventral striatum; with the integration of autonomic and visceral information into emotional and motivational functions have been ascribed to this region (Naqvi and Bechara, 2008). Furthermore, Craig (2002, 2003) has proposed that the serial processing of interoceptive information occurs within the insula, particularly the right anterior portion, where the conscious awareness of interoceptive stimuli arises. Activity in the insula has previously been demonstrated to occur in response to errors in behaviour (Garavan et al., 2002; Kaufman et al., 2003; Klein et al., 2007), suggesting that this region is also involved in action monitoring functions. The overall increased right insula response in ex-smokers observed herein, therefore, may represent an exaggerated processing of interoceptive effects, which are generalized to all forms of environmental stimuli in nicotine abstinence, perhaps within the context of action monitoring for errors.
Voxel-wise analyses limited to an amygdala region of interest (ROI) also revealed a group difference. Smokers exhibited significantly greater overall BOLD activity in the left amygdala compared to the control group. The animal and human literature has provided ample evidence that the amygdaloid nuclei respond to aversive, as well as appetitive stimulus qualities (Davis, 1992; Gallagher and Schoenbaum, 1999; Holland and Gallagher, 1999; Rasia-Filho et al, 2000; Paradiso et al, 1999). Previous research has also demonstrated amygdala activity in response to nicotine-related cues in cigarette smokers (Due et al, 2002; Wang et al, 2007; Franklin et al, 2007). Amygdalar-mesencephalic glutamatergic pathways have been shown to regulate DA in several brain areas, including the midbrain (Georges and Ashton-Jones, 2001), which it is surmised, may modulate craving and an attentional bias during the presentation of drug-predictive cues (Volkow et al, 2006). While this amygdala effect may have been driven by a deactivation in controls and ex-smokers, the data did demonstrate positive activation from baseline in both the control and ex-smoker groups.

We also performed an ROI analysis in the NAcc, given the effects of nicotine within the mesolimbic DA system (Corrigall et al, 1992), particularly within this structure (Benwell and Balfour, 1992; McCallum et al, 2006), which has been strongly implicated in addiction (Koob, 1992; Leshner and Koob, 1999). Compared to the ex-smoker group, smokers showed significantly more positive BOLD activity in the right NAcc in response to smoking-related stimuli. Human fMRI studies in cigarette smokers have observed elevated neural responses in the NAcc during the presentation of nicotine cues (Deu et al, 2002; David et al, 2005; Franklin et al, 2007). Increased activity in the NAcc during the processing of drug associated stimuli is thought to
underlie an excessive attribution of incentive salience to drug predictive cues, which may be responsible for the pathological "wanting" to consume drugs, such as nicotine (Robinson and Berridge, 1993). Interestingly, in smokers a positive correlation was observed between left NAcc BOLD activity and total scores on the SJWQ. While increased levels of BOLD in the left NAcc of smokers only approached significance on the drug condition, the observed association may implicate subtle symptoms of an attentional bias (e.g., craving) in the attribution of salience to nicotine cues.

As already stated, an attentional bias towards drug-related stimuli, such as smoking cues, may involve a dopaminergic response within the NAcc. This is supported by animal studies, in which both DA release at the ventral and dorsal striatum and drug-seeking behaviour, have been observed in response to stimuli previously paired with drug administration (Di Ciano and Everitt, 2004; Kiyatkin and Stein, 1996; Phillips et al, 2003; Vanderschuran et al, 2005; Weiss et al, 2000; Duvauchelle et al, 2000; Ito et al, 2002). Human drug users have additionally been shown to elicit increased DA activity in the VS/NAcc upon the presentation of drug cues (Wong et al, 2006; Zijlstra et al, 2008), which has also been found to correlate with drug craving. Glutamatergic afferents from the basolateral nucleus of the amygdala to the shell region of the NAcc are considered to be critical for the expression of motor behaviour, driven by motivationally and emotionally relevant stimuli (Cador et al, 1989; Robbins et al, 1989). Ventral tegmental area (VTA) dopaminergic projections to the shell of the NAcc signal the presence of rewarding stimuli (i.e. drugs), facilitate the acquisition of behaviours related to obtaining the reward, and are known to become desensitized with repeated drug exposure (Benwell et al, 1995). Taken together, increased NAcc activity in response to smoking cues in nicotine addiction may reflect increased
salience attribution to positively valenced stimuli (Robinson and Berridge, 1993) due to the chronic effects of nicotine on ventral striatal (McCallum et al, 2006; Fehr et al, 2008) and amygdaloid circuitry (Volkow et al, 2006).

Despite the NAcc BOLD response observed here, it is possible that this effect was driven by both significant activation in smokers and a deactivation in the ex-smoker group. Previous research in acutely abstinent smokers has shown right NAcc deactivation during the presentation of smoking cues (David et al, 2007), which these researchers attributed to a reduced burst firing of dopamine neurons, possibly as a consequence of nicotine withdrawal. In the present study, ex-smokers had been abstinent from nicotine for, on average, 21 months, ruling the out the possibility of nicotine withdrawal on NAcc neural functioning, in response to smoking cues. One possible explanation for NAcc deactivation in ex-smokers may relate to the salience of nicotine-predictive cues following the successful cessation of smoking. Determining whether certain stimuli should be approached or avoided may define either a positive or negative valence. Cues once predictive of smoking behaviour may now hold little in the way of salience for ex-smokers, signified by a deactivation in the NAcc. Furthermore, a decrease in NAcc activity in ex-smokers may be equally indicative of an absence in reward expectancy, with a simultaneous increase in current smokers, thus accounting for a large contrast in the fMRI signal between these two groups. Despite this potential limitation of deactivation in ex-smokers, these data provide additional evidence implicating the VS/NAcc as an important locus in the processing of salient drug-associated stimuli.
Both nicotine and conditioned cues have been shown to maintain cigarette smoking and trigger relapse (Henningfield and Goldberg, 1983; Rose, 2006). The maintenance of smoking behaviour, it is suggested, may be due to an increased “incentive salience” for drug-associated stimuli, which mediates drug “wanting” rather than drug “liking” (Robinson and Berridge, 1993). Furthermore, cigarette use is a chronic form of behaviour, leading to cellular adaptations in the brains of human addicts (Benwell et al, 1988; Fehr et al, 2008) which are likely to play an influential role in nicotine craving, and consequently relapse. The current study has demonstrated a number of BOLD differences between smokers, ex-smokers and controls in response to the processing of neutral, evocative, and drug-related stimuli. These differences may reflect both the effects of chronic nicotine use on VS and amygdaloid functioning in current cigarette smokers, while also revealing the functional consequences of successfully abstaining from nicotine in areas associated with error/risk detection and interoceptive processing.
References:


Abstract

Impaired cognitive control may be a core component of drug addiction, impeding drug abstinence and exacerbating continued drug use. For example, a failure to develop, or a loss of, previously developed cognitive control may impair the ability to restrain pre-potent behaviours such as cigarette smoking. Equally, a deficiency in monitoring one’s behaviour may be an important factor underlying smoking relapse during abstinence. Importantly, studying cognitive control in ex-smokers following a protracted period of nicotine abstinence may reveal activity patterns within neural networks which contribute to maintaining this state. Using a GO-NOGO paradigm, we examined the neural correlates of motor response inhibition and error-monitoring in demographically matched smokers, ex-smokers and controls during fMRI. Behaviourally, the results demonstrate significantly poorer motor response inhibition in smokers compared to ex-smokers and controls and a greater latency to respond in ex-smokers. Compared to controls, smokers demonstrated reduced BOLD activity in the right dorsolateral prefrontal cortex, superior frontal gyrus and anterior cingulate cortex (ACC) during response inhibition. The results also demonstrate that in ex-smokers, there was greater BOLD activity in the bilateral superior frontal gyri, bilateral parahippocampal gyri, left insula, right middle frontal gyrus and left middle temporal gyrus following a motor response error. Additionally, there was significantly more ACC and posterior cingulate BOLD activity in ex-smokers compared to smokers following an error. These data support previous findings in nicotine dependence, while the larger BOLD responses related to error monitoring in ex-smokers, may provide new evidence for neural activity facilitating nicotine abstinence, potentially protecting against relapse.
Introduction

Human and animal studies have demonstrated the reinforcing properties of nicotine at the level of the ventral striatum (VS) (Koob, 1992; Leshner and Koob, 1999; Brody et al, 2002; Dewey et al, 1999; Marenco et al, 2000; Tsukada et al, 2002) which are believed to maintain smoking behaviour in humans. While the majority of cigarette smokers endorse the desire to give up, it is estimated that only 14-49% will achieve full abstinence after 6 months (Holmes et al, 2004; Hughes et al, 1999; Hurt et al, 1997; Jorenby et al, 1999; Killen et al, 2000), with reported relapse rates after twelve months in the region of 5-17% (Hughes et al, 2008). Despite an increased understanding of its addictive properties, there is a paucity of research examining the relationship between the long-term neural effects of nicotine use and abstinence and “top-down” cognitive control mechanisms as they relate to nicotine addiction.

