Dissociating the Psychoactive Effects of Distinct Cannabis Compounds in the Mesocorticolimbic Circuitry

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Neuroscience
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DISSOCIATING THE PSYCHOACTIVE EFFECTS OF DISTINCT CANNABIS
COMPOUNDS IN THE MESOCORTICOLIMBIC CIRCUITRY

(Thesis Format: Integrated Article)

by

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The School of Graduate and Postdoctoral Studies

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ABSTRACT

The discovery of the endocannabinoid system propelled understanding of the mechanisms of action of cannabinoid compounds. While marijuana is the most widely used illicit substance in the world, the neuropsychopharmacological mechanisms that underlie the diffuse effects of cannabis in the brain remain poorly understood. This is because marijuana smoke represents a complex mixture of chemical components, possessing dissociable psychoactive properties. Clinical evidence suggests a functional dissociation between the two main pharmacological components of cannabis, Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD). Using a combination of cortical microinfusions during two emotional learning paradigms, and single-unit in vivo electrophysiological recording, we investigated the effects of phytocannabinoid compounds in emotional regulation neural circuits, specifically the nucleus accumbens shell. We report the first demonstration of hedonic properties of CBD; an effect mediated by 5-HT1A receptors, and decreased VTA dopaminergic activity. In olfactory fear conditioning, Δ9-THC potentiates and CBD attenuates emotionally salient stimuli similar to synthetic cannabinoids.

Keywords: Nucleus Accumbens, Ventral Tegmental Area, Cannabinoids, Δ9-tetrahydrocannabinol, Cannabidiol, Dopamine, Serotonin, 5-HT1A, 5-HT1B, GABA, Electrophysiology, Fear Conditioning, Conditioned Place preference, Emotional Processing, Schizophrenia, Addiction
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<tr>
<td>2-AG</td>
<td>2-α-arachidonoylglycerol</td>
</tr>
<tr>
<td>α-flu</td>
<td>α-flupenthixol</td>
</tr>
<tr>
<td>Δ9-THC</td>
<td>Δ9-tetrahydrocannabinol</td>
</tr>
<tr>
<td>ACC</td>
<td>anterior cingulate cortex</td>
</tr>
<tr>
<td>AEA</td>
<td>anandamide ((N-\text{arachidonylethanolamine}))</td>
</tr>
<tr>
<td>BLA</td>
<td>basolateral amygdala</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CB1</td>
<td>cannabinoid receptor-1</td>
</tr>
<tr>
<td>CB2</td>
<td>cannabinoid receptor-2</td>
</tr>
<tr>
<td>CBD</td>
<td>cannabidiol</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CPP</td>
<td>conditioned place preference</td>
</tr>
<tr>
<td>DA</td>
<td>dopamine</td>
</tr>
<tr>
<td>eCB</td>
<td>endocannabinoid</td>
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<tr>
<td>GABA</td>
<td>gamma-aminobutyric acid</td>
</tr>
<tr>
<td>GPCR</td>
<td>G-protein coupled receptor</td>
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<tr>
<td>GR-5562</td>
<td>GR-55562 dihydrochloride</td>
</tr>
<tr>
<td>i.p.</td>
<td>intra-peritoneal</td>
</tr>
<tr>
<td>i.v.</td>
<td>intra-venous</td>
</tr>
<tr>
<td>mPFC</td>
<td>medial prefrontal cortex</td>
</tr>
<tr>
<td>MSN</td>
<td>medium spiny neuron</td>
</tr>
<tr>
<td>NAcc</td>
<td>nucleus accumbens</td>
</tr>
<tr>
<td>NAD-299</td>
<td>NAD-299 hydrochloride</td>
</tr>
<tr>
<td>NASh</td>
<td>nucleus accumbens, shell subdivision</td>
</tr>
<tr>
<td>PCC</td>
<td>posterior cingulate cortex</td>
</tr>
<tr>
<td>PCP</td>
<td>phencyclidine</td>
</tr>
<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
</tr>
<tr>
<td>PTSD</td>
<td>post-traumatic stress disorder</td>
</tr>
<tr>
<td>PPI</td>
<td>prepulse inhibition</td>
</tr>
<tr>
<td>VTA</td>
<td>ventral tegmental area</td>
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<td>WIN-55</td>
<td>WIN 55,212-2</td>
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Chapter 1. General Introduction

In order to coordinate normative cognition and appropriate behavioural responses, the brain must accurately process the emotional and motivational significance of an enormous amount of incoming sensory stimuli. This requires the formation of adaptive learned associations between the emotional salience of sensory inputs with their respective environmental cues (Laviolette & Grace, 2006).

Several cortical and subcortical structures comprise the emotional processing circuitry known as the mesocorticolimbic system, including: the medial prefrontal cortex (mPFC), the basolateral amygdala (BLA), the ventral tegmental area (VTA) and the nucleus accumbens (NAcc). These areas contain high levels of dopamine (DA) and cannabinoid CB1 receptors, which are critical in emotional and motivational salience processing and associative learning (Herkenham et al., 1991b; Moldrich & Wenger, 2000). Furthermore, previous research shows that the cannabinoid and DAergic systems functionally interact, such that cortical CB1 transmission modulates subcortical mesolimbic DA activity (Bossong et al., 2008; Diana, Melis, & Gessa, 1998).

Activation of the brain’s endocannabinoid system is associated with severe distortions in emotional and motivational perceptions and with learning and memory impairments (D’Souza et al., 2005). However, delivery of cannabinoids to the brain, via drugs such as cannabis, represents a complex mixture of chemical components, possessing dissociable psychoactive properties (ElSohly & Slade, 2005; Radwan et al., 2009). Indeed, recent evidence suggests a functional dissociation between the two main pharmacological components of cannabis, cannabidiol (CBD) and Δ9-tetrahydrocannabinol (Δ9-THC). Furthermore, human brain imaging evidence suggests that Δ9-THC and CBD may exert differential psychological effects in distinct mesocorticolimbic substrates (Bhattacharyya & Sendt, 2012; Bhattacharyya et al., 2012; Bhattacharyya, Fusar-Poli, et al., 2009a; Bhattacharyya, et al., 2009b). The pharmacology of these compounds is also quite distinct. CBD has been shown to be a weak antagonist of the CB1 receptor (Thomas et al., 2009) as well as an agonist of the serotonin 5-HT1A receptor (Russo, Burnett, Hall, & Parker, 2005); in contrast, Δ9-THC acts as a partial CB1 agonist (Petitet, Jeantaud, Reibaud, Imperato, & Dubroeucq, 1998). While the pharmacological properties of Δ9-THC and CBD are relatively well characterized, the
differential effects of these distinct compounds on emotional processing in the brain remain poorly understood. Furthermore, there are very few studies investigating the effects of distinct Δ9-THC and CBD compounds in specific anatomical structures of the brain.

The work presented in this thesis attempts to address some of the gaps in the current understanding of the very complex nature of the major phytocannabinoids (plant-derived) in the brain and the effects they have on emotional information processing and memory formation. Specifically, this work investigates the dissociable psychoactive effects of Δ9-THC and CBD microinfusions into the shell subdivision of the NAcc during two emotional memory formation paradigms: conditioned place preference (CPP) and olfactory fear conditioning. Through a series of behavioural, pharmacological and electrophysiological experiments, I have also examined the interactions of these compounds with the serotonergic and dopaminergic neurotransmitter systems to reveal novel insights into the underlying neurobiological basis of exogenous cannabinoid signaling in the mammalian brain.

1.1 The endocannabinoid system

The endocannabinoid (eCB) system is a ubiquitous lipid-based neuromodulatory system within the nervous system that has been highly conserved across mammalian species and plays diverse roles in normative physiological and psychological functioning (Elphick & Egertova, 2001). Two main cannabinoid (CB) receptor subtypes exist in the nervous system: the CB1 receptor, which is highly expressed in the central nervous system (CNS), and the CB2 receptor which is expressed primarily in peripheral immune cells (Pertwee & Ross, 2002). Given the neuroscientific nature of this work, this thesis will focus on CB1 receptors. CB1 receptors are primarily expressed on the presynaptic axon terminals of glutamatergic principal neurons as well as GABAergic projection and interneurons (Katona & Freund, 2012), and are inhibitory G-protein coupled receptors (GPCR). Activation of these receptors by their endogenous ligands anandamide (AEA) and 2-arachidonoylglycerol (2-AG), or by exogenous cannabinoids such as those found in cannabis, initiates a number of intra- and extracellular signaling events that serve to
inhibit the cell, including: decreasing cytosolic cyclic adenosine monophosphate (cAMP) production by inhibiting adenylate cyclase activity, closure of Ca\(^{2+}\) channels, and opening of K\(^+\) channels (Lupica, Riegel, & Hoffman, 2004; Piomelli, 2003). The CB1 receptor is the most ubiquitously expressed GPCR in the brains of both humans (Glass, Dragunow, & Faull, 1997) and rats (Herkenham et al., 1991b) and, due to their location on the primary excitatory and inhibitory cells in the brain, they play a crucial role in the modulation of a number of other neurotransmitter systems including the dopaminergic (DA), serotonergic (5-HT), GABAergic and glutamatergic systems (López-Moreno, González-Cuevas, Moreno, & Navarro, 2008). The interaction between the eCB and DA systems is of particular importance in the consideration of emotional processing and will be discussed below.

1.1.1 Role of CB1 receptors in emotional processing

The eCB system, as mediated by CB1 receptors, plays a central role in the processing of emotionally salient stimuli and related behaviours (Laviolette, 2014). The CB1 receptor is highly expressed in the striatum, a series of structures involved in emotional and motivational information processing, of both rats (Moldrich & Wenger, 2000) and humans (Burns et al., 2007). In humans, use of cannabis as well as synthetic cannabinoid analogues, both of which act on CB1 receptors, have profound effects on emotional processing. Users report a wide array of emotional experiences ranging from euphoria and sedation to anxiety, paranoia and agitation. Even acute exposure can result in mood-related disturbances and alter the significance of external sensory input (Green, Kavanagh, & Young, 2003; Rosenbaum, Carreiro, & Babu, 2012; Wachtel, ElSohly, Ross, Ambre, & de Wit, 2002). In animal studies, mice lacking the CB1 receptor show impairments in extinction learning of an aversive conditioned fear memory (Cannich et al., 2004; Marsicano et al., 2002), and display an abnormal emotional phenotype characterized by heightened aggressive, depressive and anxiety responses, in some behavioural stress paradigms (Martin, Ledent, Parmentier, Maldonado, & Valverde, 2002). Pharmacological manipulations of CB1 receptors in various regions of the mesocorticolimbic system also result in profound alterations of the salience of incoming sensory stimuli. In olfactory fear conditioning experiments, rats receive an electric
footshock during presentation of one of two distinct odours. Rats receiving intra-mPFC microinfusions of the CB1 receptor agonist WIN-55,212-2 (WIN-55) prior to conditioning with a normally non-salient subthreshold footshock display heightened levels of freezing in response to the shock-paired odour, a measure of conditioned fear. This effect was completely abolished using the CB1 receptor antagonist AM-251 (Laviolette, 2006). CB1 receptor transmission within the BLA—mPFC pathway is also critical for the acquisition of emotionally salient associative memories. CB1 receptor antagonism, administered systemically and as targeted microinfusions, blocks both the behavioural acquisition of conditioned fear memories and LTP along the BLA—mPFC pathway, a correlate of exposure to emotionally salient events (Tan, Lauzon, Bishop, Bechard, & Laviolette, 2010). Follow-up electrophysiology studies show a bidirectional effect of CB1 receptor modulation in the BLA, with mPFC neurons showing increased neuronal activity in response to intra-BLA CB1 activation, and a decrease in activity following CB1 antagonism (Tan et al., 2011). The studies outlined above serve to illustrate the central role of CB1 receptors in the emotional processing structures of the mesocorticolimbic pathway, however cannabinoid transmission within the NAcc remains poorly understood and represents a potentially productive avenue of future research.

1.1.2 eCB interactions with the dopamine system

DAergic signaling also plays a critical role in emotional regulation and coordinated behaviour in response to emotionally salient stimuli (Laviolette, 2007). While DA receptors are expressed throughout the CNS, they display high levels of expression in the limbic regions of the brain, suggesting a central role of DA in emotional and motivational processes (Wise, 2004). Of particular note are DA efferents originating in the VTA that project to higher level cortical structures of emotional regulation including the NAcc, BLA and PFC (Grace, Floresco, Goto, & Lodge, 2007; Ikemoto, 2007). There are profound anatomical and functional interactions between the eCB and DAergic systems. CB1 receptors are expressed in the substantia nigra and striatum, regions that are highly innervated by DAergic projections and express high levels of the DA receptor (Moldrich & Wenger, 2000). Medium spiny neurons (MSN), the primary output neurons of the
striatum, also express both CB1 and DA receptors (Glass & Felder, 1997; Herkenham et al., 1991b). Furthermore, substantial evidence suggests that the CB1 and DA receptors are coexpressed on synaptic terminals (Chiu, Puente, Grandes, & Castillo, 2010), and may even form CB1-DA heterodimer receptor complexes in some brain regions (Kearn, 2005). These data suggest that CB1 signaling may act as an important modulator of DAergic transmission in emotional regulation circuits (Giuffrida et al., 1999).

