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Graduate Program in Anatomy and Cell Biology
A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy
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DOPAMINE MODULATION OF EMOTIONAL LEARNING IN THE
MEDIAL PREFRONTAL CORTEX

(Thesis format: Integrated Article)

By

Nicole Marie Lauzon

Graduate Program in Anatomy and Cell Biology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

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Abstract

Dopamine (DA) transmission plays a critical role in the processing of emotionally salient information and in associative learning and memory processes. Within the mammalian brain, neurons within the medial prefrontal cortex (mPFC) are involved critically in the encoding, expression, and extinction of emotionally salient learned information. Within the mPFC, DAergic transmission is involved importantly in controlling attention related and motivational processes, particularly within the context of emotionally salient sensory information. Considerable evidence suggests differential roles for DA D₁-like versus D₂-like receptors, including the D₄-receptor subtype, in the regulation of neuronal activity and emotional processing within the mPFC. Using behavioural models of emotional learning and memory in rats, including olfactory fear-conditioning and conditioned place preference assays, we compared the roles of DA D₁-receptor versus D₄-receptor activation during the encoding and recall phases of emotional learning and memory. We report that specific activation of DA D₄-receptors within the mPFC strongly potentiates the salience of normally nonsalient emotional associative fear memories and blocks the encoding of suprathreshold conditioned fear associations and has no effect on memory recall. In addition, the bidirectional effect demonstrated by D₄-receptor activation in the mPFC depends upon downstream signaling via CaMKII, cyclic-AMP/PKA, and PP1 substrates. In contrast, intra-mPFC D₁-receptor activation failed to increase the emotional salience of subthreshold fear stimuli but completely blocked the expression of previously learned aversive and rewarding memories. Interestingly, both intra-PLC

D₁-receptor mediated block of either fear-related or reward-related associative memories were dependent upon downstream cAMP signaling as both effects were rescued by co-administration of a cAMP inhibitor. Taken together these results demonstrate that DA D₄ versus D₁ subtype receptor transmission within the mPFC plays distinct functional roles in the processing of emotionally salient versus nonsalient associative information and differentially modulates the encoding versus recall phases of emotional memory within the mPFC through distinct molecular signaling pathways. A clearer understanding of the specific roles of DA D₁ and D₄ receptor transmission during emotional learning and memory may help elucidate how abnormalities in the mPFC neural circuitry may lead to aberrant associative learning and memory processes in disorders such as schizophrenia, drug addiction, PTSD and ADHD.

Keywords:

medial prefrontal cortex, dopamine, D₄-receptor, D₁-receptor, basolateral amygdala, ventral tegmental area, emotional learning, associative memory, fear conditioning, reward learning, post-traumatic stress disorder, schizophrenia, attention deficit hyperactivity disorder, drug addiction

Co-Authorship Statement

Chapter 1:

Entitled “Dopamine receptor modulation of cortical neuronal network activity and emotional processing: Implications for neuropsychiatric disorders” has been adapted from an article written by Nicole M. Lauzon with intellectual input by Dr. Steven R. Laviolette.

Chapter 2:

Entitled “Dopamine D₁ versus D₄ receptors differentially Modulate the encoding of salient versus nonsalient emotional information in the medial prefrontal cortex” was written by Nicole M. Lauzon with inputs from Stephanie F. Bishop and Dr. Steven R. Laviolette, experimental procedures and data analysis were preformed by Nicole M. Lauzon and Stephanie F. Bishop. Dr. Steven R. Laviolette provided intellectual input.

Chapter 3:

Entitled “Dopamine D₄ receptor transmission in the prefrontal cortex controls the salience of emotional memory via modulation of calcium calmodulin-dependent kinase II” was written by Nicole M. Lauzon with inputs from Tasha Ahmad and Dr. Steven R. Laviolette, experimental procedures and data analysis were preformed by Nicole M. Lauzon and Tasha Ahmad. Dr. Steven R. Laviolette provided intellectual input

Chapter 4:

Entitled “Supra-normal stimulation of dopamine D₁ receptors in the prelimbic cortex blocks spontaneous recall of both aversive and rewarding associative memories through a cyclic-AMP dependent signaling pathway” was written by Nicole M. Lauzon with inputs from Tasha Ahmad, Melanie Bechard and Dr. Steven R. Laviolette, experimental procedures and data analysis were performed by Nicole M. Lauzon, Tasha Ahmad and Melanie Bechard. Dr. Steven R. Laviolette provided intellectual input.

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List of Abbreviations

ADHD, attention deficit hyperactivity disorder
AC, adenylate cyclase
AIP, autocalmitide-2-related inhibitory peptide
ANOVA, analysis of variance
AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
BLA, basolateral amygdala
CaMKII, Ca^{2+} /calmodulin-dependent protein kinases II
cAMP, Cyclic adenosine monophosphate
CPP, conditioned place preference
CS+, positive conditioned stimulus, associated with footshock (US)
CS-, negative conditioned stimulus, not associated with footshock (US)
DA, Dopamine
GABA, gamma-Aminobutyric acid
GLUT, glutamine
NMDA, N-Methyl-D-aspartic acid
mPFC, medial prefrontal cortex
PKA, protein kinase A
PLC, prelimbic cortex
PP1, protein phosphatase 1
PTSD, post-traumatic stress disorder
US, unconditioned stimulus
VNTR, variable number tandem repeats
VTA, ventral tegmental area

Chapter 1: General Introduction

- 1 Dopamine receptor modulation of cortical neuronal network activity and emotional processing: Implications for neuropsychiatric disorders¹

¹ This chapter has been adapted from a published journal article: **Lauzon NM**, Laviolette SR. (2010) Dopamine D₄-receptor modulation of cortical neuronal network activity and emotional processing: Implications for neuropsychiatric disorders. *Behavioural Brain Research* 208, 12-22.

1.1 INTRODUCTION

The neural processing, encoding, and filtering of incoming sensory information are disturbed in a number of neuropsychiatric disorders. Common features underlying these deficits are the distortion of the emotional meaning and salience of sensory information, and perhaps more importantly, the ability to form adaptive associative memories between environmental stimuli and their appropriate motivational and emotional context. Thus, not only must sensory information be appropriately categorized and attended to according to emotional relevance and salience, we must be able also to accurately remember what these sensory cues mean, and associate them with appropriate behavioural and/or psychological responses, based on their emotional context. When these processes go awry, inappropriate assignment of emotional salience to normally unimportant sensory inputs may lead to misguided attention to these inputs; impulsive reactions to sensory cues; and in the extreme, psychotic ideation, delusional thinking and the persistence of maladaptive associative memories.