Impairments in cognitive control may be a core component of addiction (Lyvers, 2000; Goldstein et al, 2002; Lubman et al, 2004) contributing to continued drug use. The failure to develop, or a loss of, previously developed cognitive control, such as behavioural inhibition, may affect the ability to restrain the immediate pursuit of pleasurable stimuli such as drugs. Equally, monitoring one’s behaviour, as assayed by the detection of performance errors, may be important for detecting risky circumstances or behaviours, including those that might precipitate relapse (Garavan and Stout, 2005). The prefrontal cortex (PFC) and anterior cingulate (ACC) have been implicated in response inhibition and error-monitoring (Garavan et al, 1999, 2002; Aron et al, 2003; Watanabe et al, 2002; Carter et al, 1998; Ullsperger and von Cramon, 2001), with previous research in addicted populations providing evidence for diminished neural activity in these regions (Ridderinkhof et al, 2002; Kaufman et al, 2002; Kaufman et al, 2004; Lubman et al, 2004).
2003; Forman et al, 2004; London et al, 2005; Neuhaus et al, 2006; Musso et al, 2007). Therefore, behavioural deficits in drug addiction may indicate a dysfunction in neural systems involved in “top-down” cognitive control, the result of which is the progression of drug use and the inability to remain abstinent.

Inhibitory functioning and error monitoring neural activity in addiction are of particular interest, especially during long-term abstinence, as it may indicate specific adaptations within neural networks which promote this state. For example, reduced cognitive control performance, together with prefrontal, cingulate and temporal lobe activity have been shown to predict drug relapse (Carpenter et al, 2006; Cox et al, 2002; Paulus et al, 2005). In cigarette smokers, there is existing evidence of poor inhibitory control (Mitchell, 2004), together with reduced ACC functioning during attention processing (Neuhaus et al, 2006; Musso et al, 2007). Therefore, investigating behavioural and neural differences in current and ex-smokers, who are well matched with respect to nicotine use history, may elucidate neural characteristics of cognitive control which develop to facilitate nicotine abstinence and protect against relapse.

The aim of the present study, therefore, was to examine the neural correlates of inhibitory functioning and error-monitoring in smokers, ex-smokers and controls, using a cognitive control task previously shown to engage PFC and ACC functioning (Garavan et al, 2002).
Material and Methods

Participants

13 current cigarette smokers, 10 ex-smokers and 10 controls completed the current investigation. For a full description of participant screening, questionnaires and other particulars, please refer to the material and methods section in chapter 4, pages 148-150.

Go/No-go Response Inhibition Task

The Go/Nogo task performed by the three groups consisted of alternating target stimuli, each of which were presented for 900 milliseconds, immediately followed by a 100 millisecond inter-stimulus interval. The target stimuli ("go" trials) consisted of the letter "X" and letter "Y", alternately presented, at the centre of the screen. During the presentation of each alternating target stimulus, participants were instructed to make a response (on a right hand-held key pad) as quickly as possible. Participants were additionally instructed to inhibit their response ("no-go" trials) when the target stimuli did not alternate (i.e. the second of two identical, successively presented target stimuli). Participants recommenced responding to alternating stimuli once again, following the presentation of the "no-go" stimulus (See Fig 1 below). There were a total of 250 stimuli presented in each run of the task, of which 25 were "no-go" trials. Participants completed a total of two runs of the task, with each run lasting 254 seconds.
Figure 1. Participants were presented with a 1 second serial stream of alternating Xs and Ys, during which they responded with a single button press. Periodic presentations of non-alternating stimuli ("no-go" trials) required the inhibition (STOP) of a response.
fMRI acquisition

All scanning was conducted on a Philips Intera Achieva 3.0 Tesla MR system (Best, The Netherlands). Please refer to pages 82-83 in chapter 2 (Learning and memory in cannabis users) for a full description of imaging acquisitions.

Data processing and analyses

All analyses were conducted using AFNI software (Cox, 1996). Following image reconstruction, the two time series datasets were concatenated and motion-corrected using 3-D volume registration (least-squares alignment of three translational and three rotational parameters). Activation outside the brain was also removed using edge detection techniques.

To examine neural activations in response to successful inhibitions (STOPs) and ERRORs in all groups, an event-related analysis was performed. Here, separate regressors identifying the locations of STOPs and ERRORs were used in a deconvolution analysis to calculate impulse response functions (IRFs) for each regressor (Murphy and Garavan, 2005). Using a nonlinear regression programme, we determined the best-fitting gamma-variate function for each IRF (Cohen, 1997) as described previously (Garavan et al, 1999). The area under the curve of the gamma-variate function was expressed as a percentage of the area under the baseline. The baseline for both the STOP and ERROR measures reflected tonic task-related processes ("go" trials) of the task.
The percentage area (event-related activation) voxels were re-sampled at 1 mm$^3$ resolution, before being warped into standard Talairach space (Talairach and Tournoux, 1988) and spatially blurred with a 3-mm isotropic rms Gaussian kernel. Group activation maps for each measure were determined with one-sample $t$-tests against the null hypothesis of zero activation change (i.e. no change relative to the ongoing “go” trial period baseline). For ERRORS, significant voxels passed a voxelwise statistical threshold ($t = 3.4, p \leq 0.005$) and were required to be part of a 274μl cluster of contiguous significant voxels. This cluster size criterion was determined through Monte Carlo simulations and resulted in a posterior statistical threshold of $p \leq 0.05$, corrected. Due to the very robust BOLD response observed across all groups for STOPs, we opted for an omnibus threshold of 0.01. Here significant voxels which passed a voxelwise statistical threshold ($t = 3.6, p \leq 0.001$) were required to be part of a 328μl cluster of contiguous significant voxels. The activation maps for the control, ex-smoker and smoker groups were combined by condition (i.e. separate comparisons for STOPs and ERRORs) as OR maps (either/or maps in which a voxel is included if significant in any of the separate group maps). Between-group comparisons were performed on the mean activations of these clusters using a one-way analysis of variance (ANOVA). Bonferroni post hoc analyses were conducted upon the observation of a significant ANOVA group difference.
Results

Demographics and drug use

Table 1 shows the demographic, nicotine and drug use histories for the control, ex-smoker and smoker groups. The groups did not significantly differ on age, years of education, verbal intelligence or alcohol and other drug use. Furthermore, there were no differences between smokers and ex-smokers with respect to nicotine use demographics, such as years of use, pack-years and the number of cigarettes smoked per day. Expired CO levels were significantly lower (0-6 ppm; non-smoker level) in controls and ex-smokers compared to smokers (11-20 ppm; moderate-heavy smoker level), confirming nicotine abstinence in both these groups prior to testing.

Table 2 shows that on withdrawal and craving measures, only the appetite construct of the SJWQ significantly differed between the smoker and ex-smoker groups. Here, ex-smokers appeared to have less appetite compared to smokers. Smokers demonstrated a significantly greater UTS score at the testing session.
Table 1. Mean and SEM for the control, ex-smoker and smoker groups on demographic, nicotine use and drug use history (‘‘denotes usage prior to abstinence: ***p<0.001 smoker versus ex-smoker and control).

<table>
<thead>
<tr>
<th></th>
<th>Control (n=13)</th>
<th>Ex-smoker (n=10)</th>
<th>Smoker (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>23.6 ± 1.3</td>
<td>25.4 ± 1.6</td>
<td>24.3 ± 1.2</td>
</tr>
<tr>
<td>Years of Education</td>
<td>17.3 ± 0.8</td>
<td>17.9 ± 0.9</td>
<td>16.8 ± 0.6</td>
</tr>
<tr>
<td>Verbal Intelligence Score (NART)</td>
<td>122.9 ± 1.2</td>
<td>123.2 ±1.0</td>
<td>121.0 ± 1.0</td>
</tr>
<tr>
<td>Females/Males</td>
<td>8/5</td>
<td>7/5</td>
<td>5/5</td>
</tr>
<tr>
<td>Years of Alcohol Use</td>
<td>6.3 ± 1.3</td>
<td>9.0 ± 1.5</td>
<td>8.0 ± 1.2</td>
</tr>
<tr>
<td>Alcohol Use in the Last Month (no. days)</td>
<td>6.4 ± 1.2</td>
<td>6.6 ± 1.6</td>
<td>8.8 ± 1.3</td>
</tr>
<tr>
<td>Alcohol Use Age Onset (Years)</td>
<td>16.4 ± 0.4</td>
<td>16.4 ± 0.6</td>
<td>16.3 ± 0.5</td>
</tr>
<tr>
<td>Years of Nicotine Use</td>
<td>0.0 ± 0.0</td>
<td>7.1 ± 1.7</td>
<td>6.7 ± 1.2</td>
</tr>
<tr>
<td>Pack-Years</td>
<td>0.0 ± 0.0</td>
<td>5.9 ± 1.5</td>
<td>5.4 ± 1.7</td>
</tr>
<tr>
<td>Number of Cigarettes/day</td>
<td>0.0 ± 0.0</td>
<td>16.0 ± 2.5^</td>
<td>15.0 ± 1.5</td>
</tr>
<tr>
<td>Nicotine Use in the Last Month (no. days)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>29.6 ± 0.4</td>
</tr>
<tr>
<td>Number of Packs in the Last Month</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>21.3 ± 2.3</td>
</tr>
<tr>
<td>Nicotine Abstinence (wks)</td>
<td>84.8 ± 13.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amphetamine Use (no. times)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Amphetamine Use in the Last Month (no. times)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Cocaine Use (no. times)</td>
<td>0.0 ± 0.0</td>
<td>0.4 ± 0.3</td>
<td>0.5 ± 0.4</td>
</tr>
<tr>
<td>Cocaine Use in the Last Month (no. times)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>MDMA Use (no. times)</td>
<td>0.1 ± 0.1</td>
<td>1.0 ± 0.7</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>MDMA Use in the Last Month (no. times)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Hallucinogenic Use (no. times)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
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<tr>
<td>Hallucinogenic Use in the Last Month (no. times)</td>
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<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
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<tr>
<td>Cannabis Use (no. times)</td>
<td>4.7 ± 2.5</td>
<td>9.8 ± 3.1</td>
<td>10.6 ± 2.0</td>
</tr>
<tr>
<td>Cannabis Use in the Last Month (no. times)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Expired Carbon Monoxide (ppm)</td>
<td>3.0 ± 0.0</td>
<td>3.0 ± 0.0</td>
<td>15.2 ± 0.9</td>
</tr>
</tbody>
</table>
Table 2. Mean and SEM for withdrawal, dependence and craving measures in the ex-smoker and smoker groups (**p<0.01).