Functionally, CB1 receptor modulation has been shown to play an important role in DAergic transmission. Systemic administration of CB1 receptor agonists such as Δ9-THC and WIN-55 increase DAergic neuronal activity in the VTA (Diana et al., 1998; French, Dillon, & Wu, 1997), and also enhance the firing rate of PFC pyramidal neurons to the VTA (Pistis, Porcu, Melis, Diana, & Gessa, 2001). Furthermore, a recent study found that Δ9-THC and WIN-55 administration increase DA synthesis in key emotional processing regions such as the dorsal striatum, NAcc, PFC and amygdala (Polissidis et al., 2009), suggesting an active role of eCB transmission in the synthesis and activity of subcortical DA signaling.

1.1.3 The eCB system in neuropsychiatric disorders

Given the importance of the eCB system in emotional information processing, it is not surprising that dysfunctions in the eCB system have been implicated in a range of neuropsychiatric disorders that are characterized by deficits in emotional regulation (Parolaro, Realini, Viganò, Guidali, & Rubino, 2010). Much of the evidence supporting this hypothesis comes from studies using CB1 receptor knockout (KO) animals. Mice lacking the CB1 receptor show increased anxiety responses in response to chronic stress (Martin et al., 2002), and display a depressive phenotype in the forced swim test (Steiner et al., 2008) and tail suspension test (Aso et al., 2008), both behavioural models of depression. CB1 KO animals also exhibit elevated serum levels of glucocorticoids (Urigüen, Pérez-Rial, Ledent, Palomo, & Manzanares, 2004), suggesting dysfunction of the hypothalamus-pituitary-adrenal (HPA) axis, a physiological correlate found in major depressive disorder (Pariante & Lightman, 2008; Stokes, 1995), anxiety (Pariante, 2003) and post-traumatic stress disorder (PTSD; Yehuda, 2002).
Deficits in circulating eCB levels are also observed in these disorders, further implicating eCB system dysfunction. Basal serum levels of AEA and 2-AG have been found to be significantly lower in individuals diagnosed with major depressive disorder in comparison to healthy controls (Hill, Miller, Carrier, Gorzalka, & Hillard, 2009). The same group has shown the plasma levels of 2-AG are also significantly reduced in a population of PTSD patients in comparison to normal controls (Hill et al., 2013).

Evidence from pharmacological studies also provides compelling evidence for the role of the eCB system in various neuropsychiatric disorders. Both systemic injections and targeted microinfusions of CB1 agonists into specific brain regions critically involved in emotional processing have been found to be effective at reversing depressive-like behaviours in various animal models (Bambico, Katz, Debonnel, & Gobbi, 2007; Hill & Gorzalka, 2005). Furthermore, pharmacological inhibition of fatty acid amide hydrolase (FAAH), the enzyme responsible for degrading the endogenous AEA, shows efficacy as an antidepressant and anxiolytic (Bortolato et al., 2007; Haller et al., 2009; Scherma et al., 2008), further supporting the hypothesis that a reduction in eCB tone, in addition to CB1 receptor deficits, may underlie depressive and anxiety-related symptoms.

1.1.4 Schizophrenia

There is also substantial evidence for severe dysfunction of the eCB system in individuals suffering from schizophrenia. This dysfunction manifests both at the level of the CB1 receptor as well as a dysregulation of eCB tone. In addition, heavy cannabis use constitutes a significant environmental risk factor for the development of schizophrenia and related psychotic disorders (this will be discussed in more detail below, see section 1.2.1).

Neuroanatomical studies have revealed that the CB1 receptor is highly expressed in brain regions implicated in the pathophysiology of schizophrenia including the PFC, hippocampus, basal ganglia and anterior cingulate cortex (Glass et al., 1997). More recent studies suggest that expression of the CB1 receptor in these areas may be altered in schizophrenia. Binding of the cannabinoid radioligand \[^{3}H\]CP55940 to the CB1 receptor was shown to be higher in the dorsolateral-PFC of schizophrenic patients than in healthy
controls (Dean, Sundram, Bradbury, Scarr, & Copolov, 2001). Dalton et al. (2011) subsequently confirmed these findings and additionally provided evidence that CB1 receptor expression is significantly higher in paranoid schizophrenics as compared to non-paranoid schizophrenics and healthy controls. Furthermore, both the anterior and posterior cingulate cortex (ACC and PCC, respectively) display increased CB1 receptor expression in individuals suffering from schizophrenia in comparison to normal controls (Newell, Deng, & Huang, 2006; Zavitsanou, Garrick, & Huang, 2004). Studies employing pharmacological manipulation of the eCB system also support a role for the CB1 receptor in schizophrenia. Administration of the CB1 receptor agonist WIN-55 results in impairments in sensorimotor gating such as prepulse inhibition (PPI), an information processing and attentional deficit that is evident in patients with schizophrenia (Martin et al., 2003; Schneider & Koch, 2002). Furthermore, administration of CB1 receptor agonists such as Δ9-THC, the main psychoactive ingredient in cannabis, can produce a constellation of effects in humans that are reminiscent of schizophrenic symptomatology (Bhattacharyya et al., 2012; Green et al., 2003; Wachtel et al., 2002) and elicit hyperactivity of mesolimbic DA circuits (Diana et al., 1998; French et al., 1997), a core pathophysiological feature of schizophrenia (see section 1.2.1). These data suggest that dysfunction of the CB1 receptor may play a critical role in the aberrant emotional processing observed in schizophrenia. Finally, there is evidence to suggest that polymorphisms in the gene coding the CB1 receptor (CNR1) may be correlated with an increased susceptibility to developing schizophrenia (Chavarrí-Siles et al., 2008), although the nature of this relationship is not fully understood (Seifert, Ossege, Emrich, Schneider, & Stuhrmann, 2007).

Dysregulation of eCB tone is also evident in schizophrenia. In a population of first-episode paranoid schizophrenic patients, researchers found a significant elevation in cerebrospinal fluid levels of AEA as compared to healthy controls (Giuffrida et al., 2004; Leweke, Giuffrida, Wurster, Emrich, & Piomelli, 1999), and clinical remission was associated with a significant reduction in AEA levels (De Marchi et al., 2003). There is also some evidence from animal studies, using a PCP model of schizophrenia, of increased AEA levels in some brain regions (Seillier, Advani, Cassano, Hensler, & Giuffrida, 2009; Viganò et al., 2008). Somewhat paradoxically, clinical studies have
shown that AEA levels are inversely correlated with the severity of psychotic symptoms suggesting that elevated eCB tone may be a compensatory adaptation in response to abnormal neurotransmission in schizophrenia-related psychoses (Giuffrida et al., 2004; Leweke et al., 2012).

### 1.2 Cannabis and phytocannabinoids

Although cannabis has been used for spiritual, medicinal and industrial purposes for thousands of years, it wasn't until the isolation of Δ9-tetrahydrocannabinol (Δ9-THC), the major psychoactive cannabinoid in cannabis, in 1964 that scientific understanding of phytocannabinoids (plant-derived) truly began (Mechoulam & Gaoni, 1964). In 1990, a group of researchers elucidated the structure and expression of an endogenous cannabinoid receptor, which was dubbed the CB1 receptor (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990), indicating an endogenous target on which Δ9-THC acts in the brain. This was quickly followed by the discovery of arachidonylethanolamide (AEA), also known as “anandamide”, an endogenous ligand for the CB1 receptor (Devane et al., 1992). Since these discoveries, research interest in the endocannabinoid system has accelerated dramatically and the endocannabinoid system has been implicated in a wide range of physiological and psychological functions (Elphick & Egertova, 2001).

However despite these advances, the neurobiological mechanisms through which cannabis exerts its neuropsychological effects, especially at the level of discrete neural circuits, remains poorly understood. This is in part because cannabis contains hundreds of chemical compounds and over 70 known cannabinoids, each with distinct, dissociable and complex pharmacological profiles (ElSohly & Slade, 2005; Radwan et al., 2009). The two predominant cannabinoid compounds contained in cannabis are the psychoactive Δ9-THC and the non-psychoactive cannabidiol (CBD; Russo & Guy, 2006), which will be the focus of the work presented in this thesis. Recent basic and clinical evidence suggests that these two compounds have very different pharmacological profiles and dissociable neuropsychological properties (Bhattacharyya et al., 2009b). While Δ9-THC administration generally produces subjective states of anxiety and agitation (Bhattacharyya et al., 2012; Fusar-Poli et al., 2009), CBD has potent anxiolytic, and
antipsychotic effects (Bhattacharyya et al., 2009a; Crippa et al., 2003; Crippa et al., 2010; Schubart et al., 2013; Zuardi et al., 2012). Although the neurobiological basis for these dissociable effects is not fully understood, it is likely a result of significant pharmacological differences between Δ9-THC and CBD. While Δ9-THC is a partial agonist of the CB1 receptor (Petitet et al., 1998), CBD is a weak CB1 receptor antagonist (Thomas et al., 2009), and displays relatively high binding affinity for serotonin 5-HT1A receptors (Russo et al., 2005). CBD also displays antagonistic properties at the putative new cannabinoid receptor GPR-55 (Ryberg et al., 2009), and is an effective inhibitor of FAAH, and thus indirectly increases AEA tone (Bisogno et al., 2001).

1.2.1 Cannabis use as a risk factor for schizophrenia

The link between cannabis use and an increased risk of developing schizophrenia is well established in the scientific literature (Bossong & Niesink, 2010; Semple, 2005; Smit, Bolier, & Cuijpers, 2004; Wilkinson, Radhakrishnan, & D’Souza, 2014). A seminal 1987 study established a correlation between lifetime prevalence of cannabis use and an increased likelihood of developing schizophrenia later in life (Andersson et al., 1987). Since then, numerous longitudinal studies have explored the nature of this relationship. In general, the main findings demonstrate that while cannabis use alone is neither necessary nor sufficient to induce psychotic disorders, prolonged and excessive use especially during critical developmental periods of adolescence represents a significant risk factor in individuals genetically and environmentally predisposed to developing psychotic disorders and other mental illnesses (Wilkinson et al., 2014). Indeed, neuroimaging studies have found that individuals who start using cannabis before 17 years of age show reductions in cortical grey matter and increased percentage of white matter in comparison to those who initiate use later (Wilson et al., 2000). Other imaging studies show that chronic cannabis users also display reduced hippocampal and amygdalar volumes in comparison to non-using controls (Yücel et al., 2008). Both cortical grey matter reductions and structural abnormalities in hippocampal and amygdalar structures are commonly observed pathophysiological features of schizophrenia (Ellison-Wright &
Bullmore, 2009), however whether these neural alterations are a cause or effect of early and extensive cannabis use has yet to be elucidated.

Additional evidence for the association between cannabis and schizophrenia comes from challenge studies involving acute administration of Δ9-THC or other cannabinoids, in healthy and schizophrenic individuals. In healthy volunteers, acute Δ9-THC exposure has been shown to transiently induce a range of psychotomimetic symptoms reminiscent of those observed in schizophrenia, including cognitive and attentional impairments as well as feelings of paranoia and anxiety (Bhattacharyya et al., 2012; Bhattacharyya et al., 2009a; D’Souza et al., 2012). In individuals with a confirmed diagnosis of schizophrenia, treatment with Δ9-THC resulted in a transient exacerbation of core psychotic symptoms, cognitive deficits and perceptual alterations (D’Souza et al., 2005). Interestingly, studies investigating the effects of CBD show starkly opposite results to those found with Δ9-THC treatment. In contrast to Δ9-THC, CBD administration does not elicit any psychotomimetic effects, and is not associated with perceptual alterations or cognitive deficits in healthy volunteers (Bhattacharyya et al., 2009b). In individuals with schizophrenia, CBD actually displays antipsychotic efficacy comparable to conventional antipsychotic medications (Leweke et al., 2012), likely due to its ability to inhibit FAAH and indirectly elevated endogenous levels of AEA (Giuffrida et al., 2004; Leweke et al., 1999).

The relationship between cannabis use and schizophrenia is thus complex and further investigation is needed to elucidate the neurobiological mechanisms underlying this association.