Given the complexity of these psychological processes, it is not surprising that a plethora of neurochemical systems and neuroanatomical substrates have been implicated as being critical for emotional processing and memory formation. A considerable body of evidence points to functional interactions between cortical and subcortical structures underlying the neuroplastic processes responsible for emotional processing, learning, and memory. Specifically, the neurotransmitter dopamine (DA) is well recognized as an essential modulator of these events. In the following chapter,

we will discuss evidence from both human and animal-based research demonstrating a role for functional interactions between the medial prefrontal cortex (mPFC) and associated subcortical inputs, the amygdala, and ventral tegmental area (VTA). We will examine how modulation of this neural circuitry, specifically through different DA receptor subtype populations, may be a critical regulator of emotional salience, associative memory encoding and neuronal plasticity events associated with normal and aberrant emotional processing and memory encoding.

The mPFC is well recognized as an important integrative centre for executive cognitive functioning and is crucial in the processing and filtering of emotionally relevant sensory information. Disturbances in the mPFC are evident in various neuropsychiatric conditions such as schizophrenia, addiction, ADHD, and post-traumatic stress disorder (PTSD) (Goldman-Rakic, 1994; Shin et al., 2006). In terms of emotional integration, the mPFC receives critical inputs from both the VTA and amygdala, and DA receptor transmission within the PFC can modulate the inputs from both of these regions (Rubinstein et al., 1997; Ceci et al., 1999; Yuen et al., 2009). The amygdala has been widely investigated for its role in normal emotional processing and has been implicated in a variety of psychiatric conditions. Specifically, the basolateral region of the amygdala (BLA) has been generally viewed as a sensory integration site, receiving inputs from the visual, auditory, somatosensory (including pain), and olfactory systems (LeDoux, 2007). Human studies have implicated the BLA-mPFC pathway in fear conditioning and extinction of fear memories (Phelps et al., 2004). Previous reports have demonstrated that functional

interactions between the BLA and mPFC in rodents are critical for encoding learned emotional associations at the level of the single neuron, and in behaving animals (Laviolette et al., 2005; Laviolette & Grace, 2006a; Rosenkranz & Grace, 2002; Rosenkranz et al., 2003). In terms of emotional processing and perception, structural and functional abnormalities in the BLA and mPFC regions have been reported in schizophrenic subjects (Lawrie et al., 2003; Crespo-Facarro et al., 2000; Taylor et al., 2002; Williams et al., 2004; Gur et al., 2002). Specifically, human imaging studies have found significant contribution of these areas to altered brain activity in patients with psychiatric conditions such as schizophrenia and PTSD (Herbener et al., 2007; Whitfield-Gabrieli et al., 2009; Gilboa et al., 2004; Shin et al., 2004).

Electrophysiological investigations have demonstrated a subpopulation of neurons within the mPFC is capable of encoding and expressing emotional conditioned associations via inputs from the BLA (Laviolette, 2005; Laviolette & Grace, 2006a, b). Using *in vivo*, intracellular recordings, Rosenkranz & Grace (2002) demonstrated that single neurons within the rodent BLA could encode emotional associative learning through a DA-dependent mechanism. At the neurochemical level, abnormalities in DAergic signaling within the mesocorticolimbic system have been implicated in the underlying pathology of aberrant emotional processing observed in numerous psychiatric conditions (Moore et al., 1999; Hall et al., 2004). Consequently, increasing evidence from clinical, genetic, behavioural, and electrophysiological investigations demonstrates critical and distinct roles for DA D₁-like versus D₂-like

receptors, including the D₄ receptor subtype, in the regulation of neuronal activity and emotional processing within the mPFC neural circuitry.

1.2 Dopamine Modulation of mPFC Neuronal Networks

Various anatomical mapping studies using human, rodent and primate models have localized the D₁, D₄ receptor subtypes to cortical, and subcortical neural regions involved in emotional processing, learning, and memory. In particular, several studies have reported relatively higher concentrations of the D₄-receptor subtype within the PFC, amygdala, hippocampus, thalamus, caudate putamen, and cerebellum, with relatively less distribution within the mid-brain and striatal regions (O'Malley et al., 1992; Meador et al., 1994; Primus et al., 1997; Ariano et al., 1997; Khan et al., 1998; Defagot et al., 1997) while the D₁ receptor has been localized within PFC, caudate, putamen, substantia nigra, nucleus accumbens and olfactory bulb (Goldman-Rakic et al., 1992; Farde et al., 1987; Beaulieu et al., 2011). Therefore, both human and rodent localization of D₁ and D₄ receptors strongly suggest a distribution within neural regions important in executive function and emotional regulation (Ariano et al., 1997; Khan et al., 1998; Defagot et al., 1997; O'Malley et al., 1992; Meador et al., 1994; Primus et al., 1997). The following chapter will review clinical, genetic, behavioural, and electrophysiological evidence for the specific roles of the DA D₁ and D₄ receptor subtypes in mPFC and the implications for cognitive and emotional processes.

1.2.1 Evidence linking the D₁-receptor to learning and memory

DAergic modulation of mPFC neuronal networks is known to control the encoding of working memory and the temporal sequencing of behavioural output (Goldman-Rakic, 1995; Goldman-Rakic et al., 1989; Seamans et al., 1998). Previous investigations have demonstrated that intra-mPFC activation of D₁ receptors impairs spatial working memory performance (Zahrt et al., 1997) and delayed response performance (Arnsten et al., 1994; Cai & Arnsten, 1997). In addition, Takahashi et al (2008 & 2012) have demonstrated that individual differences in D₁ receptor availability in the brain predicted differences in working memory, emotional reaction and decision making better than that of D₂ receptor availability. Current theories of mPFC DAergic function have suggested that D₁ receptor transmission may be critical for the preservation and expression of memories over temporal delay periods (Durstewitz & Seamans, 2008), although few studies have examined directly the role of mPFC D₁ receptor transmission during the expression of emotionally salient associative memories. Seamans et al. (1998) reported that blockade of mPFC D₁ receptors blocked the ability of rodents to use previously acquired spatial memory during a delayed performance radial arm task, while having no effect on non-delayed memory expression. These studies suggest that executive memory functions within mPFC neuronal networks may require an optimal level of DA D₁ signaling, specifically during periods of memory expression, rather than during associative memory encoding. Several rodent and primate studies have shown that too much or too little D₁ receptor stimulation impairs mPFC function, demonstrating an inverted U-shaped dose response curve (Arnsten et al., 1994; Sawaguchi and Goldman-Rakic,