<table>
<thead>
<tr>
<th>Subscales of Shiffman/Javik Withdrawal Scale</th>
<th>Ex-smoker (n=10)</th>
<th>Smoker (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Craving</td>
<td>3.4 ± 0.2</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>Physical Symptoms</td>
<td>2.0 ± 0.3</td>
<td>2.0 ± 0.4</td>
</tr>
<tr>
<td>Psychological Symptoms</td>
<td>3.4 ± 0.1</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>Sedation</td>
<td>3.8 ± 0.2</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>Appetite</td>
<td>2.0 ± 0.3</td>
<td>3.5 ± 0.4**</td>
</tr>
<tr>
<td><strong>Total Score</strong></td>
<td>14.4 ± 0.7</td>
<td>14.9 ± 0.9</td>
</tr>
<tr>
<td>Fagerström Score</td>
<td></td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>Urge to Smoke Scale Score (UTS)</td>
<td>12.3 ± 1.1</td>
<td>38.0 ± 6.1**</td>
</tr>
</tbody>
</table>

Go/No-go Response Inhibition Task Performance

For behavioural performance, a between-group one-way ANOVA revealed significant group differences for STOPs ($F_{(2,30)} = 7.6$, $p<0.01$). Post hoc analyses showed that smokers were poorer at inhibiting compared to controls ($p<0.05$) and ex-smokers ($p<0.01$; See Fig 2a). For percentage omission errors (See Fig 2c), there was no significant difference between the groups ($F_{(2,30)} = 0.4$, $p=0.7$). For GO trial reaction time (See Fig 2d), there was a significant difference between the groups ($F_{(2,30)} = 4.6$, $p<0.05$). Post hoc analyses revealed that ex-smokers were significantly slower than both controls and smokers ($p<0.05$). There was a similar difference between the groups for ERROR trial reaction time ($F_{(2,30)} = 4.6$, $p<0.05$; See Fig 2b) with post hoc analyses revealing that ex-smokers were significantly slower than both controls and smokers ($p<0.05$).
Figure 2. Control, ex-smoker and smoker groups performance scores on a) percentage STOPs (*p<0.05 versus control, **p<0.01 versus ex-smoker); b) the mean ERROR reaction time (*p<0.05 versus control and smoker); c) mean omission errors and d) mean GO trial reaction time (*p<0.05 versus control and smoker). Data expressed as means and SEM.

**fMRI**

**STOP analyses**

Activated areas were predominantly in the right hemisphere, but also included some bilateral activity (See Table 3 below). These areas included the bilateral frontal and temporal gyri, cingulate and inferior parietal lobule, as well as insula, striatal and cerebellar regions (See Fig 3 below).
Between-group, one-way ANOVA analyses revealed that the smoker group had less BOLD activity than controls in almost all areas where significant group differences were observed. Particularly significant, given the evidence of their role in inhibitory control, was the observation of group differences in the right dlPFC [BA9/46] ($F_{(2,30)} = 8.9, p<0.001$) and the right ACC [BA32] ($F_{(2,30)} = 7.1, p<0.01$), with smokers significantly lower ($p<0.01$) than controls in both regions (See Figs 4a and Fig 4b below). Also particularly significant, given evidence for its role in inhibitory control was a significant group effect in the left inferior frontal gyrus (IFG) ($F_{(2,30)} = 9.0, p<0.001$), where ex-smokers also demonstrated significantly lower BOLD activity compared to controls ($p<0.05$) along with smokers ($p<0.001$) (See Fig 5b below). We additionally observed a significant group effect for a cluster in the right superior temporal gyrus (STG) ($F_{(2,30)} = 7.9, p<0.01$), where ex-smokers also demonstrated significantly lower BOLD activity compared to controls ($p<0.05$) along with smokers ($p<0.01$) (See Fig 5a below). We also observed a group difference for clusters in the right insula [BA13] ($F_{(2,30)} = 9.1, p<0.001$) and the left insula [BA13] ($F_{(2,30)} = 7.7, p<0.01$); where ex-smokers had significantly less BOLD compared to controls ($p<0.05$) (See Figs 6a and 6b below), together with smokers ($p<0.001, p<0.01$ respectively).
Table 3. Regions activated for STOP trials in the control, ex-smoker and smoker groups. Positive values for x, y and z Talairach coordinates denote, respectively, locations that are left, posterior and inferior relative to the anterior commissure. Table abbreviations: BA=Brodmann area; HS=hemisphere; Vol=activity cluster volume in microliters, Ctrl=controls; Ex=ex-smokers; Smk=smokers; FG=frontal gyrus; dIPFC=dorsolateral prefrontal cortex; TG=temporal gyrus; ACC=anterior cingulate; PCC=posterior cingulate; IPL=inferior parietal lobule; PHG=parahippocampal gyrus (*p<0.05, **p<0.01, ***p<0.001 - Post hoc Bonferroni multiple comparisons).

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Figure 3. Sagittal sections showing midline regions activated during STOP trials on the go/no-go task. Shown are $t$-test maps ($p<0.01$, corrected) for the control, ex-smoker and smoker groups.
**Figure 4.** BOLD response (mean % change from baseline) for the control, ex-smoker and smoker groups during STOP trials in a) the right dlPFC (shown in red, **p<0.01 versus control) and b) the right ACC (shown in orange, **p<0.01 versus control).
Figure 5. BOLD response (mean % change from baseline) for the control, ex-smoker and smoker groups during STOP trials in a) right superior temporal gyrus (*p<0.05 versus control; **p<0.01 versus control) and b) the left inferior frontal gyrus [BA44] (*p<0.05 versus control; ***p<0.001 versus control).
Figure 6. BOLD response (mean % change from baseline) for the control, ex-smoker and smoker groups during STOP trials in a) the right insula [BA13] (*p<0.05 versus control; ***p<0.001 versus control) and b) left insula [BA13] (*p<0.05 versus control; **p<0.01 versus control).
**ERROR analyses**

Activated areas were bilateral and in the left hemisphere (See Table 4 below). Areas included bilateral frontal and temporal gyri, insula, parahipocampal gyri, as well as left cingulate and cerebellar regions (See Fig 7 below). Between-group one-way ANOVA analyses revealed that the ex-smoker group had more BOLD activity in most areas where significant group differences were observed, a pattern in contrast to that observed for the STOP trial comparisons reported above. For the right superior frontal gyrus [BA8], there was a significant effect of group ($F_{(2,30)} = 6.9, p<0.01$), where ex-smokers had significantly more BOLD activity compared to both controls and smokers ($p<0.05$) (See Fig 8a below). Similarly, for a cluster in the left superior frontal gyrus [BA9], there was a significant effect of group ($F_{(2,30)} = 6.2, p<0.01$), where ex-smokers demonstrated significantly greater BOLD activity compared to both controls and smokers ($p<0.05$) (See Fig 8b below).

In the right parahippocampal gyrus [BA35], a significant group effect was observed ($F_{(2,30)} = 10.3, p<0.001$). Ex-smokers demonstrated significantly more BOLD activity compared to both controls and smokers ($p<0.01$) (See Fig 9a below). In the left parahippocampal gyrus [BA35], there was also a significant effect of group ($F_{(2,30)} = 9.9, p<0.001$), with post hoc tests showing ex-smokers had more BOLD activity than both controls and smokers ($p<0.01$) (See Fig 9b below). In the left insula, a significant group difference ($F_{(2,30)} = 6.9, p<0.01$) showed that ex-smokers had significantly greater BOLD activity compared to controls ($p<0.05$) and smokers ($p<0.05$) (See Fig 10 below). Significant group differences in both the left ACC [BA24] ($F_{(2,30)} = 3.5, p<0.05$) and left PCC ($F_{(2,30)} = 2.7, p<0.05$) were driven by significantly greater BOLD activity in ex-smokers compared to smokers ($p<0.05$; See Figs 11a and 11b below).
Table 4. Regions activated for ERROR trials in the control, ex-smoker and smoker groups. Table abbreviations are the same as for table 3 (*p<0.05, **p<0.01, ***p<0.001 - post hoc Bonferroni multiple comparisons).