1.3 Research Purpose and Hypotheses

The studies reviewed above illustrate the central role of the eCB system in emotional processing circuits, specifically within the mesocorticolimbic system, and describe how dysfunctions in this system may represent a neurobiological mechanism that contributes to the development of various neuropsychiatric disorders. However, little is known about the role that CB1 receptor signaling within the NAcc plays in the processing of appetitive and aversive emotional stimuli, specifically in response to phytocannabinoid
administration. The work presented in this thesis attempts to address this, by pursuing the following objectives:

*Objective 1: To characterize the reinforcing properties of intra-NASh CBD microinfusions in an unbiased conditioned place preference paradigm.*

*Objective 2: To characterize the behavioural effects of intra-NASh Δ9-THC and CBD microinfusions during the encoding and recall of emotionally aversive associative learning.*

Based on clinical data, work from our own laboratory and the distinct pharmacological profiles of Δ9-THC and CBD, I hypothesized that these two cannabinoid compounds would exhibit dissociable and opposite effects in our behavioural paradigms.
Chapter 2. Cannabidiol produces rewarding effects in the nucleus accumbens shell via serotonergic and dopaminergic signaling mechanisms

2.1 Introduction

Marijuana is the most widely used illicit drug in the world (Murray, Morrison, Henquet, & Di Forti, 2007; United Nations, 2010) and although our understanding of phytocannabinoids is growing, the debate over marijuana’s addiction potential and abuse liability remains controversial. While traditionally not considered an addictive drug, recent research suggests the presence of distinct marijuana dependence and withdrawal syndromes in heavy users (Budney & Hughes, 2006; Ramesh, Schlosburg, Wiebelhaus, & Lichtman, 2012). However, cannabis contains over 70 known cannabinoid compounds, each with unique pharmacological properties (ElSohly & Slade, 2005; Radwan et al., 2009). Thus, the neuropsychopharmacological mechanism by which cannabis, or its constituent compounds, elicits a behaviourally rewarding response remains poorly understood.

Indeed, emerging clinical evidence suggests a functional dissociation between the two main pharmacological components of cannabis, the psychoactive Δ9-tetrahydrocannabinol (Δ9-THC) and the non-psychoactive cannabidiol (CBD), in distinct mesocorticolimbic substrates (Bhattacharyya et al., 2012; Bhattacharyya, Fusar-Poli, et al., 2009a; Bhattacharyya et al., 2009b; Fusar-Poli et al., 2009). While Δ9-THC exhibits propsychotic effects (Bhattacharyya et al., 2012), CBD displays antipsychotic (Leweke et al., 2012; Mechoulam, Peters, Murillo-Rodriguez, & Hanus, 2007; Schubart et al., 2013; Zuardi et al., 2012; Zuardi, Crippa, Hallak, Moreira, & Guimarães, 2006) and anxiolytic (Almeida et al., 2013; Campos & Guimarães, 2008; Casarotto, Gomes, Resstel, & Guimarães, 2010; Crippa et al., 2010; Fogaça, Reis, Campos, & Guimarães, 2014) properties comparable to conventional antipsychotic medications.

Cannabinoids exert their neuropsychological effects by acting on the body’s endocannabinoid (eCB) system at centralized cannabinoid CB1 receptors. Both Δ9-THC and CBD resemble endogenous CB1R ligands, however their pharmacology is distinct. Furthermore, in situ hybridization studies show high levels of colocalization between
CB1 receptors and dopaminergic (DA) and serotonergic (5-HT) receptors, suggesting the eCB system may play a crucial role in modulating activity of these systems (Hermann, Marsicano, & Lutz, 2002; Maroteaux et al., 1992).

Until recently, research efforts have focused almost exclusively on determining the reinforcing properties of $\Delta^9$-THC with mixed results (see Tzschentke, 2007 for a review). Animal studies reveal rewarding and aversive effects of $\Delta^9$-THC in the conditioned place preference and self-administration paradigms (Gardner, 2002; Tanda & Goldberg, 2003; Valjent & Maldonado, 2000). However, there is little evidence that CBD itself possesses hedonic properties (Parker, Burton, Sorge, Yakiwchuk, & Mechoulam, 2004). The aim of the current study was to investigate the potential psychoactive properties of CBD in discrete anatomical regions of the mesocorticolimbic circuitry. Specifically, we conducted a series of behavioural pharmacological experiments examining the rewarding effects of CBD microinfusions into the shell subdivision of the nucleus accumbens (NASh), a structure which highly expresses CB1 receptors (Fusco et al., 2004; Herkenham, Lynn, de Costa, & Richfield, 1991a; Moldrich & Wenger, 2000) and is sensitive to synthetic cannabinoid reward in animal models (Mahler, Smith, & Berridge, 2007; Zangen, Solinas, Ikemoto, Goldberg, & Wise, 2006). We report here the first evidence of the hedonic psychoactive properties of CBD in a conditioned place preference paradigm.

2.2 Materials and Methods

2.2.1 Animals and Surgery
Male Sprague Dawley rats (300-350g) were obtained from Charles River Laboratories (Quebec, CAN). Rats were anesthetized with an intraperitoneal (i.p.) injection of a ketamine (80mg/ml)-xylazine (6mg/kg) mixture and meloxicam (1.0 mg/kg; s.c.) was administered preoperatively in order to reduce pain and post-surgical inflammation. Following an assessment of anesthetic depth, rats were immobilized using a Kopf stereotaxic device. Using sterile technique, an incision was made to expose the skull. Guide cannulae (22-gauge) were implanted bilaterally into the NASh using flat skull stereotaxic coordinates as follows (12° angle, in mm from bregma): anteroposterior (AP)
Cannulae were implanted in the VTA using the following stereotaxic coordinates (10° angle, in mm from bregma): anteroposterior (AP) + 1.8, lateral (LAT) ± 2.6, ventral (V) -7.4 from the dural surface. All stereotaxic coordinates were based upon the atlas of Paxinos and Watson (1996). Cannulae were secured to the skull using four small jeweler’s screws and dental acrylic. Dust-caps were used to ensure the patency of the cannulae. All procedures were performed in accordance with the Canadian Council on Animal Care (CCAC) and were approved by Western University’s Council on Animal Care.

2.2.2 Post-Surgery SOP

Rats were injected subcutaneously with 3 – 10 mL of physiological saline in order to prevent dehydration. Rats recovered in a clean home cage filled with wood shavings and warmed under a heat lamp. Behaviour was monitored until recovery from the anesthetics and sternal recumbency attained. A maintenance dose of meloxicam (1.0 mg/kg) was administered (s.c.) 24 hours post-surgery in order to reduce pain and inflammation. Following at least one week of recovery, rats were then tested in behavioral paradigms.

2.2.3 Conditioned Place Preference

All rats were conditioned using an unbiased, fully counterbalanced conditioned place preference (CPP) procedure, as described previously (Laviolette & van der Kooy, 2003; Laviolette, Nader, & van der Kooy, 2002). Rats received drug microinfusions, and were then conditioned in one of two environments that differed in colour, texture and scent. One environment was black constructed with a smooth Plexiglas floor, wiped down with 2% acetic acid solution before each conditioning session. The other environment was white with a wire mesh floor covered with wood chips. Rats display no baseline preference for either of these two environments (Laviolette & van der Kooy, 2003). Rats were preconditioned (24 hrs prior to conditioning) in a motivationally neutral grey box for 30 min, in order to minimize stress during behavioural conditioning. Immediately prior to conditioning, rats received intra-NASh microinfusions of CBD (50, 100, 500ng/0.5µl) alone, or coadministered with the DA antagonist α-flupenthixol (100ng –
1.0μg/0.5μl), 5-HT\textsubscript{1A} antagonist NAD-299 hydrochloride (100 – 500ng/0.5μl), or 5-HT\textsubscript{1B} antagonist GR-55562 (10 – 100ng/0.5μl). For animals implanted with cannulae in the NASh and the VTA, the GABA\textsubscript{A} antagonist bicuculline methiodide (50ng/0.5μl) and the GABA\textsubscript{B} antagonist hydroxysaclofen (100ng/0.5μl), or their respective vehicle, were coadministered into the VTA 2 min prior to intra-NASh CBD infusion.

Following microinfusions, rats were placed in one of the two conditioning environments for 30 min. Experimental treatments were counterbalanced such that each animal was randomly assigned to receive either drug or vehicle infusions in either the white or the black environment. During conditioning, rats received four drug-environment and four saline-environment conditioning sessions. During testing, rats were placed on a narrow, neutral grey zone separating the two test environments and times spent in each environment was digitally recorded and scored separately for each animal over a 10 min test session. All rats were tested in a drug free state.

2.2.4 Drug administration

The broad-spectrum DA receptor antagonist, α-flupenthixol, the highly specific 5-HT\textsubscript{1B} receptor antagonist, GR-55562, the GABA\textsubscript{A} antagonist, bicuculline methiodide, and the GABA\textsubscript{B} antagonist, hydroxysaclofen, were dissolved in physiological saline (pH adjusted to 7.4). The 5-HT\textsubscript{1A} receptor antagonist, NAD-299 hydrochloride, and CBD were dissolved in dimethyl sulfoxide (DMSO, Fisher Scientific). For NASh and VTA microinfusions, stainless steel guide cannulae (22-gauge) were implanted bilaterally into the NASh through a 28-gauge microinfusion injector (Plastics One). Bilateral microinfusions were performed over 1 min through a Hamilton microsyringe. In order to ensure the diffusion of the drug away from the injector tip, the internal cannulae were left in place for an additional minute. All drugs were purchased from Tocris Biosciences.

2.2.5 VTA neuronal activity recording

In vivo single-cell extracellular recordings in the VTA were performed as described previously (Tan, Bishop, Lauzon, Sun, & Laviolette, 2009). Briefly, 18 rats were anesthetized with urethane (1.4 g/kg, i.p.) and placed in a stereotaxic frame with
body temperature maintained at 37°C. A scalp incision was made and a hole was drilled in the skull above the NASh and the VTA. For intra-NASh microinfusion of CBD (100 ng/0.5 µL), a 1 µL Hamilton syringe was slowly lowered at the same coordinates used for behavioral studies. For intra-VTA extracellular recording, glass micro-electrodes (with an average impedance of 6-8 MΩ) filled with a 2% Pontamine Sky Blue solution were lowered using a hydraulic micro-positioner (Kopf 640) at the following flat skull stereotaxic coordinates (in mm from bregma): anteroposterior (AP) -5.3, lateral (LAT) ± 0.7 from midline, ventral (V) -7.0 to -8.5 from the dural surface. Extracellular signals were amplified using a MultiClamp 700B amplifier (Molecular Devices) and recorded through a Digidata 1440A acquisition system (Molecular Devices) using pClamp 10 software. Extracellular recordings were filtered at 1 kHz and sampled at 5 kHz. VTA DA neurons were identified according to well-established electrophysiological features (Jalabert et al., 2011; Ungless, Magill, & Bolam, 2004): (1) a relatively long action potential width (>2.5 ms); (2) a slow spontaneous firing rate (2 - 5 Hz) (3) a triphasic waveform consisting of a notch on the rising phase followed by a delayed after potential; and (4) a single irregular or bursting firing pattern. VTA GABA interneurons were also characterized based upon previously reported criteria: (1) a narrow action potential width (<1 ms), (2) a biphasic waveform; (3) relatively fast firing rates (typically between 10 and 20 Hz); and (4) the absence of bursting activity. Neurons that failed to clearly meet the aforementioned criteria for VTA DA or VTA non-DA electrophysiological neuronal properties were excluded from further study.

Recordings analyses were accomplished using the Clampfit 10 software package. The response patterns of isolated VTA neurons to intra-NASh CBD microinfusions were determined by comparing the neuronal frequency rates between the 5 min pre-infusion versus post-infusion epochs. Classification of drug-infusion effects used a criterion of a ≥ 10% increase in firing frequency post-infusion to be classified as an “increase” effect; a ≤ 10 % decrease to be classified as a “decrease” effect. Neurons showing firing frequency parameters within these cut-off points were classified as “no change”. Within identified VTA DA neurons, we also analyzed the burst rate (number of burst events over time) and the number of spikes within each burst. The onset of a burst was defined as the occurrence of two consecutive spikes with an inter-spike interval of <80 ms.
For histological analysis of extracellular VTA neuronal recording sites, recording electrode positions were marked with an iontophoretic deposit of Pontamine Sky Blue dye (~20 μA, continuous current for 12–15 minutes). Then, rats were perfused transcardially with isotonic saline followed by 10% formalin. Brains were removed and stored in a 25% sucrose-formalin solution before sectioning (40 μm sections) on a freezing cryostat. Following this, sections were stained with cresyl violet and infusion and/or neuronal recording sites were confirmed with light microscopy.

2.2.6 Histology
Following completion of experiments, rats were transcardially perfused, brains were extracted and stored in a specimen vial containing formalin with 25% sucrose solution for at least 24hrs. The region of the projected cannulae placements were cut into 40 μm coronal sections, mounted and stained using cresyl violet to allow for histological examination of the injection site. The majority of NASh and VTA placements were localized within the region of the shell of the nucleus accumbens or the ventral tegmental area, respectively. Rats found to have injectors situated outside the anatomical boundaries of the NASh or VTA were excluded from experimental analysis.

2.2.7 Data analysis
Behavioural data were analyzed with a multivariate ANOVA followed by paired-samples t-tests or Fisher’s Least Significant Difference (LSD) tests, where appropriate. Electrophysiological data were analyzed using a paired-sampled t-test (SPSS Statistics 21 software) and only values with \( p \leq 0.05 \) were considered as significant.