1994; Williams and Goldman-Rakic, 1995; Zahrt et al., 1997; Goldman-Rakic, 2000; Williams & Castner, 2006; Cools & D'Esposito, 2011). Thus, while low level D₁ receptor stimulation tended to enhance neuronal spatial tuning during a spatial working memory task, high stimulation levels impaired performance both at the neuronal and behavioural levels. Similarly, Vijayraghavan et al. (2007) demonstrated that excessive D₁ receptor stimulation induces nonselective decrease of mPFC neural activity in a spatial working memory task. This is consistent with the finding that a D₁ receptor agonist increased interneuron excitability and the frequency of inhibitory postsynaptic current in amygdala projection neurons, as will be discussed in detail to follow (Kröner et al., 2005). Taken together, these investigations provide evidence of a crucial role of the DA D₁ receptor in adaptive learning and memory processes. Next, we will outline a potential role for DA D₁ receptor signaling in memory related neuropsychiatric disorders such as drug addiction, PTSD, and schizophrenia.

1.2.2 D₁ receptor: potential implications for memory-related neuropsychiatric disorders

Perturbations in DA transmission are well-established correlates related to both the rewarding and dependence producing properties of most drugs of abuse (Wise, 2004). D₁ receptor signaling may be critical for the expression of drug related memories. Previous research has suggested that the retrieval of morphine-related associative memories is more likely to occur in the presence of the morphine-conditioning cue during expression testing (Izquierdo & Dias, 1983; Darbandi et al., 2008). In addition, a human imaging study suggests that reduced D₁ receptor binding in the mPFC is

associated with an increased risk of relapse in cocaine addiction (Martinez et al., 2009). In the context of addiction-related memories, neurons within the mPFC both encode and express associative memories linked to the rewarding properties of opiates (Sun et al., 2011; Sun and Laviolette, 2012). Furthermore, activation of mPFC neuronal circuits is linked to various drug-seeking behaviours (Daglish et al., 2001; Luo et al., 2004; Koya et al., 2006; Langleben et al., 2008). The formation and persistence of these drug-related associative memories can trigger drug-seeking behaviours or relapse in response to drug-associated stimuli (de Wit and Stewart, 1981; Ehrman et al., 1992). Indeed, considerable evidence has linked D₁ receptor transmission to psychostimulant drug-related memory expression. For example, D₁ receptor activation has been shown to decrease cocaine-seeking behaviour in rats, whereas D₂ receptor activation triggers cocaine-seeking behaviour and relapse (Self et al., 1996). In addition, D₁ receptor activation has been reported to induce extinction-like behaviours in rats that have previously acquired cocaine-related self-administration behaviours, suggesting that stimulation of D₁ transmission may degrade previously acquired drug-related associative memories (Self et al., 2000).

The obtrusive and spontaneous expression of associative memories linked to either traumatic or highly rewarding experiences may be a critical factor underlying the pathological features of disorders such as PTSD or addiction. PTSD patients may associate a wide variety of sensory stimuli with intense fear (Rothbaum & Davis, 2003). While the specific role of D₁ signaling in the context of PTSD has not been extensively examined in clinical populations, abnormally low levels of cyclic

adenosine monophosphate (cAMP) signaling have been found to be a persistent correlate of PTSD in clinical populations (Kolb, 1989) leading to the suggestion that lowered cAMP signal transduction may serve as a clinical biomarker for PTSD (Lerer et al., 1987). DA D₁ receptor stimulation is critically linked to downstream regulation of cAMP (Senogles et al., 1988). Together with the well-established profound decreases in cAMP levels observed in PTSD clinical populations, the present results may have implications for understanding how DA D₁-mediated inhibition of cAMP levels may modulate spontaneous expression of traumatic associative memories through mPFC-dependent memory circuits. Indeed, considerable evidence points to neuropathological abnormalities within frontal cortical regions of PTSD patients as potential clinical correlates of PTSD-related memory disturbances and lack of control over traumatic memory expression (Tavanti et al., 2012 and Carrion et al., 2009).

Considerably less research has been completed on the implications of D₁ receptors in schizophrenia than D₂ receptors; however, evidence may suggest a role for D₁ receptors in the cognitive deficits experienced by schizophrenic patients. Abi-Dargham et al. (2002) discovered that an increase in D₁ receptors in the PFC was associated with working memory impairment in schizophrenia. An additional human imaging study by the same researcher found an increase in D₁ receptor binding in drug naïve schizophrenic patients, but not in patients who had previously been treated with antipsychotics (Abi-Dargham et al., 2012). This study suggests that the increase is due to schizophrenia itself and may be normalized during drug treatment of the illness. In addition, Golman-Rakic et al. (2004) found working memory deficits could

be ameliorated by treatments that increase D₁ receptor stimulation, indicating that this target presents a unique opportunity for the restoration of cognitive function in schizophrenia. Taken together, the above provide evidence for the crucial role of DA D₁ receptors in prefrontal cortex function, including working memory. In addition, D₁ receptor aberrant transmission implicating D₁ receptor transmission as a possible correlate of the memory related cognitive deficits seen in drug addiction, PTSD, and schizophrenia. These findings further implicate the PFC as a critical neural substrate, wherein transmission via the D₁ receptor may play a crucial during the expression of emotionally salient associative memories.

1.2.3 D₄ receptor: potential implications for schizophrenia

Since the advent of the atypical antipsychotic clozapine, and subsequent discovery of the D₄ receptor, a wide variety of clinical genetic linkage studies have examined possible correlations with D₄ receptor expression and neuropsychiatric disorders including schizophrenia, attention-deficit disorder (ADHD), addiction and depression. Although most research attention surrounding the DA theory of schizophrenia has concentrated on the D₂ receptor, there is increasing evidence to support an important role specifically for the DA D₄ receptor subtype in various neuropsychiatric disorders. Human postmortem analyses have conflicting results, with some reporting a significant increase in D₄ receptor binding in the putamen, nucleus accumbens, caudate nucleus and entorhinal cortex of patients with schizophrenia, relative to controls (Seeman et al., 1993; Murray et al., 1995; Sumiyoshi et al., 1995; Lahti et al., 1998). While others have failed to show a difference in D₄ binding levels in the

frontal cortex or striatal regions of patients with schizophrenia versus control subjects (Mulcrone & Kerwin, 1996; Reynolds & Mason, 1995).