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Figure 7. Sagittal sections show midline regions activated during ERRORs on the go/no-go task. Shown are t-test maps ($p \leq 0.05$, corrected) for the control, ex-smoker and smoker groups.
Figure 8. BOLD response (mean % change from baseline) for the control, ex-smoker and smoker groups during ERRORS in a) the right superior frontal gyrus [BA8] (shown in red) and b) the left superior frontal gyrus [BA9] (shown in yellow) (*p<0.05 versus control and smoker).
Figure 9. BOLD response (mean % change from baseline) for the control, ex-smoker and smoker groups during ERRORs in a) the right parahippocampal gyrus [BA35] (shown in red) and b) the left parahippocampal gyrus [BA35] (shown in orange) (**p<0.01 versus control and smoker).
Figure 10. BOLD response (mean % change from baseline) for the control, ex-smoker and smoker groups during ERRORs in the left insula/inferior frontal gyrus (*p<0.05 versus control and smoker).
Figure 11. BOLD response (mean % change from baseline) for the control, ex-smoker and smoker groups during ERRORS in a) the left PCC [BA24] and b) the left ACC [BA24] (*p<0.05 versus smoker).

**Left PCC/BA24 (-1; -24; 35)**

- Control
- Ex-smoker
- Smoker

**Left ACC/BA24 (-1; 31; 24)**

- Control
- Ex-smoker
- Smoker
Figure 12. Fagerström test of nicotine dependence (FTND) scores were significantly negatively correlated with left insula/inferior frontal gyrus BOLD (r= -.8, p<0.01) in the smoker group.

Correlations

(i) Smokers

Fagerström test of nicotine dependence (FTND) scores were negatively correlated with BOLD activity in the left parahippocampal gyrus during response inhibition (r= -.7, p<0.05). Urge to smoke (UTS) scores were negatively correlated with BOLD activity in the left inferior frontal gyrus [BA44] during response inhibition (r= -.7, p<0.05). During ERRORs there were also a number of correlations in the smoker group. FTND scores were found to be negatively correlated with left insula/inferior frontal gyrus BOLD (r= -.8, p<0.01) (See Fig 12 above) and right superior frontal gyrus [BA10] BOLD activity (r= -.7, p<0.05). Years of nicotine use was negatively correlated with left superior frontal (r= -.7, p<0.05) and left middle temporal gyrus
BOLD ($r = -0.7, p<0.01$). Finally, UTS scores were negatively correlated with BOLD activity in the left parahippocampal gyrus [BA35] ($r = -0.8, p<0.01$).

(ii) *Ex-smokers*

Nicotine use age onset was found to be positively correlated with right middle frontal gyrus BOLD during ERRORs ($r = 0.6, p<0.05$).
Discussion

The current study investigated neural activity in response to behavioural inhibition and error monitoring in demographically well matched controls, ex-smokers and current smokers using a GO/NOGO task. Behaviourally, smokers demonstrated significantly poorer motor response inhibition compared to both the ex-smoker and control groups. Smokers have been shown to exhibit higher rates of impulsivity than the general population (Mitchell, 1999; Waldeck and Miller, 1997), with the current findings appearing to corroborate existing evidence (Mitchell, 2004) of poorer response inhibition, possibly as a consequence of chronic nicotine exposure. Importantly, the mean percentage of omission errors was not significantly different in the smoker group, suggesting that the effect of impulsive responding during ERROR trials was not confounded by an overall inability to perform the task. The current study also revealed that in ex-smokers, there was a significantly longer latency to respond during both GO and ERROR trials, possibly suggesting an overall more conservative or cautious response style.

During motor response inhibition, all groups activated areas consistently shown to be involved in inhibitory control; namely the right dorsolateral prefrontal cortex (dIPFC), ACC, superior temporal gyrus, inferior parietal lobule (IPL), insula and left inferior frontal gyrus (Garavan et al, 1999; Konishi et al, 1999; Fuster et al, 2001; Rubia et al, 2001; Kaufman et al, 2003; Novick et al, 2005; Swick et al, 2008; Horn et al, 2003). One of the defining features of cognitive control is the ability to inhibit responses that are inappropriate, such as restraining the immediate pursuit of pleasurable stimuli. The standard model for the GO/NOGO task, used in the present study, posits that regions of the brain are responsible for inhibiting a prepoent tendency to respond to
inappropriate stimuli; a model invoked within a framework of “top-down” control over impulsive behaviour (Miller and Cohen, 2001). The present study observed significant reductions in the right dlPFC of smokers compared to controls during successful motor response inhibition, consistent with previous research findings of deficits in this region during cognitive processing in smokers (Xu et al, 2006a, 2006b; Musso et al, 2007; Loughead et al, 2008). Importantly, long-term nicotine use has been associated with a reduced grey matter volume in this region (Brody et al, 2004; Gazdzinski et al, 2005; Gallinat et al, 2006), potentially implicating changes in dlPFC volume integrity with respect to “top-down” cognitive control mechanisms; an effect, which functionally, may abate following long-term nicotine abstinence.

The ACC has previously been implicated in response inhibition (Garavan et al, 1999, 2002; Aron et al, 2003; Watanabe et al, 2002), with suggestions that this structure plays a critical role in urgent inhibitions, when time pressures preclude the involvement of the dlPFC (Casey et al, 1996; Ponesse et al, 1998). Compared to controls, cigarette smokers also showed a significant reduction in the right ACC [BA32] BOLD response during STOP trials. ACC dysfunction has been implicated in the progress and maintenance of addictive behaviour (Goldstein and Volkow, 2002; Peoples et al, 2002; Volkow et al, 2002), with cigarette smokers (Neuhaus et al, 2006; Musso et al, 2007), cannabis (Gruber and Yurgelun-Todd, 2005), cocaine (Hester et al, 2004; Kaufman et al, 2003) and methamphetamine users (London et al, 2005) manifesting diminished activity in this region during attention and inhibitory control tasks. Therefore, chronic nicotine use (and drug use in general) may have significant implications for reductions in ACC “top-down” cognitive control over impulsive
responding; with ACC deficits potentially remitting, following a protracted period of abstinence in former nicotine users.

Investigating neural activity in former substance users during a prolonged period of drug abstinence may expose the residual effects of chronic drug exposure on the neural mechanisms of cognitive control. The present study revealed evidence for deficits in the left inferior frontal gyrus (IFG) of ex-smokers during STOP trials. The left IFG is considered critical to cognitive functioning and has been strongly implicated in the suppression of prepotent responses (Novick et al, 2005; Swick et al, 2008) and proactive interference within working memory (Thompson-Schill et al, 2002). During STOP trials, ex-smokers also demonstrated a deficit within the right superior temporal gyrus (STG). Previous research has alluded to the role of the right STG during the inhibitory demands of the GO/NOGO task, which it is conceived, may reflect processing within paralimbic areas in response to the motivational aspects of response inhibition, and their interaction with cognition (Horn et al, 2003). Compared to controls, ex-smokers additionally revealed a significant deficit, bilaterally, in the insula, which is known to respond under conditions of inhibitory control, as well as in the learning and acquisition of inhibitory avoidance behaviour (Garavan et al, 1999; Kaufman et al, 2003; Buchsbaum et al, 2005; Blakemore et al, 1998). These deficits in ex-smokers, which appear common to those observed in current smokers, may suggest a possible disrupting effect of nicotine within selective prefrontal, temporal and subcortical circuits. The failure of these areas to improve, therefore, may still have significant implications for the residual effects of nicotine on response inhibition during abstinence.
The ability to detect errors in one’s behaviour during drug abstinence, one would assume, is a crucial prerequisite necessitating behavioural adjustment, especially when there is a need to detect risky circumstances or behaviours, including those that might precipitate relapse (Garavan and Stout, 2005). Neural responses evoked during error monitoring in addiction are of particular interest, especially during a protracted period of drug abstinence, as they may elucidate specific adaptations within neural networks which promote cognitive control over addictive processes.