2.3 Results
2.3.1 Histological analysis
Histological analysis revealed microinfusion injector cannulae placements to be bilaterally localized within the anatomical boundaries of the shell subdivision of the nucleus accumbens and the VTA, according to the Rat Brain Atlas (Paxinos & Watson,
Figure 1A presents a microphotograph displaying a typical injector placement within the NASh. Figure 1B displays a schematic illustration showing representative intra-NASh bilateral cannulae placements along the rostral-caudal axis of the nucleus accumbens. Figure 1C shows a microphotograph displaying a typical injector placement within the VTA. Figure 1D displays a schematic illustration showing representative intra-VTA bilateral cannulae placements along the rostral-caudal axis of the ventral tegmental area. Rats with cannulae placements located outside of the NASh or VTA were removed from data analysis.
Figure 1. Histological analysis of intra-NASh and intra-VTA microinjection sites. A, Microphotograph of a representative injector placement within the shell subdivision (AcbSh) of the NAcc. B, Schematic representation of select intra-NASh injector locations; • = 100ng CBD group, ♦ = 100ng CBD + 500ng NAD299 group. C, Microphotograph of a representative injector placement in the VTA. D, Schematic representation of intra-VTA injector locations; o = 100ng saclofen + 50ng bicuculline.

2.3.2 Intra-NASh CBD microinfusions display behaviourally rewarding properties
In order to establish a dose-response curve to examine the motivational properties of CBD, we performed a conditioned place preference experiment using a range of doses of CBD, 50ng (n = 8), 100ng (n = 8), 500ng (n = 6). Multivariate ANOVA revealed a
significant effect of treatment, \( F(4, 36) = 3.38, p = .019 \). Follow-up paired samples \( t \)-tests revealed that rats spent significantly more time, \( t(7) = -5.45, p = .001 \), in the environment paired with 100ng CBD microinfusion (\( M = 284.10s, SD = 32.99 \)) than the environment paired with vehicle administration (\( M = 172.24s, SD = 42.83 \)). In contrast, there were no significant differences in the amount of time rats spent in the CBD or vehicle-paired environments for the 50ng dose, \( t(7) = 0.47, p = .66 \), or the 500ng dose, \( t(5) = -0.38, p = .72 \) (see Figure 2).

Based upon this dose-dependent effect, we chose the highest behaviourally effective dose (i.e., 100ng/0.5µl) of CBD for subsequent behavioural experiments. For the sake of simplicity, data from all follow-up experimental groups will be compared to the data of our 100ng CBD group.

**Figure 2.** Dose response curve for intra-NASh CBD microinjections. Data represent mean (±SEM) time spent in drug- and vehicle-paired environments for rats conditioned with intra-NASh CBD. A moderate dose of 100ng/0.5µl CBD produces a significant conditioned place preference for the drug-paired environment, suggesting that CBD has reinforcing properties in moderate doses.
2.3.3 Intra-NASh 5-HT\textsubscript{1A} receptor blockade switches the motivational valence of CBD from rewarding to aversive

Based on the large body of evidence that implicates the serotonergic system, particularly 5-HT\textsubscript{1A} receptors, in the pharmacological action of CBD we challenged our observed place preference effect with intra-NASh NAD-299 hydrochloride, a potent 5-HT\textsubscript{1A} antagonist. Multivariate ANOVA revealed a significant effect of treatment, $F_{(6, 46)} = 3.29$, $p = .009$. Follow-up paired samples $t$-tests revealed that 100ng NAD-299 ($n = 7$) was effective at blocking the place preference induced by CBD (100ng), $t_{(6)} = 0.90$, $p = .40$. At the higher dose of 500ng NAD-299 ($n = 6$), 5-HT\textsubscript{1A} antagonism switched CBD’s motivational valence from rewarding to aversive, $t_{(5)} = 2.682$, $p = .044$, rats receiving co-administration of CBD with 500ng NAD-299 spent significantly more time in the vehicle-paired environment ($M = 327.70s$, $SD = 67.17$) than the CBD-paired environment ($M = 182.07s$, $SD = 70.29$) (see Figure 3A). In order to rule out the possibility that NAD-299 may possess aversive properties on its own, we performed a control experiment in which rats were conditioned with vehicle or 100ng NAD-299 ($n = 7$). Paired samples $t$-test revealed that rats did not form a significant preference or aversion for either the vehicle- ($M = 224.09s$, $SD = 167.89$) or NAD-299 ($M = 301.03s$, $SD = 168.74$) -paired environments, $t_{(6)} = -0.607$, $p = .57$, suggesting that NAD-299 alone is not responsible for the conditioned aversion observed with rats receiving 500ng NAD-299 in combination with CBD. (see Figure 3A). Post hoc analysis using Fisher’s LSD revealed that rats conditioned with 100ng CBD co-administered with 100ng and 500ng NAD-299 spent significantly ($p$’s < .05) more time in the vehicle-paired environment ($M_{100ng \text{ NAD}} = 310.53s$, $SD_{100ng \text{ NAD}} = 146.71$; $M_{500ng \text{ NAD}} = 327.70s$, $SD_{500ng \text{ NAD}} = 67.17$) compared to rats conditioned with 100ng CBD alone ($M = 172.24$, $SD = 42.83$).

2.3.4 Intra-NASh 5-HT\textsubscript{1B} receptor antagonism blocks the acquisition of CBD reward memory

Given our results with 5-HT\textsubscript{1A} receptor antagonism, and based upon evidence showing that CB1 receptors are colocalized with 5-HT\textsubscript{1B} receptors, we next examined whether blockade of intra-NASh 5-HT\textsubscript{1B} receptors would yield similar results. In a separate experiment, rats were conditioned with CBD (100ng) co-administered with GR-55562
dihydrochloride, a potent 5-HT$_{1B}$ antagonist. Multivariate ANOVA revealed a significant effect of treatment, $F_{(4, 36)} = 3.29$, $p = .021$. Follow-up $t$-tests revealed that intra-NASh 5-HT$_{1B}$ antagonism was effective at blocking CBD-induced place preference at a dose of 10ng ($n = 7$), $t_{(6)} = 0.558$, $p = .60$, and 100ng ($n = 7$), $t_{(6)} = -0.176$, $p = .87$. Post hoc analysis using Fisher’s LSD revealed that rats conditioned with 100ng CBD co-administered with 10ng GR-55562 spent significantly ($p = .044$) more time in the vehicle-paired environment ($M = 290.53$s, $SD = 141.23$) compared to rats conditioned with 100ng CBD alone ($M = 172.24$, $SD = 42.83$) (see Figure 3B). Together, these data suggest that serotonergic transmission at both 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors is necessary for the behaviourally hedonic properties of CBD.

**Figure 3.** Intra-NASh 5-HT antagonism blocks CBD reward memory acquisition. **A,** Intra-NASh 5-HT$_{1A}$ antagonism with 100ng/0.5μl NAD299 is sufficient to block
acquisition of CBD reward memory, while 500ng/0.5µl NAD299 switches CBD’s motivational valence from rewarding to aversive. Intra-NASh NAD299 alone does not produce a preference or an aversion. B, Intra-NASh 5-HT₁B antagonism (10ng and 100ng/0.5µl) is sufficient to block acquisition of intra-NASh CBD reward memory.

2.3.5 Intra-NASh DA receptor antagonism blocks the acquisition of CBD reward memory

Given that most drugs of abuse evoke phasic DA release from the VTA, we challenged our behaviourally effective dose of CBD (100ng) with α-flupenthixol (α-flu), a broad spectrum DA antagonist, in order to block signaling from DAergic VTA projections to the NASh. Although CBD is not known to interact with the DAergic system, multivariate ANOVA revealed a significant effect of treatment, $F(4, 46) = 6.93, p = .00$. Follow-up $t$-tests revealed that intra-NASh DA antagonism was effective at blocking CBD-induced place preference at a dose of 100ng ($n = 9$), $t(8) = -1.26, p = .24$, and 1.0µg ($n = 10$), $t(9) = -1.06, p = .32$. Rats conditioned with CBD co-administered with 100ng or 1.0µg α-flu did not develop significant preferences for either the CBD- or vehicle-paired environments (see Figure 4). Thus, the data suggest that acquisition of CBD reward memory is dependent on intra-NASh DAergic transmission, presumably originating from VTA projections.

**Figure 4. Intra-NASh DA antagonisms blocks acquisition of CBD reward memory.** Intra-NASh DA antagonism with α-flupenthixol (100ng and 1.0µg/0.5µl) is sufficient to block acquisition of intra-NASh CBD reward memory.
2.3.6 Intra-NASh CBD decreases DAergic neuronal activity in the VTA

In order to test the hypothesis that intra-NASh CBD elicits phasic increases in VTA DA release, we performed *in vivo* electrophysiology experiments in which the rewarding dose of CBD (100ng) was microinfused into the NASh while simultaneously recording single-cell extracellular neuronal activity in the VTA. A total of 15 VTA-DA neurons were recorded and analyzed following intra-NASh CBD microinfusion. A cell was considered to have changed its firing rate if there was a minimum of 10% difference from baseline. According to this criterion, 60% (*n* = 9) of DA neurons showed a decrease in activity, 7% (*n* = 1) showed an increase, and 33% (*n* = 5) demonstrated no change in activity levels.

Statistical analyses of the average frequency recorded during the 5min before and 5min after the intra-NASh microinfusion showed that CBD significantly decreased the activity of VTA DA neurons (*t*(14) = 3.13, *p* < 0.01). Furthermore, bursting analysis revealed that CBD significantly reduced the number of burst events over time (*t*(14) = 2.76, *p* < 0.05) without significantly decreasing the number of spikes within each burst (*t*(14) = 2.06, *p* = 0.06) (see Figure 5). Taken together, these data suggest that CBD (100 ng) microinfused in the NASh induces an inhibition of VTA-DA neuronal activity. The reduction of the firing frequencies and burst events suggest that the tonic and phasic release of DA in the NASh are reduced following the microinfusion of CBD.
2.3.7 Intra-NASH CBD modulates GABAergic neuronal activity in the VTA

We found that CBD (100ng) was able to significantly decrease VTA DAergic neuronal activity. GABA interneurons are one of the major sources of inhibitory neurotransmitters within the VTA and are known to control reward signaling between DAergic and non-
DAergic neural motivational systems (Laviolette & van der Kooy, 2001). As a result, it is highly probable that the observed CBD effect on VTA DAergic cell activity was due to an increase in VTA GABA interneuron activity. To test this hypothesis, we recorded and analyzed VTA GABA neurons \((n = 15)\) before and after a microinfusion of CBD \((100\ ng)\) into the NASh. Analysis of the general activity changes revealed that \(27\%\ (n = 4)\) of interneurons decreased their activity, \(40\%\ (n = 6)\) of interneurons increased their activity, and \(33\%\ (n = 5)\) demonstrated no change in activity levels. Statistical analyses of the frequencies recorded during the 5 min before and 5 min after the intra-NASh microinfusion showed that CBD did not significantly modify the average firing frequency of VTA GABA interneurons, \(t_{(14)} = -0.04; p = 0.97\). Collectively, these data suggest that CBD microinfused in the NASh induce heterogeneous effects on VTA GABA cells (see Figure 6). However, the majority of interneurons \((40\%)\) increased their firing. Therefore, it seems reasonable that the effect of CBD on VTA DAergic neuronal activity was attributable to the sub-population of GABA cells that increased their firing frequency.
Figure 6. Intra-NASh CBD modulates GABAergic interneuron neuronal activity in the VTA. A. Representative rastergram showing firing frequency patterns of sampled VTA GABAergic neurons during intra-NASh CBD microinfusion. VTA GABAergic neuronal recording sample demonstrates a typical decrease in neuronal activity following intra-NASh CBD (100ng/0.5μl). B. Representative rastergram showing firing frequency patterns of sampled VTA GABAergic neurons during intra-NASh CBD microinfusion. VTA GABAergic neuronal recording sample demonstrates a typical increase in neuronal activity following intra-NASh CBD (100ng/0.5μl). C. Number of GABAergic cells sampled, 27% of cells decreased neuronal activity, 33% displayed no change in activity, and 40% exhibited an increase in activity. D. Frequency (Hz) of VTA GABAergic cell firing before and after intra-NASh microinfusion. Following CBD administration, the subpopulation of cells that demonstrated an increase in neuronal activity displayed a significant increase in firing frequency following intra-NASh CBD.
2.3.8 Intra-VTA GABA<sub>A/B</sub> receptor antagonism blocks the acquisition of intra-NASh CBD reward memory