The DA D₄ receptor displays highly polymorphic variations in the coding sequence in the human population (Van Tol et al., 1992). One polymorphism linked to schizophrenia is located in the third exon of the gene, which forms the third intracellular loop and may contribute to the binding characteristics of the receptor. The 48-base-pair element encoding a 16 amino acid chain within the third exon has a variable number of tandem repeats (VNTR) ranging from 2 to 11 in humans (Litcher et al., 1993; Ding et al., 2002). There is a significantly higher frequency of the long form of the D₄ allele in patients with schizophrenia relative to normal controls (Weiss et al., 1996; Lung et al., 2006). Aguirre et al. (2007) performed an association analysis of exon III and I polymorphisms of the D₄ receptor locus and found an excess of “rare” alleles (3-, 5-, 6- and 8–48 repeats) in a sample of psychotic Mexican patients. In addition, Nakajima et al. (2007) reported that upstream gene promoter variants appeared to confer susceptibility to schizophrenia in Japanese psychiatric patients. In contrast, several reports have suggested a lack of genetic linkage between the D₄ receptor and schizophrenia (Mitsuyasu et al., 2007; Lee et al., 2007; Ambrosio et al., 2004). Clearly, genetic linkage studies examining the potential etiological role of the D₄ receptor in clinical populations with schizophrenia are controversial at best. However, some recent evidence using physiogenomic analysis of D₄-related genes combined with functional magnetic resonance imaging (fMRI) have yielded some intriguing results. For example, Windemuth et al. (2008) reported

that abnormalities in fMRI responses during an auditory ‘oddball’ task in patients with schizophrenia were related to single nucleotide polymorphisms including the DA beta-hydroxylase gene and the D₄ receptor gene. Given the rather overwhelming variability of these studies, clearly further studies are required to accurately characterize the potential functional role of D₄ receptor gene expression in the symptomology and/or etiological course of schizophrenia.

1.2.4 The role of the D₄ receptor in impulsivity and novelty seeking: implications for ADHD

Increasing evidence from both human and animal-based research links disturbances in D₄ receptor transmission to psychiatric disorders associated with impulsivity, most notably, attention-deficit hyperactivity disorder (ADHD). Although ADHD is a complex syndrome, cardinal features of the disorder are characterized by inappropriate attention or lack thereof, hyperactivity, and impulsivity (APA DSM IV, 2000). Brain imaging studies have shown that subjects with ADHD have a characteristically smaller brain volume (Rapoport et al., 2001) specifically, within the bilateral mPFC and anterior temporal cortices (Sowell et al., 2003), both regions implicated in the processing of emotionally salient information (Laviolette et al., 2007; Laviolette & Grace, 2006a). It has also been shown that in adults with ADHD there is a decrease in DOPA decarboxylase activity in the mPFC, which is responsible for converting the precursor of DA into functional DA (Ernst et al., 1998). Increased levels of DA transporter expression have been reported in patients with ADHD (Dougherty et al., 1999), further implicating a role for disturbed DA transmission as

an underlying factor in this disorder. In line with this, considerable evidence now links dysregulated expression and signaling of the DA D₄ receptor in the pathophysiology of ADHD (Leung et al., 2005).

Psychological measures of novelty seeking have been conceptually linked to impulsive behavioural traits and several studies have linked the D₄ receptor to measures of novelty seeking. One polymorphism in particular that has been investigated for possible association with novelty seeking traits is the 5' region -521C/T polymorphism, which reduces D₄ receptor transcriptional efficiency (Okuyama et al., 2000). The C-521T polymorphism has also been associated with approach-related behaviours such as extroversion, sensation seeking, impulsivity, and novelty seeking (Okuyama et al., 2000; Mitsuyasu et al., 2001; Ronai et al., 2004; Golimbet et al., 2007). In two high impact back-to-back publications, the long allele of the D₄ exon III polymorphism was correlated with a significant increase in the frequency of novelty seeking as a personality trait (Benjamin et al., 1996; Ebstein et al., 1996). Although some subsequent studies have also found an association between personality traits and the length of the VNTR (Nobel et al., 1998; Strobel et al., 1999; Tomitaka et al., 1999; Lee et al., 2003; Lynn et al., 2005; Kim et al., 2006; Serretti et al., 2001), others have failed to find such associations (Jonsson et al., 1997; Hill et al., 1999; Persson et al., 2000; Schinka et al., 2003; Munafo et al., 2008). Thus, similar to genetic linkage studies examining correlations between D₄-receptor expression and schizophrenia, there is considerable controversy concerning the potential role of the D₄-receptor gene in the etiology of ADHD.

Various animal models have similarly reported functional roles for the D₄ receptor in novelty/impulsive behavioural phenomena. Duwala et al. (1999) reported that mice with genetic deletion of the D₄ receptor demonstrated decreased exploration and approach towards novel stimuli. However, a subsequent report using this genetic knockout model reported no effects of D₄-receptor deletion on other impulsivity assays such as 'Go/No-Go' and measures of response inhibition (Helms et al., 2008). Nevertheless, Bailey et al. (2007) used a non-human primate model (the vervet monkey) to correlate polymorphisms in the D₄-receptor gene with novel object approach assays and found that variants in D₄ receptor expression could account for a significant portion of the total variance in novelty seeking measures. Thus, although controversial, evidence at the genetic and behavioural levels of analysis suggests a link between variations in D₄ receptor expression, novelty/impulsivity and the presence of behavioural/psychological traits associated with ADHD.

1.2.5 Evidence linking the D₄ receptor to addictive behaviours

Despite the complexity of addiction in terms of underlying psychological variables and neuropathological correlates, it is reasonable to suggest that, similar to other neuropsychiatric disorders such as schizophrenia and ADHD, an important feature common to addictive behaviours are disturbances in emotional processing, learning, memory and impulsive behaviours. Similar to human and animal investigations examining schizophrenia and ADHD-related phenomena, increasing evidence points to a role for the D₄ receptor as an underlying factor related to addiction. Using a genetic knockout mouse model, Rubinstein et al. (2001) reported that genetic deletion

of the D₄ receptor led to a behavioural phenotype comprising locomotor supersensitivity to several drugs of abuse, including ethanol, cocaine, and methamphetamine. Genetic deletion of the D₄ receptor gene was also reported to potentiate the discriminative effects of cocaine (Ktaz et al., 2003), as well as a decreased reward sensitivity to methamphetamine and amphetamine (Thanos et al., 2009). In addition to these animal models of reward and addiction, a number of clinical studies using human patient populations have suggested a link between D₄ polymorphisms and the occurrence of opiate and/or alcohol abuse. For example, two early studies revealed alleles of the D₄ receptor exon III VNTR, as being positively correlated with heroin abuse in both Chinese psychiatric patients (Li et al., 1997) and in heroin addicted subjects in Israel (Kotler et al., 1997). However, a later report from Franke et al. (2000) failed to observe any differences in this D₄ receptor polymorphism in samples of heroin-dependent addicts relative to controls in German population samples. A more recent study reported that significantly greater cue-induced craving for heroin was observed in heroin-dependent Chinese subjects carrying the D₄ VNTR long-type allele, relative to non-dependent controls (Shao et al., 2006). This finding is particularly interesting given the well established role for DA transmission in drug-related memory recall (Wise, 2004; Goldstein & Volkow, 2002) and evidence demonstrating that D₄-receptor transmission within the PFC is essential for the encoding of emotionally salient associative information (Laviolette et al., 2005).