The current study observed significantly greater BOLD activity in the right and left superior frontal gyri (SFG) of ex-smokers during ERROR trials compared to both the control and smoker groups. Specifically observed was a greater BOLD response in the right SFG [BA8], which may be sensitive to conditions involving uncertainty surrounding behavioural responses (Volz et al, 2005). This may suggest in that in long-term drug abstinence, there evolves some degree of cognitive control which responds to uncertainty in the presence of potentially erroneous behavioural actions. This effect may, therefore, serve to facilitate in the readjustment to more beneficial and appropriate behavioural patterns and for more optimal error monitoring. Significantly increased BOLD activity in the left SFG [BA9] in response to an ERROR trial was also observed in the ex-smoker group. Research suggests that BA9 may be important in higher level working memory processing (Du Boisgueheneue et al, 2006), where information may be maintained by working memory in the dPFC [BA9/46] and then monitored for error responses in the SFG [BA8] and ACC [BA32]. Therefore, the ability to maintain drug abstinence may arise from neural hyperactivity within an assemblage of cognitive networks, which are exploited in addiction recovery to encode and monitor error behaviour more effectively.
We additionally observed that ex-smokers had significantly greater BOLD activity than both controls and smokers in the left insula/inferior frontal gyrus. Error-related activity in the insula has previously been demonstrated in healthy controls (Garavan et al, 2002; Klein et al, 2007), with Klein and colleagues highlighting that insula activity reflects interoceptive (i.e. bodily) awareness (Critchley et al, 2004). Furthermore, recent work suggests that the insula and interoceptive awareness are critical to drug craving and dependence (Paulus, 2007; Naqvi et al, 2007; Gray and Critchley, 2007), whereby the insula monitors interoceptive "urges" for rewarding stimuli such as drugs. This may suggest that heightened insula monitoring in ex-smokers facilitates the maintenance of nicotine abstinence, particularly in the presence of error behaviour or drug-related urges. Appreciably, we observed an inverse correlation between nicotine dependence scores on the FTND in current smokers and BOLD activity subsequent to an ERROR trial in this same cluster; an effect which may also speaks to the insula's role when initiating drug abstinence.

The current study also provided evidence for an elevated BOLD response in ex-smokers, bilaterally, in the parahippocampal gyrus [BA35] following ERROR trials. The parahippocampal gyrus (PHG) receives convergent inputs from unimodal and polymodal association cortices in the temporal, frontal and parietal lobes (Van Hoesen and Pandya, 1975; Van Hoesen et al, 1975; Tranel et al, 1988; Suzuki and Amaral, 1994) and has been shown to respond robustly during error rate decision making in healthy individuals (Paulus et al, 2002). Furthermore, research has shown that activity within the (para)hippocampal region also predicts performance where people must learn from their errors (Hester et al, 2008). These findings of increased PHG activity observed in ex-smokers may, therefore, provide evidence for an increased encoding of
errors within subcortical memory circuits during action monitoring, which may guide and influence the future direction of behaviour within the context of drug abstinence.

Research implicates the ACC in monitoring one’s performance, especially during situations where risky decision-making, high response conflict or high error likelihood are involved (Carter et al, 1998; Botvinick et al, 2001; Paulus et al, 2006; Magno et al, 2006; Scheffers et al, 1996; Coles et al, 2001). Significantly greater activity in the left ACC [BA24] of ex-smokers, compared to smokers, was observed following ERROR trials in the present study. The ACC has been associated with addiction and its cognitive sequelae (Goldstein and Volkow, 2002; Peoples et al, 2002; Volkow et al, 2002), with previous research demonstrating reduced BOLD activity in this area following error responses in cocaine (Bolla et al, 2004; Kaufman et al, 2004), cannabis (Eldreth et al, 2004) and opiate users (Forman et al, 2004). We additionally observed that ex-smokers elicited more activity in the left posterior cingulate (PCC)/BA24, compared to smokers. The PCC has also been shown to respond to errors during the GO/NOGO task (Menon et al, 2001), with previous evidence for PCC hypofunctioning in cigarette smokers (Neuhaus et al, 2006); suggesting this region may also be particularly sensitive to committing behavioural errors. Taken together, these findings may signify both poorer error monitoring throughout the cingulate gyrus as a consequence of ongoing and chronic nicotine use; with an increase within this region concomitant with a greater attentiveness to errors during extended drug abstinence.
Drug-addicted individuals often appear incapable of exerting sufficient control over their drug-taking urges, suggesting that cognitive processes involved in controlling human behaviour may be compromised in substance dependence. The current study has shown that in chronic cigarette smokers, there is significant evidence for hypofunctioning in brain areas traditionally associated with inhibitory control. Furthermore, these findings were observed in the absence of significant behavioural and functional differences between the control and ex-smoker groups. Although it is feasible that these behavioural and neural differences in current smokers preceded nicotine use, the findings may suggest that cigarette smokers show reductions in inhibitory control, which may serve to exacerbate further nicotine consumption. Equally, ex-smokers were found to demonstrate significantly more neural activity than both controls and smokers, in response to errors, within neural networks consistently shown to be under responsive in various drug-using populations. This increased activity in response to committing an error, may suggest that ex-smokers are “hyper vigilant” with respect to monitoring their behavioural errors. Notwithstanding the possibility that such effects could have arisen from the practicing and monitoring of such behaviours over a prolonged period of abstinence; the current findings may explicate ways in which treatment approaches may augment drug abstinence through pharmacotherapeutic modulation and psychological interventions.
References


Chapter 7 - General Discussion
Summary

The major neural substrates believed to be affected by drugs of addiction are made up of a series of independent and overlapping brain circuits related to reward, motivation and/or drive, learning and memory and cognitive control. Functional neuroimaging studies that focus on distinct, well-characterized cognitive processes related to the integrity of these circuits, afford cognitive neuroscience the chance to better understand behavioural and neuronal processes in drug users. The research above has provided strong evidence for alterations in cognitive neuronal processing in chronic cannabis and nicotine users. These alterations were observed in cannabis users with respect to learning and memory functioning and non-drug reward processing. In nicotine users, distinct behavioural and neuronal differences were observed in current and former cigarette smokers in response to non-drug reinforcement, drug-predictive cues, inhibitory control and error monitoring. The following section will discuss the main research findings of this thesis with a view to addressing the implications of these results in relation to the current literature, the understanding of addiction and future empirical directions for such investigations.

Cannabis

Chapter 2 first presented the research findings of experiment 1 and provided evidence for learning and memory deficits in chronic recreational cannabis users, appearing to corroborate the current literature (Grant et al, 2003; Pope et al, 2001; Pope, 2002; Rodgers et al, 2001; Solowij et al, 2002; Fisk and Montgomery, 2008; McHale and Hunt, 2008). These findings that showed impairments in learning and memory appear to expand upon the existing literature of verbal memory deficits in cannabis users,
with a reduced capacity for face-name learning and memory perhaps holding greater ecological validity with respect to day-to-day cognitive operations (i.e. real world memory). The memory-impairing effects of cannabis are believed to be mediated by the cannabinoid CB₁ receptor, which is expressed at especially high densities in the dentate gyrus (DG) and cornu amonis (CA) 3 regions of the hippocampus (Herkenham et al, 1991; Tsou et al, 1998). The task used in experiment 1 was based on that developed by Zeineh and colleagues (2003) who showed that learning associations between faces and names most prominently activate the DG and anterior CA 2 and 3 fields of the hippocampus. Therefore, the current literature provides evidence for an important overlap between the distribution of CB₁ receptors targeted by cannabis and the neuroanatomy of face-name learning. The hypothesis of experiment 1, stating expected deficits in face-name learning and memory as a consequence of chronic cannabis exposure, therefore, was well founded.

We cannot, however, infer from the findings of inferior learning and memory capacity in cannabis users observed in experiment 1, that such differences were specifically a consequence of cannabis at CB₁ receptors in the (para)hippocampus. Future research, which employs tasks sensitive to (para)hippocampal functioning during fMRI studies, also involving the controlled administration of cannabinoids in both healthy participants and cannabis users, may help in providing additional, more informative evidence regarding the exact location of their effects on learning and memory in the brain. Only by conducting such research will one be able to infer whether or not cannabis exclusively targets the integrity of (para)hippocampal-dependent memory, or whether the effects of cannabis on memory consolidation and retrieval are more pervasive in nature.
Experiment 2 of chapter 2 documented the results of learning and memory-related brain functioning in cannabis users. The implementation of experiment 2 was based on the research findings of experiment 1. Here it was hypothesized that if the learning and memory deficits observed in cannabis users in experiment 1 were hippocampal in origin, then BOLD differences between cannabis users and drug naïve controls would emerge in this region. Using a task which required the learning and recall of faces in similar paired associate form to experiment 1, there was evidence for right parahippocampal gyrus hyperactivity, concomitant with fronto/temporocortical hypoactivity in cannabis users. The results from this experiment appear to concord with the current literature demonstrating increased activity in the parahippocampus of cannabis users (Eldreth et al, 2004; Bolla et al, 2005), together with prefrontal and temporal hypoactivity (Amen and Waugh, 1998; Yurgelun-Todd et al, 1999; Lundqvist et al, 2001; Block et al, 2002; Eldreth et al, 2004; Bolla et al, 2005; Pillay et al, 2004; Gruber and Yurgelun-Todd, 2005; Chang et al, 2006; Jager et al, 2007).