In order to further investigate the effects of CBD’s modulation of VTA GABAergic activity at the behavioural level, we performed experiments in which rats were bilaterally implanted with cannulae into both the NASh and VTA. Rats received infusions of intra-NASh CBD (100ng) and a combination of intra-VTA bicuculline (50ng) and saclofen (100ng) in order to antagonize both GABA<sub>A</sub> and GABA<sub>B</sub> receptors in the VTA. Rats were then conditioned in the CPP protocol outlined above. A paired samples t-test revealed intra-VTA GABA<sub>A/B</sub> antagonism blocked the acquisition of intra-NASh CBD reward memory, t<sub>(8)</sub> = 1.42, p = .195. Rats did not spend significantly more time in the CBD-paired environment (M = 209.95, SD = 139.32) compared to the vehicle-paired environment (M = 343.41, SD = 145.03). An independent samples t-test revealed that rats conditioned with intra-VTA GABA<sub>A/B</sub> antagonists spent significantly more time in the vehicle-paired environment (M = 343.41, SD = 145.03) compared to rats conditioned with intra-NASh CBD alone (M = 172.24, SD = 42.83), t<sub>(15)</sub> = -3.379, p = .007. In contrast independent samples t-test revealed that rats conditioned with intra-VTA GABA<sub>A/B</sub> antagonists did not spend significantly more time in the drug-paired environment (M = 209.95, SD = 139.32) compared to rats conditioned with intra-NASh CBD alone (M = 284.10, SD = 32.99), t<sub>(15)</sub> = 1.55, p = .156. These data indicate that antagonism of VTA GABA<sub>A/B</sub> receptors blocks the acquisition of intra-NASh CBD reward memory (Figure 7). Together with our electrophysiology data, these results suggest that a reduction in VTA DAergic signaling due to a general increase in VTA GABAergic interneuron activity is necessary for the acquisition of intra-NASh CBD reward memory. By blocking GABA<sub>A/B</sub> receptors in the VTA, GABA interneurons are unable to suppress DAergic projection neurons to the NASh. Thus, the intra-NASh CBD-induced decrease in VTA DAergic activity is blocked, and the behavioural reward effect is abolished.
Figure 7. Intra-VTA GABA<sub>A/B</sub> antagonism blocks acquisition of intra-NASh CBD reward memory. Intra-VTA antagonism of GABA<sub>A</sub> receptors, with bicuculline (50ng/0.5µl), and GABA<sub>B</sub> receptors, with saclofen (100ng/0.5µl), is sufficient to block acquisition of intra-NASh CBD reward memory.

2.4 Discussion

This work represents the first demonstration that cannabidiol does indeed possess reinforcing psychoactive properties in the shell of the nucleus accumbens (NASh) at both the behavioural and electrophysiological levels. Intra-NASh CBD microinfusions produced a significant place preference reward memory in an unbiased CPP paradigm. This effect was dependent on 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub> and DA receptor signaling within the NASh, and elicited a modulation of DAergic and GABAergic neuronal activity within the VTA.

2.4.1 Intra-NASh CBD produces a reward memory in a dose-dependent manner

The conditioned place preference paradigm is an animal model for investigating the rewarding or aversive properties of various chemical compounds. It has particular utility when examining the effects of drugs of abuse, because of the many parallels it shares with human drug use. Both humans and animals will seek out environmental cues that have been associated with drug administration and for many addicts coming into contact
with cues that predict drug use (e.g., drug paraphernalia, a particular spatial location where drugs were used), can be major contributing factors to relapse (Carter & Tiffany, 1999; Young et al., 2014). In addition, many drugs of abuse, such as nicotine (Laviolette & van der Kooy, 2004; Tan et al., 2009), alcohol (Philpot, Badanich, & Kirstein, 2003), and cannabinoids (Katsidoni, Kastellakis, & Panagis, 2013), exhibit dose-dependent effects in animal models such as CPP.

The current study is the first to systematically establish a dose-response curve for the rewarding properties of CBD using a CPP paradigm. Our results show that while very low (50ng) and very high (500ng) doses of CBD did not produce any appetitive or aversive effects, a moderate dose of CBD (100ng) produced a significant preference for the environment paired with intra-NASH CBD microinfusions (Figure 2). These data suggest that CBD does possess hedonic psychoactive properties and may give us insight into the behavioural and cellular mechanisms that precipitate and maintain cannabis abuse.

2.4.2 CBD reward signals through 5-HT receptor transmission in the NASH

In addition to its antagonistic action at CB1 receptors, CBD has been shown to have agonistic properties at serotonin (5-HT) receptors, particularly at 5-HT1A receptors (Russo et al., 2005). We therefore performed experiments to investigate whether the observed rewarding effect of CBD was dependent on 5-HT1A receptor transmission within the NASH. We found that co-administration of CBD with NAD-299, a 5-HT1A receptor antagonist, was able to block CBD reward memory acquisition at the low dose (100ng). However, at the high dose (500ng), 5-HT1A receptor antagonism switched CBD’s motivational valence from rewarding to aversive (Figure 3A). Together these data suggest that 5-HT1A signaling is crucial for the hedonic properties of intra-NASH CBD, and that a decrease in 5-HT1A signaling may precipitate an aversive response. Recent studies implicate the 5-HT1A receptor as playing a critical role in the anxiolytic, antidepressant and antipsychotic actions of CBD (Almeida et al., 2013; Campos & Guimarães, 2008; Campos, Ferreira, & Guimarães, 2012; De Almeida & Mengod, 2008; Fogaça et al., 2014; Zanelati, Biojone, Moreira, Guimarães, & Joca, 2009). Interestingly, deficits in 5-HT signaling have been implicated in a number of neuropsychiatric disorders
including anxiety, depression and addiction (see Gingrich & Hen, 2001 for a review). Indeed, mice lacking the 5-HT$_{1A}$ receptor display altered emotional states and reactivity to environmental cues (Zhuang et al., 1999). Furthermore, 5-HTergic neurotransmission through 5-HT$_{1A}$ receptors has been suggested to modulate brain reward processes (Harrison & Markou, 2001), and CBD has been shown to inhibit synaptic transmission in the hippocampus through 5-HT$_{1A}$ receptors (Ledgerwood, Greenwood, Brett, Pratt, & Bushell, 2010). Together, these data suggest that 5-HT$_{1A}$ receptor transmission may be required for the accurate processing of emotional and motivational information. It is therefore possible that antagonism of 5-HT$_{1A}$ receptors in our study prevented CBD from exerting the full range of its psychopharmacological effects. Another possibility is that 5-HT$_{1A}$ antagonism alone produced an aversive response. However this seems unlikely, as rats conditioned with NAD-299 alone did not develop a significant aversion to the environment paired with NAD-299 administration. Furthermore, 5-HT$_{1A}$ antagonism is not known to produce either appetitive or aversive effects in the CPP paradigm (Risinger & Boyce, 2002).

The 5-HT$_{1B}$ receptor is another well-characterized 5-HT receptor subtype and like the 5-HT$_{1A}$ receptor, it has been implicated in a number of physiological functions and neuropsychiatric disorders (Sari, 2004; Ujike et al., 2011). Furthermore, 5-HT$_{1B}$ receptors are highly co-expressed with CB1 receptors in the striatum (Hermann et al., 2002; Maroteaux et al., 1992) suggesting a modulatory role of this receptor in motivational and emotional processing. Based on this information, we performed a separate experiment to examine whether the rewarding effect of CBD was dependent on 5-HT$_{1B}$ receptor signaling. We found that intra-NASh co-administration of CBD with GR-55562, a 5-HT$_{1B}$ receptor antagonist, was able to block CBD reward memory acquisition (Figure 3B). Previous studies have found that 5-HT$_{1B}$ receptors are expressed at the nerve terminals of GABAergic projection neurons from the nucleus accumbens to the VTA as well as DAergic projection neurons from the VTA to the nucleus accumbens (Boschert, Amara, Segu, & Hen, 1994; Bruinvels et al., 1994; Maroteaux et al., 1992; Sari, 2004; Voigt, Laurie, Seeburg, & Bach, 1991). Additionally, both the NASh and the VTA are heavily innervated by serotonergic projections (Lavoie & Parent, 1990; Phelix & Broderick, 1995), implicating the serotonergic system as a critical modulator of brain...
reward pathways. Given that 5-HT$_{1B}$ receptors are inhibitory G-protein coupled receptors, it is possible that antagonism of the receptor inhibited the ability of DAergic projection neurons from the VTA to be modulated by endogenous 5-HT, thereby raising the overall DAergic tone in the NASh. In light of our electrophysiology results (discussed below) that indicate that intra-NASh CBD microinfusions result in a decrease in VTA DAergic neuronal activity, it is possible that artificially elevating NASh DAergic tone abolishes the behaviourally rewarding effect of CBD, which may depend on reduced NASh DA originating from the VTA. Together, the results suggest that 5-HT receptor signaling may play a crucial role in the rewarding effects of cannabinoids in general, and of CBD in particular.

2.4.3 CBD reward signals through DA receptor transmission in the NASh
Given that most drugs of abuse elicit increased DA release from the VTA, which reinforces drug use behaviours (Adinoff, 2004; Laviolette & Grace, 2006; Margolis, Lock, Hjelmstad, & Fields, 2006), we performed separate experiments in order to investigate whether the behaviourally rewarding dose of CBD (100ng) was dependent on DAergic signaling within the NASh. We found that co-administration of CBD with $\alpha$-flupenthixol (100ng, 1.0µg), a DA receptor antagonist, was sufficient to block the acquisition of intra-NASh CBD reward memory (Figure 4).

Previous studies have found DA receptor antagonism, using $\alpha$-flupenthixol, to be effective at blocking the acquisition of drug reward memory in the CPP procedure (García Horsman & Paredes, 2004). Given that the NASh receives DAergic input from the VTA (Ferreira, Del-Fava, Hasue, & Shammah-Lagnado, 2008; Ikemoto, 2007), it is highly probable that our intra-NASh DA receptor antagonism prevented increased DAergic reward signals from the VTA and thus blocked the acquisition of intra-NASh CBD reward memory.

2.4.4 CBD modulates intra-VTA DA and GABA neuronal activity
In order to elucidate the mechanism of intra-NASh CBD reward in greater detail, we performed single unit in vivo electrophysiology experiments in which rats received CBD
microinfusion into the NASh while we simultaneously recorded single cell neuronal activity in the VTA. Given that drugs of abuse elicit phasic DA release from the VTA, and that we were successful in blocking intra-NASh CBD reward memory acquisition using DA receptor antagonists, we predicted that our behaviourally effective dose of CBD (100ng) would elicit a similar increase in DAergic neuronal activity in the VTA, providing a mechanism to explain the rewarding effect observed in the CPP experiments. Contrary to our hypothesis, the majority of DA cells sampled showed a significant decrease in neuronal activity as measured by a decrease in firing frequency (Fig. 5C) and a decrease in the number of burst events per minute (Fig. 5D). Of the non-DA neurons (presumably GABAergic interneurons) sampled, we observed a trend towards increased neuronal activity as measured by an increase in firing frequency (Fig. 6B). Of the subpopulation of neurons exhibiting increased activity, there was a significant increase in firing frequency following intra-NASh CBD microinfusion (Fig. 6D). In contrast, only a small sample of non-DA neurons showed a decrease in firing frequency (Fig. 6A). It is therefore highly probable that the observed decrease in DAergic activity is due to the increased inhibitory influence of GABAergic interneurons that modulate the activity of DAergic projection neurons to the NASh and other mesocorticolimbic structures.

Based on these results, we performed a separate experiment in which rats received bilateral cannulae implantations into the NASh as well as the VTA, in order to examine whether intra-NASh CBD reward was dependent on intra-VTA GABAergic signaling. We found that antagonism of both GABA_A and GABA_B receptors in the VTA was sufficient to block the acquisition of intra-NASh CBD reward memory (Figure 7). Together with the results from our electrophysiology experiments, these data suggest that modulation of DAergic activity by GABAergic interneurons is critical for the acquisition of intra-NASh CBD reward memory. These results seem at odds with the results of our behavioural experiment using DA antagonists, which suggested that intra-NASh DA signaling was necessary for the behavioural expression of CBD reward. It is possible that while intra-VTA DAergic neuronal activity was significantly reduced following intra-NASh CBD microinfusion, the reduced VTA DA signals were still necessary for the acquisition of intra-NASh CBD reward memory. Thus, by blocking intra-NASh DA
receptors, we had prevented the VTA from being able to send reward-related DA signals to the NASh.

Many recent research efforts have focused on elucidating the mechanism by which CBD may exert its antipsychotic properties (Mechoulam et al., 2007). One recent study (Leweke et al., 2012) suggests that the ability of CBD to prevent the degradation of the endocannabinoid anandamide may be at least partially responsible for CBD’s antipsychotic efficacy. In the present study, we present evidence that CBD can modulate mesolimbic DA activity by decreasing DAergic activity in the VTA. Given that mesolimbic DA hyperactivity has been implicated as a major etiological factor in a number of neuropsychiatric disorders including addiction and schizophrenia (Laviolette & Grace, 2006; Mehler-Wex, Riederer, & Gerlach, 2006), these data represent another possible pharmacological mechanism that may be responsible for CBD’s antipsychotic actions.