D₄ receptor polymorphisms have also been associated with alcoholism. Genetic linkage studies performed by Hill et al. (1999) found evidence of modest linkage between polymorphisms near the D₂ and D₄ receptor genes between measures of physical dependence and early onset of alcoholism in families with histories of chronic alcoholism. In addition, Hutchison et al. (2002) revealed that subjects with polymorphisms in the D₄ receptor long variant, displayed higher levels of alcohol craving following consumption of alcoholic beverages. In terms of interactions between psychometric measures of novelty seeking and co-morbidity with alcoholism, Sander et al. (1997) reported no significant correlation between expression of the D₄ receptor exon III and measures of novelty seeking amongst alcoholic subjects. However, a subsequent study by the same group found a significantly higher prevalence of the 7-repeat allele alcoholic individuals compared to the control sample (Franke et al., 2000). Furthermore, a study by Ray et al. (2009) reported a significant direct path between the D₄ receptor VNTR genotype and alcohol abuse, with novelty seeking traits as the qualifying mediator between variables. Thus, similar to previously described linkage studies between D₄ receptor polymorphisms and the occurrence of schizophrenia and ADHD, further studies are required to elucidate the potential role of D₄ receptor expression abnormalities in addiction related phenomena. Given the predominant localization of the D₄ receptor within PFC circuits that are essential to executive control and response inhibition, these studies collectively, suggest an important role for D₄ receptor transmission within this cortical circuitry for the processing and filtering of emotionally salient

sensory information and in the control of appropriate behavioural responding to such inputs.

1.2.6 Dopamine D₄ receptor transmission modulates emotional memory encoding and learning in cortical and subcortical circuits

Early behavioural studies using D₄ receptor knockout mice demonstrated motivational/emotional abnormalities such as decreased expression of behavioural inhibition when tested in open field tests or the elevated plus maze, and enhanced reactivity in response to unconditioned (but not conditioned) fear stimuli (Avale et al., 2004; Falzone et al., 2002). In addition to rodent behavioural studies employing genetic knockout techniques, several studies have used more traditional behavioural pharmacological approaches to examine the role of D₄ receptor signaling within the PFC during neuronal encoding of emotional associative learning and memory. Using a cognitive set-shifting task, Floresco et al. (2006) reported that intra-PFC microinfusions of a D₄ receptor agonist impaired, whereas microinfusions of a D₄ receptor antagonist improved behavioural measures of set shifting in rats. Their findings suggested that executive cognitive flexibility requires an optimal level of D₄ receptor activation within the PFC. Laviolette et al. (2005), using single-unit extracellular recordings, isolated single neurons within the rat PFC that responded to electrical stimulation of the BLA. Rats were then given an olfactory fear conditioning procedure wherein specific olfactory cues (CS) were paired with footshock (US). Systemic pre-treatment with a competitive antagonist of the D₄ receptor, L-741,741 completely blocked the ability of PFC neurons to associatively encode olfactory cues

paired with footshock, suggesting that D₄ receptor transmission within the PFC is essential for the encoding of emotionally salient memories. Furthermore, direct microinfusions of the same D₄ antagonist into the PFC prevented also the encoding and behavioural expression of associative olfactory fear learning, while having no effect on the behavioural or neuronal sensitivity to footshock presentation, in and of itself. One report using the atypical antipsychotic drug clozapine showed a dose-dependent attenuation in the acquisition of behavioural response to fear conditioning at significantly lower doses than those required for typical antipsychotics (Inoue et al., 1996). This inhibition of conditioned fear response was significantly correlated with K_i values for the DA D₄ receptors, but not with other acetylcholine or monoamine receptors. This study demonstrated also that clozapine did not affect the expression phase of fear conditioning. These results further suggest an important role specifically for the D₄ receptor, in the mediation of clozapine's effects on emotional processing and memory encoding. Taken together with previously noted studies implicating abnormal expression patterns for the D₄ receptor as a possible correlate of emotional processing disorders like schizophrenia, ADHD and depression, these findings further implicate the mPFC as a critical neural substrate wherein transmission via the D₄ receptor may control not only the emotional amplitude of incoming sensory information, but may be crucial for the normal associative encoding of emotionally salient versus non-salient sensory information with their related environmental cues.

Thus far, we have presented electrophysiological, clinical, genetic, and behavioural evidence that the mPFC is a critical area involved in normal cognitive functioning, and that disruptions involving DA receptors modulation within the mPFC have been implicated in several psychological disorders. In addition, the regulation of pyramidal neuron activity by the DA receptors is considered a fundamental aspect of associative learning and memory processes (Goldman-Rakic 1995; Seamans et al., 2004; Sobotka et al. 2005; Nowak et al. 2012). We will now examine evidence demonstrating different roles for the DA D₁ and D₄ receptors in the regulations of neuronal activity within the mPFC circuitry.