Experiment 2, using a similar learning and memory paradigm to experiment 1 provided evidence to suggest that deficits in learning and memory in cannabis users may not be exclusively (para)hippocampal in origin, but originate within prefrontal and temporal cortical circuits. Regions within the medial temporal lobe receive convergent inputs from unimodal and polymodal association cortices in the temporal, frontal and parietal lobes (Van Hoesen and Pandya, 1975; Van Hoesen et al, 1975; Tranel et al, 1988; Suzuki and Amaral, 1994). Therefore, it is quite possible that the deficits in learning and memory observed in chronic cannabis users in experiment 1, and those observed in previous studies (Grant et al, 2003; Pope et al, 2001; Pope,
2002; Rodgers et al, 2001; Solowij et al, 2002; Fisk and Montgomery, 2008; McHale and Hunt, 2008) are the result of related neuronal adaptations within a number of structures, given the cortical and (para)hippocampal distribution of CB₁ receptors (Herkenham et al, 1991; Tsou et al, 1998). Therefore, the use of fMRI in experiment 2 appears to support the value of cognitive functional imaging when attempting to unravel the precise neural correlates of chronic cannabis use upon learning and memory, potentially suggesting that such deficits in encoding and retrieval may be more ubiquitous in the brain than previously conceived.

One likely limitation of experiment 2, however, is related to the type of analysis conducted, in which a simple block (ON-OFF) design was utilized to examine BOLD activity during learning and recall periods. This limitation is particularly relevant to future research directions in which fMRI paradigms may be used to explore learning and memory capacity in cannabis and other substance-using populations. Such research may benefit from specifically modelling periods in which information is both successfully and unsuccessfully learned and recalled, thereby “teasing” out neuronal correlates specifically related to different levels of learning and memory performance. This approach to data analysis is likely to better explicate where functional learning and memory deficits are located as a consequence chronic drug exposure.

Chapter 3 documented results examining the long-term effects of recreational cannabis use on neural activity during the processing of cues predicting non-drug rewards. The initial hypothesis posited that if all substance use is associated with a reward deficiency syndrome (RDS) (Blum et al, 2000), then cannabis users, like alcoholics in a previous study (Wrase et al, 2007) would also demonstrate ventral
striatal (VS) deficits during the processing of cues indicative of non-drug incentives. The findings from this experiment appeared to contradict the RDS and previous literature in alcoholics (Wrase et al, 2007), in which a cohort of cannabis users demonstrated increased BOLD responses in the right VS, as well as in the right declive of vermis during the presentation of non-drug reward cues. Cannabis users also exhibited an increased VS response to cues predicting non-drug losses, albeit not significantly, suggesting a similar effect of loss and win anticipation in this region, perhaps indicative of a general heightened sensitivity to reward and loss expectancy.

These results demonstrating VS hyperactivity in cannabis users do not concur with theoretical assumptions, such as the RDS, regarding the effects of addictive drugs. Alternatively, the results of this study appear to suggest that cannabis users are not hypoactive, but in fact hyper-responsive to non-drug reward-predicting stimuli, an effect which appears to conform to the reward hypersensitivity hypothesis of drug use (Bechara et al, 2001, 2002). This finding may corroborate research which has demonstrated that cannabis users do not exhibit deficits in striatal DA functioning (Sevy et al, 2008), potentially ruling out a dopaminergic reward deficiency. Taken together, these results provide some evidence for mesolimbic reward hyperactivity in cannabis users mediated by the magnitude of previous cannabis exposure (i.e. years of cannabis use and cannabis joints consumed).

**Nicotine**

Because of the well documented reinforcing effects of nicotine on the DA mesolimbic system (Koob, 1992; Leshner and Koob, 1999; Benwell and Balfour, 1992; Corrigall et al, 1992; Brody et al, 2002; Dewey et al, 1999; Marenco et al, 2000; Tsukada et al,
2002; Nakamura et al 2000; Stein et al 1998), and the observed long-term effects of recreational cannabis use on VS processing described above, research was additionally conducted to investigate long-term current nicotine use and abstinence with respect to non-drug VS reward functioning.

The research hypothesis of chapter 4 similarly stated that if substance use is associated with a RDS (Blum et al, 2000), then current cigarette smokers would also demonstrate VS hypofunctioning during the anticipation of non-drug rewards. This hypothesis was well founded given evidence for long-lasting reductions in striatal DA D2 receptors in chronic cigarette smokers (Fehr et al, 2008), potentially suggestive of a hypodopaminergic state as a consequence of chronic nicotine use. Furthermore, it was hypothesized that if successful nicotine abstinence is associated with changes in reward processing for non-drug incentives, then a cohort of ex-smokers would demonstrate similar patterns of VS activity to a nicotine naïve control group. While the results of chapter 4 demonstrated significant BOLD changes from baseline in the VS (ventral putamen) and dorsal striatum (caudate), the research findings failed to provide any evidence for alterations in the striatal functioning of current long-term cigarette smokers or of ex-smokers.

While no differences were observed during the presentation of reward cues there was evidence for reward and loss feedback-related neural hyperactivity in the NAcc of former cigarette smokers; an effect which may still be indicative of long-term alterations in striatal dopaminergic functioning in this population. This may suggest that in ex-smokers, there is not a hypersensitivity to reward predicting cues, but alternatively to reward and loss feedback. This finding, together with the lack of
group differences in cue-elicited VS functioning is in contrast to those observations documented in chapter 3. Cannabis users demonstrated VS hyperactivity in the absence of any feedback-related neural response differences, an effect which may speak to the distinct psychopharmacological effects of cannabis and nicotine on reward-related brain circuitry. Chapter 4 additionally documented evidence of blunted neural activity in the insula of ex-smokers and smokers, with a reduction in the dorsal ACC of smokers in response reward and loss feedback. Reductions in smokers and ex-smokers it was suggested, may be indicative of long-term alterations in neural functioning as a consequence of chronic nicotine use, effects which may fail to subside following long-term abstinence.

Chapters 3 and 4 have presented research findings for VS functioning in response to cues predictive of non-drug rewards and losses in cannabis and nicotine users. Results from chapter 3 in cannabis users strongly suggested that chronic cannabis use is associated with increased VS responses during reward anticipation, an effect which appeared to be significantly associated with life-time cannabis exposure. Chapter 4 demonstrated no such evidence for alterations in either current nicotine or former nicotine users with respect to reward anticipation, but did reveal distinct differences in the processing of reward and loss feedback in ex-smokers and smokers. As mentioned above, the neural responses observed in these two studies may reveal important information regarding the distinct long-term psychopharmacological effects of cannabis and nicotine on VS reward circuitry.
Traditionally, drugs of abuse have been viewed as potential toxins to the integrity of DA functioning. This is supported by studies in alcohol (Heinz et al, 2004; Martinez et al, 2005; Volkow et al, 1996), cocaine (Volkow et al, 1993; Volkow et al, 1997), methamphetamine (Volkow et al, 2001) and nicotine addiction (Fehr et al, 2008), where either a downregulation of DA D<sub>2</sub> receptors, or a reduction in presynaptic DA release has been observed. These reductions in DA functioning, possibly as a consequence of addiction to these particular substances, suggests that in these substance dependent populations, the threshold required for non-drug reinforcers to activate the VS is likely to be increased (Martin-Solch et al, 2001), leading to a reward deficiency syndrome, in which only these drugs of abuse are able to normalize DA within the VS (Blum et al, 2000). Because of these effects on striatal DA integrity, it is likely that these groups would show a blunted VS response during the processing of non-drug rewards. Indeed, research in alcohol (Wrase et al, 2007) and nicotine dependence (Martin-Solch et al, 2001) has provided some evidence for reductions in VS activity in response to non-drug incentives, appearing to corroborate the RDS (Blum et al, 2000) in these drug-dependent populations. As already stated, striatal DA integrity has previously been investigated in chronic cannabis users (Sevy et al, 2008), with this group failing to observe any deficit in D<sub>2</sub> receptor numbers. The results of Sevy and those observed in cannabis users reported in chapter 3, therefore, may suggest that chronic cannabis is not toxic to striatal DA functioning, in which mesolimbic hypoactivity in response to non-drug rewards would be expected. Despite previous research in cigarette smokers showing reductions in DA D<sub>2</sub> receptors (Fehr et al, 2008), chapter 4 could provide no evidence for a RDS in either current or former cigarette smokers; suggesting that reductions in DA integrity may not necessarily translate into deficiencies within the reward circuitry of nicotine users.
As stated, the incentive-sensitization theory of addiction proposes that sensitized neural circuits function to attribute incentive salience to reward-related stimuli, allowing reward cues to trigger excessive “wanting” for the reward (Robinson and Berridge, 1998). In drug addiction, however, the focus of sensitized “wanting” is believed to be primarily towards drug cues and drug rewards, rather than natural rewards (Robinson and Berridge, 2000, 2001). Despite this belief, sensitization has been shown to enhance the pursuit of natural rewards in animals, where pre-treatment with drugs of abuse have been shown to significantly increase cue-elicited approach behaviour for food, water and sexual contact (Mitchell and Stewart, 1990; Fiorino and Phillips, 1999; Harmer and Phillips, 1999; Taylor and Horger, 1999; Wyvell and Berridge, 2001). This may suggest that chronic pre-exposure to a drug of abuse, such as cannabis, might sensitize mesolimbic neural circuits, the effect of which is manifested by cue-triggered VS responses in human cannabis users during the pursuit of non-drug rewards such as money. Therefore, if the results in chronic cannabis users in chapter 3 are indicative of sensitization within the VS during cues predictive of non-drug rewards, such findings may also have significant implications for the future use and misuse of other drugs, as well as other forms of reward-related dysfunctional behaviour.