2.5 Summary

This study presents the first evidence for hedonic properties of CBD, long believed to be devoid of reinforcing effects. Our results support past research showing that cannabinoids have a diverse range of pharmacological effects including interactions with the serotonergic, DAergic and GABAergic neurotransmitter systems. Our electrophysiology results lend further credence to the notion that cannabinoids are unique in the world of abused drugs as CBD actually drives inhibition of VTA DAergic transmission. Future studies should examine the electrophysiological changes induced by 5-HT$_{1A}$ receptor antagonism on VTA neuronal activity in order to determine the mechanism that switches CBD’s motivational valence from rewarding to aversive.
Chapter 3. Cannabidiol and $\Delta^9$-tetrahydrocannabinol differentially modulate the acquisition of an associative fear memory in the nucleus accumbens shell

3.1 Introduction

Dysfunction in emotional and motivational information processing is a hallmark of the cognitive deficits observed in schizophrenia. This can manifest as abnormal social cognition and impaired emotional expression, and may lead to aberrant salience attribution to what would normally be insignificant sensory experiences or events. In the extreme, such attribution may lead to psychotic ideation and persistent maladaptive behaviours (Laviolette & Grace, 2006). Cannabinoids can produce similarly profound effects on emotional information processing and sensory perception (D’Souza et al., 2005) by acting on centralized CB1 receptors. Acute cannabinoid exposure can lead to paranoid thought processes, anxiety and agitation even among healthy individuals and may precipitate episodes of psychosis in individuals predisposed to psychotic disorders (Bhattacharyya et al., 2012). Several neural structures comprise the emotional processing circuitry, collectively referred to as the mesocorticolimbic system. These areas contain high levels of dopamine (DA) and CB1 receptors, which are critical in emotional salience processing. Furthermore, both of these neurotransmitter systems have been implicated in the etiology and neuropathological profile of schizophrenia (Eggan, Hashimoto, & Lewis, 2008; Lauzon, Bishop, & Laviolette, 2009; Laviolette & Grace, 2006; Scatton & Sanger, 2000), and structural and functional abnormalities in mesocorticolimbic structures have been reported in patients with schizophrenia (Burns et al., 2003; Crespo-Facorro et al., 2001; Taylor, Liberzon, Decker, & Koepppe, 2002). Abnormalities in endocannabinoid signaling in particular have been implicated in the aberrant emotional processing observed in psychosis (Dalton, Long, Weickert, & Zavitsanou, 2011; Hall, Degenhardt, & Teesson, 2004), and there is also a growing body of evidence which supports the link between excessive marijuana exposure, especially during adolescence, and an increased risk of developing schizophrenia-related psychoses in later adulthood (Bossong & Niesink, 2010; Semple, 2005; Smit et al., 2004).
However, marijuana smoke represents a complex mixture of chemical components, possessing dissociable psychoactive and pharmacological properties (ElSohly & Slade, 2005; Radwan et al., 2009). While Δ9-THC may precipitate, or even exacerbate psychotic symptoms, CBD has been shown to have antipsychotic and anxiolytic properties (Bhattacharyya, et al., 2009b).

The goal of the current study was to investigate the effects of CBD and Δ9-THC on the encoding of emotionally salient information using an olfactory fear conditioning procedure. Evidence from our laboratory has shown that activation of CB1 receptors in discrete mesocorticolimbic regions can significantly potentiate the salience of an emotional memory, while blockade of CB1 receptor transmission blocks emotional associative learning in this paradigm (Lauzon et al., 2009; Laviolette, 2006; Tan et al., 2010). However, the effects of distinct, marijuana-specific, compounds on the encoding of emotionally salient associative memories are not well understood. In the current study, we performed several pharmacological behavioural experiments in which rats received microinfusions of CBD or Δ9-THC into the shell subdivision of the nucleus accumbens (NASh), a structure that is sensitive to cannabinoids (Mahler et al., 2007; Zangen et al., 2006) and plays a crucial role in emotional processing and motivated behaviour (Di Chiara, 2002; Di Chiara et al., 2004). We report that Δ9-THC microinfusions potentiated the acquisition of a subthreshold fear memory through a DA-dependent mechanism. In contrast, CBD microinfusions were effective at blocking the acquisition of a suprathreshold fear memory through a 5-HT1A-dependent mechanism. CBD was also effective at abolishing Δ9-THC-induced potentiation of a subthreshold fear memory. Implications for neuropsychiatric disorders are discussed.

3.2 Materials and Methods

3.2.1 Animals and Surgery

Male Sprague Dawley rats (300-350g) were obtained from Charles River Laboratories (Quebec, CAN). Rats were anesthetized with an intraperitoneal (i.p.) injection of a ketamine (80mg/ml)-xylazine (6mg/kg) mixture and meloxicam (1.0 mg/kg; s.c.) was administered preoperatively in order to reduce pain and post-surgical inflammation. Following an assessment of anesthetic depth, rats were immobilized using a Kopf
stereotaxic device. Using sterile technique, an incision was made to expose the skull. Guide cannulae (22-gauge) were implanted bilaterally into the NASh using flat skull stereotaxic coordinates as follows (12° angle, in mm from bregma): anteroposterior (AP) + 1.8, lateral (LAT) ± 2.6, ventral (V) -7.4 from the dural surface. All stereotaxic coordinates were based upon the atlas of Paxinos and Watson (1996). Cannulae were secured to the skull using four small jeweler’s screws and dental acrylic. Dust-caps were used to ensure the patency of the cannulae. All procedures were performed in accordance with the Canadian Council on Animal Care (CCAC) and were approved by Western University’s Council on Animal Care.

3.2.2 Post-Surgery SOP

Rats were injected subcutaneously with 3 – 10 mL of physiological saline in order to prevent dehydration. Rats recovered in a clean home cage filled with wood shavings and warmed under a heat lamp. Behaviour was monitored until recovery from the anesthetics and sternal recumbency attained. A maintenance dose of meloxicam (1.0 mg/kg) was administered (s.c.) 24 hours post-surgery in order to reduce pain and inflammation. Following at least one week of recovery, rats were then tested in behavioral paradigms.

3.2.3 Olfactory fear conditioning

All rats were conditioned using a fully counterbalanced olfactory fear conditioning procedure, as described previously (Lauzon et al., 2009; Laviolette, 2005). Rats were taken from their home cages and received sham microinfusions into the NASh before being habituated for 30 min to the conditioning apparatus, a ventilated chamber with a metallic electric grid floor. Conditioning took place 24 hrs later in one of two visually distinct environments. The first environment was a 30” x 30” Plexiglas box with black stripes on a white background and a metallic electric grid floor (shock environment A). The second environment was a 30” x 30” Plexiglas box with black dots on a white background and a metallic grid floor (shock environment B). Testing occurred 24 hrs later in one of two alternate environments in which rats did not receive electric foot
shock. The first environment was a 30” x 30” Plexiglas box with black stripes on a white background and a grey Plexiglas floor (test environment A), while the second environment had black dots on a white background with a gray Plexiglas floor (test environment B). This fear conditioning procedure is context independent as rats are conditioned and tested in distinct environments, with only olfactory conditioning cues being consistent across the experimental procedure. On day 1 (habituation), rats were habituated to a random combination of shock environment A or B and test environment A or B for 30 min in each environment. On day 2 (conditioning) rats were placed in their previously assigned shock environment. During conditioning, one of two distinct odours (almond or peppermint) were presented for 19s, followed by a 1s footshock (CS+) of either 0.4 mA or 0.8 mA. The alternate odour is presented 120s later for a duration of 20s in the absence of a footshock (CS-). This procedure is repeated five times. By modulating the intensity of foot shock administered (either a subthreshold foot shock of 0.4 mA that normally does not produce an associative fear memory, or a suprathreshold foot shock of 0.8 mA that produces robust fear memory), we can examine how specific pharmacological manipulations can either potentiate normally non-salient emotional memory events, or block the encoding of highly salient emotional memory events (Laviolette, 2006).

Testing occurred 24hrs after conditioning, when rats were placed in their previously assigned test environment. Rats were permitted to explore the testing apparatus for 1 min before odour presentation, during which time baseline levels of freezing and exploratory behaviour were recorded. Rats were then exposed to the CS+ and CS- odours for 5 minutes in a counterbalanced fashion and the percentage of time the rat spent “freezing”, a measure of conditioned fear, was recorded. Freezing was defined as complete immobility with the exception of respiratory-related movements.

3.2.4 Drug administration

The broad-spectrum DA receptor antagonist, α-flupenthixol, the highly specific 5-HT1B receptor antagonist, GR-55562, the GABA_A antagonist, bicuculline methiodide, and the GABA_B antagonist, hydroxysaclofen, were dissolved in physiological saline (pH adjusted to 7.4). The 5-HT1A receptor antagonist, NAD-299 hydrochloride, and CBD were
dissolved in dimethyl sulfoxide (DMSO, Fisher Scientific). Δ9-THC was dissolved in 100% ethanol and cremophor EL (Sigma-Aldrich). Ethanol was removed using nitrogen stream evaporation and the final solution was diluted with filtered saline. For NASH microinfusions, stainless steel guide cannulae (22-gauge) were implanted bilaterally into the NASH through a 28-gauge microinfusion injector (Plastics One). Bilateral microinfusions were performed over 1 min through a Hamilton microsyringe. In order to ensure the diffusion of the drug away from the injector tip, the internal cannulae were left in place for an additional minute. All drugs were purchased from Tocris Biosciences.

3.2.5 Histology

Following completion of experiments, rats were transcardially perfused, brains were extracted and stored in a specimen vial containing formalin with 25% sucrose solution for at least 24hrs. The region of the projected cannulae placements were cut into 40 µm coronal sections, mounted, and stained using cresyl violet to allow for histological examination of the injection site. The majority of NASH placements were localized within the region of the shell of the nucleus accumbens. Rats found to have injectors situated outside the anatomical boundaries of the NASH were excluded from experimental analysis.

3.2.6 Data analysis

Data were analyzed with a mixed-model ANOVA with a between subjects factor of treatment, and a within subjects factor of stimulus, followed by paired-samples t-tests.

3.3 Results

3.3.1 Histological analysis

Histological analysis revealed microinfusion injector cannulae placements to be bilaterally localized within the anatomical boundaries of the shell subdivision of the nucleus accumbens, according to the Rat Brain Atlas (Paxinos & Watson, 1996). Figure 8A presents a microphotograph displaying a typical injector placement within the NASH. Figure 8B displays a schematic illustration showing representative intra-NASH bilateral
cannulae placements along the rostral-caudal axis of the nucleus accumbens. Rats with cannulae placements located outside of the NASh were removed from data analysis.

Figure 8. Histological analysis of intra-NASh microinjection sites. A, Microphotograph of a representative injector placement within the shell subdivision (Sh) of the NAcc. Injectors located in the core (Co) were removed from analyses. B, Schematic representation of select intra-NASh injector locations; • = 100ng CBD group, ♦ = 100ng Δ9-THC group.

3.3.2 Intra-NASh Δ9-THC microinfusion potentiates the formation of a subthreshold fear memory through a DA-dependent mechanism

In order to test the hypothesis that intra-NASh Δ9-THC would potentiate the formation of a subthreshold fear memory, rats received microinfusions of intra-NASh Δ9-THC, 50ng ($n = 8$) or 100ng ($n = 7$), prior to olfactory fear conditioning with a subthreshold (0.4mA) footshock. In follow-up experiments, animals received 100ng Δ9-THC co-administered
with either 100ng ($n = 5$) or 1.0µg ($n = 5$) of the DA receptor antagonist $\alpha$-flupenthixol, or 100ng ($n = 5$) of the 5-HT$_{1A}$ receptor antagonist NAD-299 (Figure 9).