1.3 D₁ and D₄ receptor transmission within mPFC

Projections from the BLA and DA input from the VTA in the mPFC, forming a neural circuit important in executive function and emotional regulation. However, the role that DA plays in modulating neurotransmission and connectivity in the BLA-mPFC pathway is relatively unknown. Consistently, both human and rodent receptor mapping studies report high expression levels of the D₁ and D₄ receptors in the mPFC, strongly suggesting a localized distribution to modulate neuronal activity within this neural circuitry (Ariano et al., 1997; Khan et al., 1998; Defagot et al., 1997; O'Malley et al., 1992; Meador et al., 1994; Primus et al., 1997). The mPFC comprises a heterogeneous population consisting of various neuronal types, including intrinsic interneurons and pyramidal output neurons. Interestingly, several post-mortem schizophrenic pathology studies have found significantly decreased numbers of GABA-containing interneurons within mPFC regions (Tooney & Chahl, 2004;

Chance et al., 2005; Mirnics et al., 2000). Because these inhibitory interneurons normally provide tonic inhibitory regulation of the pyramidal output neurons within the cortex, such disturbances are believed to represent abnormal neuronal regulation within the cortex and may be related to the emotional processing and cognitive disturbances observed in schizophrenia (Whitford et al., 2005). A considerable amount of research using electrophysiological recordings *in vitro* and *in vivo*, demonstrates different roles for DA D₁ and D₄ receptor subtypes in modulating neuronal network dynamics within the PFC. For example, using *in vivo* extracellular recordings within the rat BLA-PFC pathway, Floresco and Tse (2007) reported that the ability of BLA stimulation to inhibit pyramidal neuron activity in the PFC was dependent upon both D₁ and D₂/D₄ receptor activities as pharmacological over-activation of both receptor subtypes, and led to an attenuation of BLA-evoked suppression of spontaneous neuronal activity within the PFC neurons receiving input from BLA neurons. This study reported an important dissociation between D₁-receptor and D₂/D₄ receptor-mediated modulation of BLA inputs to the PFC: D₁-receptor activation blocked the excitatory effects of BLA stimulation on spontaneous PFC neuronal activity while D₂ or D₄ receptor activation blocked the ability of BLA stimulation to inhibit spontaneous activity within the PFC. This dynamic activity is consistent with the proposed functional arrangement of D₄ versus D₁-receptor localization on pyramidal versus interneuron neuronal populations within the PFC with D₁ receptor activation increasing the intrinsic excitability of inhibitory gamma-aminobutyric acid (GABA)ergic interneurons and D₂-like receptor activation

decreasing the activity of these interneurons, presumably leading to decreased probability of tonic inhibitory GABA release within the mPFC (Seamans et al., 2001).

Indeed, at the cellular level, mPFC D₄-receptor mapping reveals the presence of D₄ receptors on both pyramidal and non-pyramidal interneurons, within the mPFC. Interestingly, Mrzljak et al. (1996) reported a preferential distribution of D₄ receptors on populations of intrinsic GABAergic interneurons within several brain regions, including the mPFC, hippocampus, and various thalamic nuclei. Similarly, D₁ receptors have been localized to both pyramidal neurons and interneuron populations within the mPFC, with a majority of studies demonstrating a greater proportion of the D₁ receptors on GABA containing inhibitory interneurons, compared to pyramidal neuron populations (Vincent et al., 1993; Sesack and Bunney, 1989; Bergson et al., 1995; Yang and Seamans, 1996; Muly III et al., 1998; Gorelova et al., 2002; Gao and Goldman-Rakic, 2003; Santana et al., 2009). Therefore, DA D₁ and D₄ receptors are functionally located to exert both direct and indirect modulation of local neuronal network activity, including inhibitory interneurons to alter excitability of pyramidal output neurons (Gao & Goldman-Rakic 2003). Therefore, our aim is to determine the mechanism underlying the DA D₁ and D₄ mediated differential control of mPFC pyramidal neuron activity in modulating emotional associative learning and memory processes. Functionally, DA transmission within the mPFC influences neuronal network activity in a biphasic manner, with D₂-like receptor activation decreasing the activity of GABAergic interneurons, and D₁ receptor activation increasing the intrinsic excitability of these inhibitory interneurons (Seamans et al., 2001).

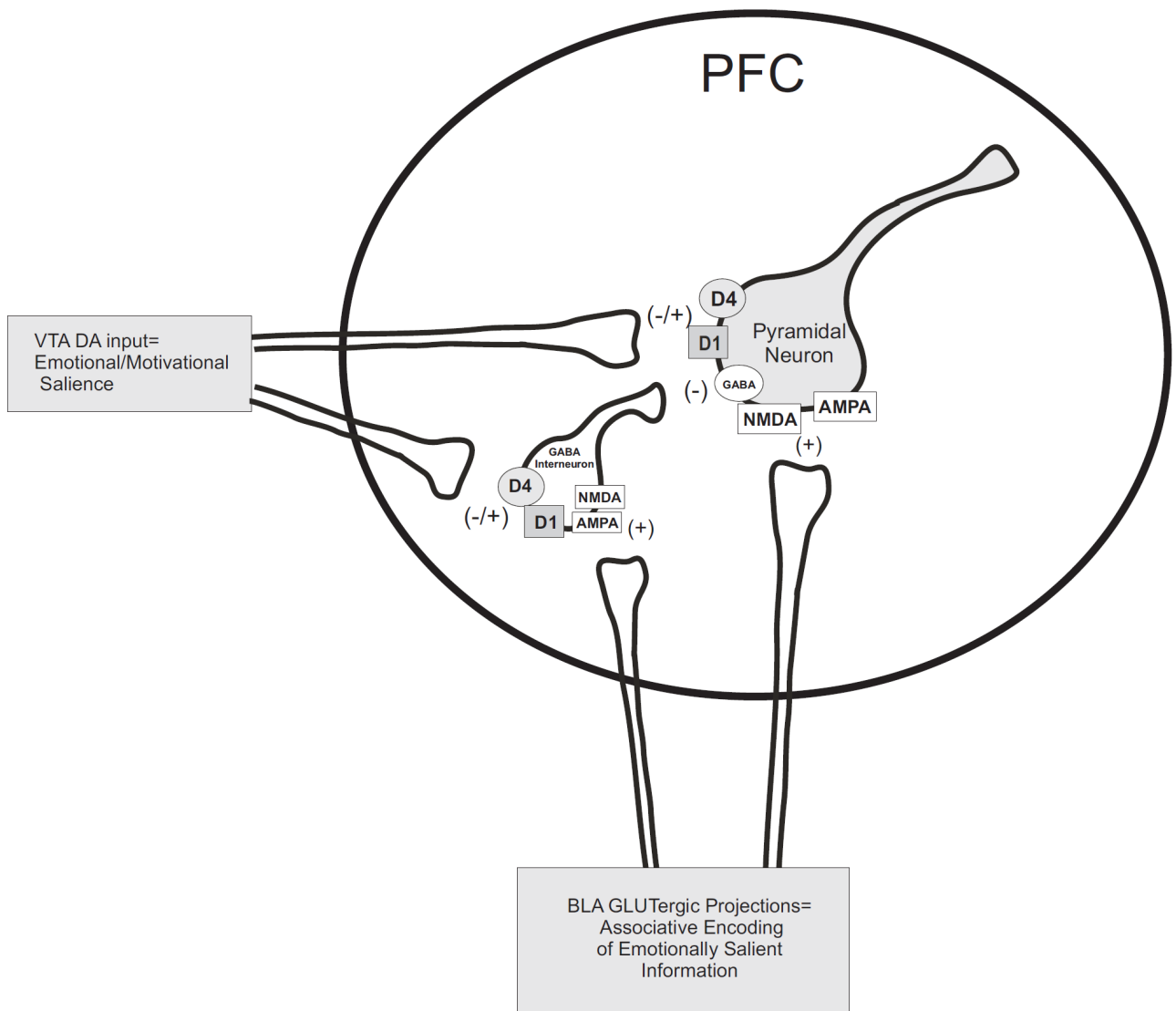
Specifically, activation of D_1 receptors increases the activity of adenylate cyclase, in turn increasing intracellular levels of cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA; Keabian & Calne, 1979). Figure 1.1 presents a simplified schematic showing this functional arrangement, whereby activation of DA receptors on inhibitory interneuron populations within mPFC may in turn increase or decrease inhibition of pyramidal output neurons from the mPFC, via network interactions with both VTA and BLA inputs converging upon mPFC pyramidal and GABAergic interneuron populations (Fig. 1.1).