There is evidence, for example, that Δ⁹THC exposure in animals affects the developmental plasticity of the reward system (Singh et al, 2006), and that the consumption of cannabis can predict a significantly higher risk for the subsequent use of other more dangerous, illicit substances (Fergusson and Horwood, 2000; Lessem et al, 2006). Cannabis users have also been shown to demonstrate more sexual risk behaviour, through a greater number of sexual partners, putting them at higher risk for
contracting HIV and other sexually transmitted diseases (Bon et al, 2001; Castilla et al, 1999; Wingood and DiClemente, 1998; Poulin and Graham, 2001). Several studies have also indicated a strong association between cannabis use and pathological gambling (Kausch, 2003; de Carvalho et al, 2005; Petry and Tawfik, 2001; Toneatto and Brennan, 2002), perhaps indicative of an inability to balance the immediate pursuit of rewards against the long-term negative consequences of such actions. Importantly, laboratory-based evidence suggests that cannabis users have a greater level of impulsivity and an increased sensitivity for small, but immediate, rewards (Whitlow et al, 2004; Simons and Arens, 2007), consistent with the notion of mesolimbic reward hyperactivity, together with reductions in frontocortical punishment-avoidance circuitry (Solomon and Corbit, 1973, 1974; Bechara, 2005; Bickel et al, 2007). Therefore, one hypothesis, arising from the findings of chapter 3, is that chronic cannabis use in humans may induce a VS hypersensitivity to other rewards (e.g., money, sex), thus increasing the likelihood of future reward seeking and risk-taking behaviour, and potentially, the pursuit of more deleterious and illicit drugs of abuse.

Future research in cannabis users, cigarette smokers and former smokers will be important if one is to demonstrate, definitively, that these substances have differential effects upon the temporal characteristics of reward processing; particularly when there is competition between drug and non-drug reinforcers. For example, do certain substance-using groups demonstrate overall differences in mesolimbic functioning, irrespective of reward type (e.g., money, drug, food)? Are there distinct differences between drug-using populations in their preferences for drug over non-drug rewards? If so, this may suggest that some drugs of abuse (e.g., alcohol) increase the liability
for a RDS while others increase the potential for a general reward hypersensitivity (e.g., cannabis). Utilizing paradigms, similar to the one employed in chapters 3 and 4, in which there is also competition between drug and non-drug rewards may reveal important differences in both current and long-term abstinent drug using groups with respect to mesolimbic reward processing circuitry. These differences will likely further our knowledge of addiction as a whole, and particularly the reward psychopharmacology of different drugs of abuse.

Chapter 5 documents the findings of an investigation into the neural correlates of attentional bias towards nicotine cues in current and former cigarette smokers. Reasons for conducting this study were based on a literature providing strong evidence for an attentional bias towards drug-related stimuli in different substance-using populations (Field et al, 2004; Field, 2005; Hester et al, 2006; Vadhan et al, 2007; Franken et al, 2000, 2004; Lubman et al, 2000; Townshend and Duka, 2001; Bradley et al, 2008; Mogg et al, 2003, 2005; Waters et al, 2003; Drobes et al, 2006; Munafo et al, 2003), including nicotine. Furthermore, previous research had shown that conditioned cigarette cues are able to maintain smoking and trigger relapse (Rose, 2006), possibly due to the potentially disrupting effects of nicotine within the mesolimbic DA system (McCallum et al, 2006a, 2006b; Fehr et al, 2008). Therefore, the hypothesis regarding an expected increased attentional bias in the brains of smokers was empirically well placed. Furthermore, the inclusion of an ex-smoker group, while probing the neural correlates of this attentional bias was well founded, given that neural differences between smokers and ex-smokers in response to nicotine cues may expound upon possible reductions in incentive salience for drug predictive stimuli, a response which may facilitate nicotine abstinence.
Cigarette smokers showed, overall, increased activity in the left amygdala compared to controls, together with an increased response to nicotine cues in the right nucleus accumbens (NAcc) compared to ex-smokers. These results appeared to conform to previous research findings (Due et al, 2002; Wang et al, 2007; Franklin et al, 2007; David et al, 2005), demonstrating an increased neural response in these structures following exposure to nicotine cues. An attentional bias towards drug-related stimuli, it is suggested, may be due to an increased “incentive salience” of drug cues, which mediate drug “wanting” (Robinson and Berridge, 1993). Therefore, the findings observed in the right NAcc of current smokers in chapter 5, may represent a sustained cue-induced change in VS DA discharge, an effect which may code possible reward availability and elicit a selective form of attentional bias or arousal (Fiorillo et al, 2003) following cue exposure. Importantly, this effect was, in part, driven by a deactivation in the NAcc of ex-smokers, which in itself, may indicate a loss of salience attribution to nicotine cues once predictive of nicotine reward. Smokers also showed a greater BOLD response in the right ventral lateral nucleus of the thalamus during the processing of drug-related compared to neutral stimuli. This finding is additionally consistent with previous research showing a thalamic response to nicotine-related cues in cigarette smokers (Due et al, 2002; Franklin et al, 2007; McBride et al, 2006).

As stated above, studying the functional brain activity of ex-smokers during the processing of nicotine-related cues afforded the opportunity to examine how nicotine abstinence may influence incentive salience for drug-predictive stimuli. Indeed, chapter 5 appears to illustrate this, showing that ex-smokers had an overall increased response in the rostral ACC (rACC) during stimulus processing. There is considerable
evidence implicating the rACC in the assessment of emotional information as well as in the regulation of emotional processing and the assessment of salience (Bush et al, 2000; Devinsky et al, 1995), where the suppression of task-irrelevant emotional information is required (Whalen et al, 1998). Chapter 5 also reported that ex-smokers had significantly greater BOLD activity than both controls and smokers in the right insula. The insula is known to have reciprocal connections with the ACC, as well as the amygdala and ventral striatum (Naqvi and Bechara, 2008); with suggestions that serial processing of interoceptive information occurs within this region, particularly the right anterior portion, where the conscious awareness of interoceptive stimuli arises (Craig, 2002, 2003). Thus, the ACC and insula findings reported in chapter 5 may suggest that, in ex-smokers, there is an overall greater implementation of task-relevant processing of environmental stimuli, conferring greater interoceptive information to the insula, resulting in an increase in ACC-dependent cognitive control during conflict resolution. Therefore, these responses may have potentially evolved to facilitate nicotine abstinence, particularly where resolution of conflict may induce a high susceptibility to relapse.

Studying attentional bias in drug abstinence is important for testing the incentive sensitization model of addiction set out by Robinson and Berridge (1993). Recruiting former drug users, such as ex-smokers used in the current studies, may help expose the long-term cognitive and behavioural consequences of addiction, particularly a propensity to drug relapse following a significant period of drug abstinence. Robinson and Berridge (1993) have proposed that neuroadaptations, which occur during chronic drug use, may be permanent or semi-permanent in nature, in which processing biases may persist long into drug abstinence, contributing to drug craving and relapse.
Furthermore, it has been suggested that there are likely to be substantial individual differences in the susceptibility to addiction, which may account, in part, for individual neuroadaptations which may hamper drug abstinence (Robinson and Berridge, 2000). The results set out in chapter 5 appeared to demonstrate that the neural activity in reward circuitry of ex-smokers were indicative of reductions in reward expectancy in response to smoking cues, an effect which may suggest that neuroadaptations induced by chronic nicotine exposure remit following long-term drug abstinence. One cannot rule out, however, that compensatory functioning within neural networks such as the ACC and insula, have evolved to mitigate failures in the NAcc to overcome the residual effects of nicotine.

Chapter 6 documented the results of inhibitory functioning and error monitoring using a GO-NOGO paradigm in smokers, ex-smokers and controls. Hypotheses generated for this experiment were based on well documented evidence taken from a number of sources. First, it has been suggested that impairments in cognitive control, such as behavioural inhibition and error monitoring may play a key role in addictive disorders (Lyvers, 2000; Goldstein et al, 2002; Lubman et al, 2004). Second, the prefrontal cortex (PFC) and anterior cingulate (ACC) regions have been implicated in response inhibition and error monitoring functions (Garavan et al, 1999, 2002; Aron et al, 2003; Watanabe et al, 2002; Carter et al, 1998; Ullsperger and von Cramon, 2001), both of which are known to be affected by drugs of abuse, such as nicotine (Stein et al, 1998). Third, the ACC in particular has been implicated in addiction and its cognitive sequelae (Goldstein and Volkow, 2002; Peoples et al, 2002; Volkow et al, 2002) and has previously been found to be hypoactive in drug users during attention switching (Kubler et al, 2005), Stroop (Bolla et al, 2004; Eldreth et al, 2004) and
inhibitory control paradigms (Hester et al, 2004; Kaufman et al, 2003; Forman et al, 2004). Therefore, it was the aim of chapter 6 to report results in both long-term cigarette smokers and ex-smokers based on the hypothesis that current and past nicotine exposure would differentially activate prefrontal, ACC and subcortical areas during inhibitory control and error monitoring.