A mixed-model ANOVA revealed that the main effect of treatment was significant, $F (5, 29) = 4.13, p = .006$, indicating that the mean percentage of time spent freezing was significantly different between treatment groups. The main effect of stimulus was significant, $F (1, 29) = 50.07, p = .000$, indicating a significant difference in the mean percentage of time rats spent freezing in response to the CS+ and CS- odours. Finally, the interaction between stimulus and treatment was significant, $F (5, 29) = 8.90, p = .000$, suggesting that the mean percentage of time spent freezing to the CS+ and CS- odours significantly differs as a function of treatment group. Follow-up paired samples $t$-tests revealed that rats receiving vehicle microinfusions did not spend a significantly higher percentage of time freezing to the CS+ odour ($M = 10.36, SD = 1.69$) than to the CS- odour ($M = 9.83, SD = 1.81$), $t (5) = -0.386, p = .72$. In contrast, intra-NASH $\Delta$9-THC microinfusions potentiated the acquisition of the subthreshold associative fear memory. Rats receiving $\Delta$9-THC at doses of 50ng ($M_{CS} = 12.88, SD_{CS} = 4.06; M_{CS+} = 30.07, SD_{CS+} = 4.54$), $t (7) = -6.631, p = .000$, and 100ng ($M_{CS} = 19.85, SD_{CS} = 3.32; M_{CS+} = 39.48, SD_{CS+} = 5.19$), $t (6) = -3.493, p = .013$, spent significantly more time freezing to the CS+ odour as compared to the CS- odour. The effect of $\Delta$9-THC was abolished when rats were co-administered with the DA receptor antagonist $\alpha$-flupenthixol. Rats receiving concurrent $\alpha$-flupenthixol at doses of 100ng ($M_{CS} = 20.86, SD_{CS} = 5.64; M_{CS+} = 22.74, SD_{CS+} = 6.59$), $t (4) = -.797, p = .47$, and 1.0µg ($M_{CS} = 12.7973, SD_{CS} = 2.04076; M_{CS+} = 12.4313, SD_{CS+} = 1.69558$), $t (4) = .371, p = .73$, did not spend significantly more time freezing to the CS+ odour as compared to the CS- odour. The effect of $\Delta$9-THC was not blocked by the 5-HT$_{1A}$ receptor antagonist NAD-299. Rats co-infused with 100ng NAD-299 spent significantly more time freezing to the CS+ odour ($M = 40.75, SD = 5.76$) as compared to the CS- odour ($M = 15.03, SD = 4.31$), $t (3) = -4.822, p = .017$. 

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Figure 9. Mean (±SEM) percentage of time spent freezing to the CS+ and CS- for rats microinfused with intra-NASH Δ9-THC. Intra-NASH Δ9-THC potentiates the formation of an associative fear memory to a normally non-salient, subthreshold (0.4mA) foot shock. Co-administration of the DA receptor antagonist α-flupenthixol blocks Δ9-THC-induced fear memory potentiation. In contrast, co-administration of the 5-HT1A receptor antagonist NAD-299 does not block Δ9-THC-induced fear memory potentiation. * p < .05.

3.3.3 Intra-NASH CBD microinfusion blocks the formation of a suprathreshold fear memory through a 5-HT1A-dependent mechanism

In order to test the hypothesis that intra-NASH CBD would block the formation of a suprathreshold fear memory, rats received microinfusions of intra-NASH CBD, 10ng (n = 8) or 100ng (n = 8), prior to olfactory fear conditioning with a suprathreshold (0.8mA) footshock. In follow-up experiments, animals received 100ng CBD co-administered with either 10ng (n = 7) or 100ng (n = 7) of the 5-HT1A receptor antagonist NAD-299, 100ng (n = 7) of the 5-HT1B receptor antagonist GR-55562, or 100ng (n = 4) of the DA receptor antagonist α-flupenthixol (Figure 10).

A mixed-model ANOVA revealed that the main effect of treatment was not significant, F (6, 41) = 1.61, p = .169, indicating that the mean percentage of time spent freezing was not significantly different between treatment groups. The main effect of
stimulus was significant, $F(1, 41) = 39.76, p = .000$, indicating a significant difference in the mean percentage of time rats spent freezing in response to the CS+ and CS- odours. Finally, the interaction between stimulus and treatment was significant, $F(6, 41) = 4.25, p = .002$, suggesting that the mean percentage of time spent freezing to the CS+ and CS- odours significantly differs as a function of treatment group. Follow-up paired samples $t$-tests revealed that rats receiving vehicle microinfusions spent a significantly higher percentage of time freezing to the CS+ odour ($M = 59.95, SD = 27.06$) than to the CS- odour ($M = 41.50, SD = 25.77$), $t(6) = -2.49, p = .047$. In contrast, intra-NASH CBD microinfusions blocked the acquisition of the suprathreshold associative fear memory. Rats receiving CBD at doses of 10ng ($M_{CS-} = 16.55, SD_{CS-} = 6.46; M_{CS+} = 22.60, SD_{CS+} = 13.31$), $t(7) = -2.11, p = .073$, and 100ng ($M_{CS-} = 30.81, SD_{CS-} = 24.37; M_{CS+} = 43.61, SD_{CS+} = 27.10$), $t(7) = -2.18, p = .065$, did not spend significantly more time freezing to the CS+ odour as compared to the CS- odour. The effect of CBD was abolished when rats were co-administered with the 5-HT$_{1A}$ receptor antagonist NAD-299. Rats receiving concurrent NAD-299 at doses of 10ng ($M_{CS-} = 23.43, SD_{CS-} = 15.75; M_{CS+} = 56.98, SD_{CS+} = 35.15$), $t(6) = -3.81, p = .009$, and 100ng ($M_{CS-} = 29.52, SD_{CS-} = 10.17; M_{CS+} = 60.43, SD_{CS+} = 21.48$), $t(6) = -4.65, p = .004$, spent significantly more time freezing to the CS+ odour as compared to the CS- odour. The effect of CBD was not blocked by the 5-HT$_{1B}$ receptor antagonist GR-55562. Rats co-infused with 100ng GR-55562 did not spend significantly more time freezing to the CS+ odour ($M = 44.13, SD = 32.61$) as compared to the CS- odour ($M = 38.47, SD = 30.79$), $t(6) = -1.63, p = .154$. Additionally, the effect of CBD was not blocked by the DA receptor antagonist α-flupenthixol. Rats co-infused with 100ng α-flupenthixol did not spend significantly more time freezing to the CS+ odour ($M = 27.44, SD = 12.07$) as compared to the CS- odour ($M = 29.36, SD = 18.07$), $t(3) = 0.335, p = .759$. 
Figure 10. Mean (±SEM) percentage of time spent freezing to the CS+ and CS- for rats microinfused with intra-NASh CBD. Intra-NASh CBD prevents the formation of an associative fear memory to a normally highly salient, suprathreshold (0.8mA) foot shock. Co-administration of the 5-HT$_{1A}$ receptor antagonist NAD-299 rescues the acquisition of suprathreshold fear memory. In contrast, co-administration of the 5-HT$_{1B}$ receptor antagonist GR-55562 or the DA receptor antagonist $\alpha$-flupenthixol does not rescue the acquisition of this fear memory. * $p < .05$

3.3.4 Intra-NASh CBD microinfusion blocks $\Delta 9$-THC-induced potentiation of a subthreshold fear memory

Based on emerging evidence, which suggests that CBD antagonizes many of the behavioural and pharmacological effects of $\Delta 9$-THC, we performed a separate experiment in which rats received intra-NASh microinfusions of 100ng CBD in combination with 100ng $\Delta 9$-THC prior to fear conditioning with a subthreshold footshock in order to examine whether CBD was able to block $\Delta 9$-THC-induced potentiation of a subthreshold fear memory (Figure 11). A paired samples $t$-test revealed that 100ng CBD was able to block the potentiation effect of 100ng $\Delta 9$-THC, $t (7) = -0.67, p = .53$. Rats did not spend significantly more time freezing to the CS+ odour ($M = 32.59, SD = 26.03$) as compared to the CS- odour ($M = 29.83, SD = 28.23$). These data
are in line with a wealth of evidence that supports a modulatory role of CBD over the psychotomimetic effects of Δ9-THC.

Figure 11. Mean (±SEM) percentage of time spent freezing to the CS+ and CS- for rats microinfused with intra-NASh CBD and Δ9-THC. Intra-NASh 100ng Δ9-THC potentiates the formation of an associative emotional memory to a normally non-salient, subthreshold (0.4mA) foot shock. This effect is abolished by co-administration with 100ng CBD, which blocks Δ9-THC-induced potentiation of a subthreshold fear memory.

3.4 Discussion

Dysfunctions in accurately processing the emotional significance of incoming sensory stimuli and coordinating normative cognition and appropriate behavioural responses are pervasive deficits in individuals suffering from schizophrenia (Kohler & Martin, 2006). This is suggestive of dysfunctions in the mesocorticolimbic system, the underlying neurocircuitry that regulates emotional information processing (Grace, 2000). Indeed, imaging studies of schizophrenia patients reveal severe structural and functional abnormalities in various mesocorticolimbic structures (Ellison-Wright & Bullmore, 2009). Furthermore, evidence suggests a positive correlation between the severity of psychotic symptoms and the degree of impairment in emotional processing (Crespo-Facorro et al., 2001). The nucleus accumbens (NAcc) plays a central role in emotional
processing as it sends and receives projections to a number of other mesocorticolimbic structures (Heimer, Zahm, Churchill, Kalivas, & Wohltmann, 1991), integrates information from these regions and produces goal-directed behavioural outputs (Goto & Grace, 2008). In particular, the shell subdivision of the nucleus accumbens (NASh) is a structure that has been implicated in the etiology of schizophrenia, and has been suggested as a novel and efficacious target for antipsychotic medications (Deutch, Lee, & Iadarola, 1992).

Although the precise mechanisms underlying cannabis-induce psychosis remain unknown, evidence is mounting to suggest alterations in cortical and limbic emotional processing regions as key factors. Previous studies have found that Δ9-THC induces elevations in DAergic tone in the VTA (French et al., 1997) and the NAcc (Gardner et al., 1988; Oleson & Cheer, 2012) and can induce transient psychotic symptoms following even acute exposure (Bhattacharyya et al., 2012). Furthermore, dysfunctions in cortical endocannabinoid signaling have been implicated in the aberrant emotional experiences in patients with paranoid schizophrenia (Dalton et al., 2011), and structural alternations have been observed in the NAcc and amygdala in regular cannabis users (Gilman et al., 2014). Taken together, these data suggest that cannabinoid action at structures involved in emotional processing may represent a potential mechanism that contributes to the emotional disturbances observed in cannabis-induced, schizophrenia-related psychosis.

Our previous work has shown that modulation of CB1 transmission in the BLA—mPFC pathway can mediate the emotional valence of an associative fear memory in an olfactory fear-conditioning paradigm. Activation of CB1 receptors in these areas results in the potentiation of normally non-salient emotional associative memories, while CB1 receptor blockade prevents the formation of conditioned fear memories (Laviolette & Grace, 2006; Tan et al., 2011). We report here that CBD and Δ9-THC can similarly modulate emotional memory formation in the NASh, and provide further insight into how cannabis-specific cannabinoids may elicit disturbances in emotional information processing.
3.4.1 Intra-NASh Δ9-THC potentiates the formation of a subthreshold fear memory through a DA-dependent mechanism

Given our previous findings that activation of CB1 receptors can potentiate non-salient emotional experiences in discrete mesocorticolimbic structures, we performed a series of behavioural experiments in which rats received microinfusions of Δ9-THC into the NASh prior to fear conditioning with a subthreshold (0.4mA) footshock. Our analyses revealed that while rats treated with vehicle microinfusions did not form a significant fear memory to the odour paired with a non-salient subthreshold footshock, those treated with Δ9-THC (50, 100ng) demonstrated a potentiation of associative fear memory formation as measured by a significant increase in the percentage of time spent freezing in response to the shock-paired odour (CS+). Based on evidence showing that Δ9-THC administration increases mesolimbic DA levels (Oleson & Cheer, 2012), we performed follow-up experiments in which we challenged our highest behaviourally effective dose of Δ9-THC (100ng) with co-administration of α-flupenthixol (100ng, 1.0µg), a DA receptor antagonist. Our results revealed that antagonism of DA receptors in the NASh was sufficient to block Δ9-THC-induced potentiation of a subthreshold footshock. A final follow-up experiment in which Δ9-THC was challenged with NAD-299, a 5-HT₁A antagonist, revealed that 5-HT₁A signaling was not necessary for Δ9-THC-induced potentiation of a subthreshold footshock (Figure 9).

These data suggest that the disturbances in emotional processing induced by Δ9-THC are in part dependent on DA signaling substrates in the NASh, and may provide a mechanism to explain the propsychotic effects of Δ9-THC and other potent cannabinoids. Abnormalities in endocannabinoid and DAergic signaling have been implicated in the aberrant emotional processing observed in schizophrenia (Dalton et al., 2011; Hall et al., 2004; Moore, West, & Grace, 1999), and previous research has shown that the endocannabinoid and DAergic systems functionally interact such that cortical CB1 transmission modulates subcortical mesolimbic DA activity (Bossong & Niesink, 2010; Diana et al., 1998). Indeed, cannabis use is known to induce alterations in sensory perception: environmental stimuli may become more salient and their emotional valence may be amplified (Green et al., 2003; Wachtel et al., 2002). These effects share many similarities with the cognitive, social and emotional disturbances observed in
schizophrenia, and CB1 receptor activation has been shown to exacerbate psychotic and cognitive symptoms in schizophrenia patients (Bossong, Jansma, Bhattacharyya, & Ramsey, 2014; D’Souza et al., 2005).