1.3.1 D_1 receptor specific modulation of neuronal activity in mPFC

Within mPFC neuronal circuits, electrophysiological evidence demonstrates that D_1 activation increases both cAMP and PKA levels, resulting in phosphorylation of K^+ channels to decrease conductance, and cause increased excitability of interneurons and increased GABA release onto the postsynaptic pyramidal cell leading to feedforward inhibition of mPFC pyramidal output neurons (Starr, 1987; Keabian & Calne, 1979; Laviolette & Grace 2006b; Seamans et al., 2001). Similarly, Kroner et al., (2005) found that a D_1 receptor agonist increased interneuron excitability and the frequency of inhibitory postsynaptic current in mPFC pyramidal neurons projecting to the BLA. In addition, Trantham-Davidson et al. (2004) found that D_1 antagonists and protein kinase A (PKA) inhibitors blocked the enhancement of GABA inhibitory currents, and that this was dependent upon the activation of the D_1 -cAMP/PKA signaling pathway.

Figure 1.1 A simplified schematic illustration demonstrating some of the known functional connections between the VTA, BLA and PFC.

The VTA provides DA input to the PFC via the mesocorticolimbic pathway. Given the importance of the VTA in emotional and motivational information processing, one possibility is that these DA inputs provide emotional and/or motivational salience information to target neurons within the PFC. Glutamatergic inputs from the BLA to the PFC are importantly involved in transmitting emotionally salient associative encoding information to target neurons within the PFC. For simplicity, we show only ascending PFC inputs from the VTA and BLA, respectively; however, it is important to note that PFC neuronal populations send functionally important descending projections to both the VTA and BLA which may in turn regulate recurrent inputs from these regions back up to the PFC. While D₁ and D₄ receptors are found on both interneurons and pyramidal neuron populations, considerable evidence suggests a functional localization on intrinsic, inhibitory interneurons within the PFC. Thus, activation of DA D₄ receptors through VTA > PFC inputs can inhibit or disinhibit feed-forward inhibitory influences on pyramidal output neurons, which may amplify or attenuate the emotional salience or associative memory magnitude of inputs from either the VTA or BLA that form integrative networks with pyramidal neurons.



Furthermore, Vijayraghavan et al., (2007) found that moderate D_1 receptor stimulation has been shown to spatially ‘tune’ PFC neurons that process target signals by preferentially suppressing non-target (noisy) neural activities, whereas excessive D_1 receptor stimulation induces nonselective suppression of PFC neural activities, irrespective of whether the neural activities are task related or not. They also demonstrated that co-administration of a cAMP activator can rescue the cognitive impairment induced by intra-mPFC D_1 receptor overstimulation in primates performing a delayed spatial memory task, further demonstrating the important functional link between intra-cortical D_1 receptor transmission and cognitive processing (Vijayraghavan et al., 2007). Given that neuronal populations within the mPFC are active during associative memory recall processes linked to both aversive and rewarding events, increasing cAMP/PKA signaling within the mPFC via D_1 receptor activation may lead to increased feedforward inhibition on mPFC pyramidal neurons, thereby impairing the expression of olfactory fear associations or opiate reward associations (Starr, 1987; Seamans et al., 2001; Laviolette & Grace, 2006b). Furthermore, evidence suggests that D_1 receptor stimulation had a suppressive effect on the PFC neural activities involved in a spatial working memory task. Taken together, we propose that D_1 receptor activation exerts its effects on mPFC pyramidal output neurons through a modulation of intrinsic inhibitory GABA containing interneurons, and that this is dependent upon a cAMP/PKA signaling pathway (Fig. 1.2).