Chapter 6 initially reported that smokers demonstrated significantly poorer motor response inhibition compared to both the ex-smoker and control groups. Research suggests that smokers exhibit higher rates of impulsivity than the general population (Mitchell, 1999; Waldeck and Miller, 1997), with the findings from chapter 6 appearing to corroborate existing evidence (Mitchell, 2004) of poorer response inhibition in this population, quite possibly as a consequence of chronic nicotine exposure. Chapter 6 additionally reported that ex-smokers demonstrated a significantly longer latency to respond during both GO and ERROR trials, possibly suggesting an overall more conservative or cautious response style in this population; a characteristic which may have emerged during both the early stages of abstinence and in the long-term to protect against impulsive actions, likely to precipitate relapse.

Cigarette smokers demonstrated significantly reduced activity in the right dorsolateral prefrontal cortex (dlPFC) and right ACC compared to smokers during inhibitory control, concurring with past research findings in substance users (Hester et al, 2004; Kaufman et al, 2003; Forman et al, 2004; Xu et al, 2006a, 2006b; Musso et al, 2007; Loughead et al, 2008). These effects were not observed in the ex-smoker group, however, suggesting an impact of current nicotine use on the ability to restrain prepotent and inappropriate behaviours. Error monitoring brain functioning showed that
ex-smokers were increased compared to both controls and smokers in the left insula and bilateral parahippocampus; while showing significantly more activity in the left ACC and left posterior cingulate (PCC) compared to smokers. These increased responses within an error monitoring system in ex-smokers may provide evidence for increased activity within neural substrates which facilitate nicotine abstinence and protects against drug relapse. Equally, an underactive monitoring system, such as the ACC in current cigarette smokers may serve to promote further nicotine use through drug relapse, due to an inability to appropriately inhibit, evaluate and adjust behaviour accordingly during situations where cognitive control is imperative.

Interestingly, alterations in BOLD functioning have also previously been observed in midline error monitoring regions in other populations. The ACC, for example, is hyperactive in patients suffering from obsessive compulsive disorder (OCD) and is hypoactive in schizophrenic patients (Carter et al, 1998; Ursu et al, 2001). These findings appear to be consistent with the clinical profiles of these groups, in which an overactive action monitoring system and heightened attentiveness to corrective behaviour are observed in OCD, with a failure to properly monitor and integrate stimuli in the environment, core symptoms of schizophrenia. Therefore, it is argued in chapter 6 that an increased activity within monitoring systems, together with improved attentiveness to corrective behaviour, may reflect the evolvement of superior executive functioning; some of which may be a necessary prerequisite to successful nicotine abstinence. This superior cognitive control, perhaps arising from the effects of practiced inhibitory and monitoring, may elucidate ways in which treatment approaches can augment drug abstinence through pharmacotherapeutic and psychological modulation within different brain regions, thus improving inhibitory
control and error monitoring behaviour. Overall the results of chapter 6 are empirically coherent, conforming to previous well established findings regarding inhibitory functional brain deficits in substance use, which are similar to those observed in clinical populations. Moreover, the results of this chapter expand upon a current literature in nicotine addiction by providing some evidence for advanced cognitive control processing in former smokers, which may facilitate drug abstinence and protect against drug relapse.

While the effects of drugs on reward-related processes appear central to drug abuse and dependence, it has been stated throughout that cognitive processes may significantly contribute to addiction. Drug dependence is often characterized as an affective or emotional phenomenon, given the roles of psychological functions such as reward, reinforcement, craving and stress. This is confirmed by empirical data on drugs of abuse in animals and humans, in which there are profound affects on brain circuitry related to reward and reinforcement in which there is a pathological desire to consume a substance. Despite this, it would also appear that drugs of abuse are able to affect those brain systems which are required to exercise control over desires to consume substances. This appears to suggest that an inability to reduce drug use, despite knowledge concerning the detrimental effects of drugs, may be related to dysfunctional behavioural control, which promotes and perpetuates further drug consumption. Particularly important are the roles of certain cognitive processes in addiction, such as inhibitory and attentional control, as well a behavioural monitoring.
When conducting research into the effects of drugs of abuse on brain and behavioural functioning, therefore, it is imperative that one considers the consequences of pre-existing differences which may act as an important prerequisite to initiating drug use, and essentially, addiction. Therefore, it is important to acknowledge some of the potential caveats with respect to the current research findings, particularly with respect to how differences which predate drug use, may just as likely have contributed to the aforementioned results. For example impairments in impulse control may predispose some individuals to their first impulsive use of an illicit substance such as cannabis or nicotine, a number of years later. Tarter et al (2003), using behavioural and cognitive measures of impulsivity has demonstrated just this in 10-12 years olds, in whom individuals showing poor impulse control were more likely to be engaged in drug use by the age of 19. This may suggest that cognitive control deficits, as a result of some neurochemical imbalance, likely promotes impulsivity in which there is an increased risk of subsequent drug abuse. Indeed, an animal model of impulsivity has been shown to exhibit elevated levels of cocaine self-administration in which there are reduced numbers of DA D₂ receptors in the VS prior to the first episode of self-administration (Dalley et al, 2007). This may confirm that a deficit in VS DA D₂ receptor numbers may increase the risk for impulsive decision making, which ultimately leads to drug use. The role of cognitive control and its participation in drug abstinence is also likely to be impeded if there are pre-existing differences in the brains of addicts. Research has shown, for example, that prefrontal, cingulate and temporal lobe activation during a two-choice prediction task 3-4 weeks into methamphetamine abstinence, can predict individuals who relapse one year later (Paulus et al, 2005).
Personality variables have also consistently been found to play a significant role in the initiation of substance use, and this association has been found across age, sex, and different cultures (Zuckerman et al, 1978). Sensation-seeking, in particular, has been shown to be an important personality predictor of both drug use and abuse (Segal et al, 1977; Brook et al, 1983; Martin et al, 1992; Luthar et al, 1992; Kosten et al, 1994). Sensation-seeking is a trait defined by the seeking of varied, novel, complex, and intense sensations and experiences, and the willingness to take physical, social, legal, and financial risks for the sake of such experience (Zuckerman, 1994). Adolescent cannabis use has been shown to strongly correlate with scores on Zuckerman’s Sensation-seeking scales of Disinhibition (DIS; rebellion against conventional social norms), Thrill and Adventure Seeking (TAS; need for physical excitement and risky behavior), and Experience Seeking (ES; arousal of the mind and the senses through a nonconformist lifestyle) (Zuckerman et al, 1984). Likewise, an association between high Sensation Seeking and smoking in general has been reported in a number of studies (Carton et al, 1994; Kopstein et al, 2001; Martin et al, 2002; Zuckerman et al, 1990), suggesting certain personality variables may be prerequisites to initiating nicotine use. These findings may potentially suggest that cannabis and nicotine use arise from personality traits which are, in part, genetically determined through influences on VS and prefrontal DA functioning (Golimbet et al, 2006, 2007). Therefore, it cannot be unequivocally stated that the VS differences observed in chapter 3, and some of the inhibitory deficits in chapter 6, are a direct consequence of chronic cannabis nicotine consumption, and their effects on DA meso-cortico-limbic circuitry. Nor can any such assertion be made regarding drugs of abuse in general, as important differences in the brains of individuals may predispose, and therefore, predate, the onset of drug consumption, and consequently addiction.
Conclusions

The excessive use of cannabis has become a growing concern in Western societies, with tobacco use, like cannabis, continuing to be a widespread public health problem. Importantly, cannabis and nicotine are often co-administered, with abusers of “hard” drugs commonly reporting cannabis and/or nicotine as their first recreational drug of use. Despite an increased understanding concerning the pharmacology of cannabis and nicotine, there is little research which has examined their long-term effects upon different domains of cognitive processing. Furthermore, little research has investigated the effects of long-term drug abstinence on cognition, specifically comparing cognitive neural substrates in current and former substance users. Such comparisons may reveal ways in which cognitive neuronal operations may contribute to avoiding drug relapse, while suggesting ways in which it is possible to develop behavioural and/or pharmacological treatments which may facilitate abstinence through improvements in cognition. The research findings presented in this thesis have provided some evidence to demonstrate differences in neural activity related to learning and memory, reward processing, attentional bias to drug-predictive stimuli, inhibitory control and error monitoring processes in cannabis and nicotine users. Not withstanding the distinct possibility that such differences may have predated, and contributed to the onset of drug use, the current research findings will hopefully have elucidated the cognitive operations of drug users, and importantly, how changes in neural functioning related to cognition, may likely promote continued drug use and ultimately, addiction.
References