In the current study, the aberrant emotional processing induced by acute Δ9-THC administration was abolished with concurrent administration of α-flupenthixol, a DA receptor antagonist and potent antipsychotic. Previous research implicates disruptions in DAergic signaling in the NAcc as an etiological factor in schizophrenia (Goto & Grace, 2008; Grace, 2000). Taken together, this suggests that intra-NASH Δ9-THC may have induced alterations in accumbal DAergic tone that manifests at the behavioural level as distortions in emotional information processing (Pezze & Feldon, 2004). Indeed, Δ9-THC, like all known drugs of abuse, induces DA release from the VTA to the NAcc (Bossong et al., 2008; French et al., 1997; Gardner, 2005; Tanda & Goldberg, 2003), presumably by inhibiting GABAergic interneuron activity in the VTA (Szabo, Siemes, & Wallmichrath, 2002).

The NAcc modulates mesolimbic DA levels by sending direct and indirect GABAergic projections to the ventral tegmental area (VTA) via medium spiny neurons (MSN; Xia et al., 2011). The VTA in turn sends DAergic projections back to the NAcc and other mesocorticolimbic structures (Ferreira et al., 2008; Grace et al., 2007; Heimer et al., 1991). CB1 receptors are highly expressed on presynaptic GABAergic interneurons in limbic structures (Elphick & Egertova, 2001; Pertwee & Ross, 2002; Rodriguez, Mackie, & Pickel, 2001). Thus it is possible that Δ9-THC’s activation of CB1 receptors located on presynaptic GABAergic interneurons in the NASH disinhibits MSN projection neurons to the VTA. This in turn would inhibit VTA GABAergic interneurons, hence releasing VTA DAergic projection neurons from tonic inhibition and, thus, exciting DA release from the VTA to the NAcc (see Figure 12). This model, in combination with our behavioural results, is in line with evidence suggesting that increased DA release from the VTA to the NASH potentiates behavioural responding to conditioned aversive stimuli (Pezze & Feldon, 2004), and provides a potential mechanism to explain the propsychotic properties of Δ9-THC.
3.4.2 Intra-NASh CBD blocks the formation of a suprathreshold fear memory through a 5-HT\textsubscript{1A}-dependent mechanism

Given CBD’s antagonistic action at CB1 receptors, we performed behavioural experiments in which rats received intra-NASh microinfusions of CBD (10, 100ng) prior to conditioning with a suprathreshold footshock in order to examine whether CBD was effective at blocking the acquisition of an associative fear memory similar to what was observed with synthetic CB1 receptor antagonists in previous studies (Laviolette & Grace, 2006; Tan et al., 2011). Our results indicate that CBD was effective at blocking the acquisition of a highly salient suprathreshold fear memory. This effect was dependent on 5-HT\textsubscript{1A} receptor signaling, however was independent of 5-HT\textsubscript{1B} and DA receptor signaling (Figure 10).
Numerous studies have shown CBD to be effective at attenuating the acquisition and expression (Gomes et al., 2012; Lemos, Resstel, & Guimarães, 2010), and facilitating the extinction (Bitencourt, Pamplona, & Takahashi, 2008), of conditioned fear. Although the current results indicate that CBD’s effects in the NASh depend on 5-HT_{1A} receptor signaling; it is not possible to rule out alternative pharmacological mechanisms. Recent studies reveal CBD to have a wide range of pharmacological actions, which may explain its profound effect in fear conditioning paradigms including: antagonism of CB1 receptors (Thomas et al., 2009), agonistic action at 5-HT_{1A} receptors (Russo et al., 2005), and inhibition of FAAH, the enzyme which catalyzes the degradation of AEA one of the major endocannabinoids and endogenous ligands of the CB1 receptor (Bisogno et al., 2001).

Interestingly, CB1 receptor transmission, 5-HT_{1A} receptor signaling and AEA tone have all been implicated as playing critical roles in the acquisition, expression and extinction of conditioned fear responses. For example, CB1 receptor antagonism has been shown to block the acquisition of a highly salient associative fear memory (Laviolette, 2006; Tan et al., 2011), and mice lacking the CB1 receptor show impairments in extinction learning in associative fear paradigms (Cannich et al., 2004; Marsicano et al., 2002). Agonists of the 5-HT_{1A} receptor facilitate fear extinction in animals conditioned to aversive stimuli (Saito et al., 2012), and mice lacking the 5-HT_{1A} receptor show increased fear responses to contextual fear cues (Klemenhagen, Gordon, David, Hen, & Gross, 2005). Furthermore, a study by Gomes et al. (2012) demonstrated that microinfusions of CBD into the bed nucleus of the stria terminalis attenuates behavioural expression in a fear conditioning paradigm and that this effect is blocked with 5-HT_{1A} receptor antagonism. Other studies found that inhibition of AEA reuptake was effective at facilitating the extinction of conditioned fear (Bitencourt et al., 2008; Chhatwal, Davis, Maguschak, & Ressler, 2004). Our results are thus in line with a growing body of research demonstrating the diverse pharmacological and behavioural effects of CBD in an associative fear conditioning paradigm. These findings are particularly notable, however, as we have extended these findings to a discrete neuroanatomical structure, specifically the shell subdivision of the NAcc.
From a mechanistic perspective, it is possible that CBD exerts its neuropsychological effects in precisely the opposite manner of Δ9-THC. While Δ9-THC presumably activates intra-NASh CB1 receptors on presynaptic GABAergic interneurons, ultimately resulting in increased VTA DAergic output to the NASh, CBD likely blocks intra-NASh presynaptic CB1 receptors on GABAergic interneurons, allowing MSN GABAergic projection neurons to be inhibited. This frees intra-VTA GABAergic interneurons from tonic inhibition allowing them in turn to inhibit DAergic projection neurons back to the NASh and thus decreasing DAergic tone in the NASh (Figure 13). This theoretical model is in line with the results from our electrophysiology studies discussed earlier (see section 2.4.4).

CBD’s modulation of the formation and recall of emotionally salient associative memories has interesting implications for its use as an effective pharmacotherapy in various neuropsychiatric disorders such as addiction and post-traumatic stress disorder (PTSD). These disorders are characterized by recurrent, persistent and intrusive recall of memories associated with emotionally salient experiences, which infringes upon normal cognition. These may be aversive traumatic experiences as in PTSD, or drug-related reward memories in the case of addiction, in which the individual is unable to suppress the spontaneous recall of memories associated with those experiences (Rothbaum & Davis, 2003). Indeed, recent studies have shown CBD to be effective at attenuating PTSD-like symptoms and facilitate extinction of aversive associative memories in fear conditioning paradigms, which model many features of the disorder (Bitencourt et al., 2008; Campos et al., 2012; Lemos et al., 2010; Resstel, Joca, Moreira, Corrêa, & Guimarães, 2006). Furthermore, individuals suffering from PTSD have been shown to have reductions in circulating endocannabinoid levels (Hill et al., 2013) and that treatment with CBD helps to alleviate many symptoms of PTSD (Greer, Grob, & Halberstadt, 2014; Passie, Emrich, Karst, Brandt, & Halpern, 2012). This effect is presumably due to CBD’s ability to raise endocannabinoid tone by inhibiting the degradation of AEA. CBD administration has also been found to be effective at attenuating opioid-seeking behaviour in rats trained to self-administer heroin, and even normalized mesolimbic neuronal disturbances induced by chronic heroin administration (Ren, Whittard, Higuera-Matas, Morris, & Hurd, 2009). Emerging basic and clinical
evidence suggests that CBD and other cannabinoids represent potentially effective therapeutic compounds in the treatment of PTSD (Greer et al., 2014; Neumeister, 2012; Passie et al., 2012) and various other neuropsychiatric disorders such as schizophrenia, anxiety and depression (Mechoulam et al., 2007; Schubart et al., 2013; Scuderi et al., 2009; Zuardi et al., 2006).

Figure 13. Proposed model for the pharmacological action of CBD in the NAcc-VTA pathway. DA: dopamine; MSN: medium spiny neuron; NAcc: nucleus accumbens; VTA: ventral tegmental area.
### 3.4.3 Intra-NASh CBD blocks Δ9-THC-induced potentiation of a subthreshold fear memory

Numerous lines of basic and clinical evidence show a dissociation between CBD and Δ9-THC at both the pharmacological and behavioural levels (Bhattacharyya et al., 2009b; Fusar-Poli et al., 2009; Pertwee, 2008; Petitet et al., 1998; Vann et al., 2008). These effects may be attributable to Δ9-THC and CBD’s differential pharmacological action at CB1 receptors. Given the opposing pharmacological and behavioral properties of Δ9-THC and CBD, and the substantial evidence which suggests that CBD attenuates many of the psychopharmacological actions of Δ9-THC, we completed a separate study in which rats received microinfusion of intra-NASh Δ9-THC (100ng) in combination with CBD (100ng) prior to being conditioned with a subthreshold foot shock, in order to determine if CBD was effective at blocking Δ9-THC-induced potentiation of fear memory. The infusion doses were chosen based on our maximally effective doses from each of the Δ9-THC and CBD experiments.

Our results (Figure. 11) reveal that co-administration of CBD is sufficient to block fear memory potentiation induced by Δ9-THC administration. These results are in line with recent evidence demonstrating that CBD is effective at antagonizing many of the psychopharmacological effects of Δ9-THC. In humans, pre-treatment with CBD has been shown to be effective in attenuating the appetitive (Morgan, Freeman, Schafer, & Curran, 2010), propsychotic (Bhattacharyya, Fusar-Poli, et al., 2009a) and anxiogenic (Englund et al., 2012; Zuardi, Shirakawa, Finkelfarb, & Karniol, 1982) effects induced by Δ9-THC administration. In animals, there is evidence that CBD can modulate the heterogenous reinforcing properties of Δ9-THC by attenuating both its aversive and rewarding effects (Vann et al., 2008).

Given the results from our electrophysiology experiments with CBD microinfusions, it seems likely that CBD antagonized the excitation of VTA DAergic neurons induced by Δ9-THC microinfusion (French et al., 1997), and therefore abolished its potentiation of a non-salient subthreshold stimulus.
3.5 Summary

This study presents evidence for the dissociable and opposite effects of \( \Delta 9 \)-THC and CBD on emotional memory acquisition. Our results support the diverse pharmacological properties of plant cannabinoids as well as their pharmacokinetic interactions, which have been established by recent research efforts, and findings presented from complimentary studies from our laboratory (see Chapter 2). Future studies should examine the electrophysiological changes induced by intra-NASH \( \Delta 9 \)-THC and CBD in emotional regulation structures (e.g., VTA, amygdala) during olfactory fear conditioning in anesthetized rats (as described in Laviolette, 2005), in order to more fully elucidate the neurophysiological mechanisms that underlie cannabinoid-mediated effects on emotional information processing.
Chapter 4. General Conclusions and Future Directions

The work presented in this thesis represents one of the first systematic evaluations of phytocannabinoid effects in discrete neural substrates during emotional learning paradigms and describes novel pharmacological mechanisms for their action. Specifically, the results from our CPP experiments are the first demonstration that CBD possess hedonic rewarding properties in the NASh, an effect that was dependent on 5-HT\textsubscript{1A}, 5-HT\textsubscript{1B} and DAergic signaling. Furthermore, intra-NASh CBD administration elicited a depression in VTA DA neural activity, a novel electrophysiological effect that suggests a potential alternative mechanism for CBD’s well-known antipsychotic properties, as well as its ability to oppose the effects of Δ9-THC.

The results from our olfactory fear conditioning experiments reveal that Δ9-THC can potentiate the formation of a subthreshold associative fear memory similar to the effect observed with synthetic CB1 receptor agonists. This effect was dependent on DAergic receptor signaling; supporting the observation that Δ9-THC elicits increased mesolimbic DA transmission. In the same paradigm, CBD was shown to be effective at blocking the formation of an aversive fear memory to a normally highly salient suprathreshold footshock in a manner similar to that observed with synthetic CB1 receptor antagonists. This effect was dependent on the 5-HT\textsubscript{1A}, but not the 5-HT\textsubscript{1B}, receptor signaling mechanism. These results suggest a functional dissociation between the neurophysiological mechanisms underlying aversive and appetitive memory formation as CBD’s rewarding properties were dependent on 5-HT\textsubscript{1B} receptor transmission in the CPP paradigm. These results also support emerging basic and clinical research suggesting the possible use of CBD as an efficacious pharmacotherapy in a number of psychiatric disorders such as schizophrenia, PTSD and addiction.

The results of these studies suggest many possible avenues for future investigation. Future studies should continue our electrophysiology experiments in order to characterize the neuroelectrophysiological nature underlying the switch in CBD’s motivational valence following 5-HT\textsubscript{1A} receptor antagonism. Extensions of our olfactory fear conditioning experiments should examine electrophysiological changes that may occur following intra-NASh Δ9-THC and CBD administration in other areas of the
mesocorticolimbic system including the BLA and the mPFC. Finally, although not presented in this thesis, preliminary work investigating Δ9-THC reward in the CPP paradigm has revealed a strong correlation between the location of Δ9-THC microinfusions along the rostral-caudal axis of the NASh and appetitive-aversive behavioural reactions to Δ9-THC administration. Follow-up studies should employ targeted microinfusions into the rostral and caudal NASh in order to further elucidate the nature of this hedonic gradient.
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