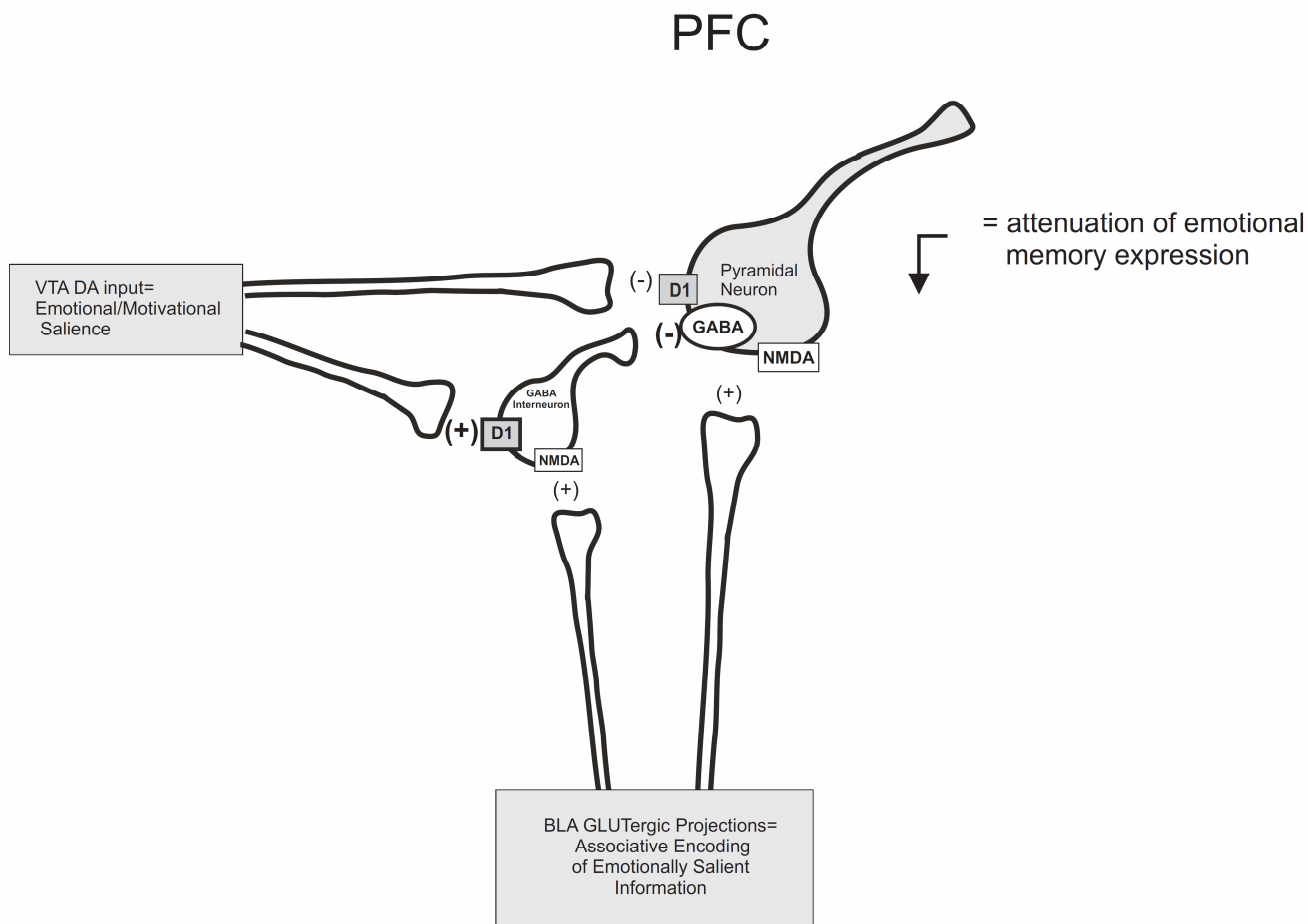


Figure 1.2 An alternative framework for PFC D₁-receptor-mediated modulation of emotional processing and memory expression based on molecular evidence demonstrating a role for PFC D₁ signaling in the control of GABAergic containing inhibitory interneurons.

A simplified hypothetical model for the relationship of D₁ receptor stimulation and expression of associative memories. D₁ receptor activation preferentially enhances glutamatergic (GLUT) inputs to GABAergic interneurons leading to a reduction in pyramidal cell activity via a GABA mediated increase of inhibitory post-synaptic currents (IPSC). Increased GABA interneuron activity would result in a feed forward inhibitions and therefore a decrease in pyramidal neuron output activity.

In this hypothetical model, we present a simplified schematic demonstrating how D₁-receptor-mediated modulation of inhibitory GABA transmission in the PFC may control the expression of emotional information. We have excluded the potential roles of D₁-receptor-mediated modulation of NMDA receptors and/or AMPA-receptor substrates associated with PFC interneurons. Although these mechanisms may be important, there is insufficient previous research to demonstrate a specific role during emotional learning and memory. Therefore, we propose that during the recall of emotionally salient memories PFC neuronal activity in emotional memory encoding networks would be susceptible to disruptions in D₁-receptor signaling (as may be present in disorders such as addiction or PTSD), which in turn could increase cAMP and PKA substrates within GABAergic interneurons, leading to an increase in activity. This could theoretically lead to an increased magnitude of inhibitory modulation of pyramidal output neurons and thus, a resultant block of emotionally associative memory recall. Interestingly, in the context of reward-related learning and memory, previous evidence has demonstrated that cAMP/PKA stimulation or inhibition directly within the nucleus accumbens can disrupt the acquisition of reward-related memory, specifically, the acquisition of amphetamine-conditioned CPP (Beninger et al., 2003). Thus, cAMP/PKA signaling may be critical not only for the expression of drug reward-related memories, but may be necessary for the acquisition of these memories as well.

1.3.2 D₄ receptor specific modulation of neuronal activity in mPFC

In addition, considerable evidence using molecular approaches demonstrates an important role for the D₄ receptor in the modulation and regulation of various downstream signaling molecules and/or functionally related receptor systems that are critical for plasticity related to learning and memory. For example, using an *in vitro* neuronal dissociation procedure, Wang et al. (2003) examined the effects of D₄-receptor transmission in isolated pyramidal neurons from PFC tissue in rats. They reported that application of a D₄ receptor agonist could strongly decrease NMDA receptor currents and induced a reduction of the amplitude of evoked NMDA excitatory post-synaptic potentials in PFC slices. Furthermore, activation of D₄ receptors reduced the membrane surface expression of NMDA receptors and induced internalization of NMDA receptors through a CaMKII-dependent mechanism. Given the well established role of NMDA receptor plasticity in various forms of neuronal synaptic plasticity related to learning and memory, these results demonstrated for the first time an important molecular link between PFC D₄ receptors and associated NMDA receptor complexes, providing a potential mechanism by which intra-PFC D₄-receptor transmission may modulate learning and memory processing (Yashiro et al., 2008). In addition, the reported ability of D₄ receptor activation to decrease NMDA receptor activity within the PFC are particularly interesting given the known psychotomimetic effects of NMDA receptor antagonist drugs, which have been proposed as a model of schizophrenia-related psychosis (Javitt et al., 1991; Jenstsch et al., 1998).

Indeed, the ability of D₄-receptor activation to produce NMDA receptor hypofunction within the PFC, further suggests a potential mechanism by which abnormalities in D₄-receptor transmission may be related to schizophrenia-like psychosis. *In vitro* evidence demonstrating a reduction in NMDA receptor expression in PFC pyramidal neurons following D₄ receptor activation (Wang et al., 2003) may suggest that D₄-receptor-mediated modulation of GLUT substrates within the PFC may act specifically via NMDA receptor substrates during the encoding of highly salient emotional associative information. However, D₄ mediated reduction in NMDA receptor expression may not play a role in the modulation of emotional salience magnitude, since D₄ receptor activation potentiates normally sub-threshold conditioning stimuli, but blocks the encoding of supra-threshold associative emotional memory. The question is what molecular mechanisms may underlie the ability of D₄-receptor activation within the PFC to potentiate the emotional salience of normally sub-threshold fear conditioning stimuli? More recent molecular evidence, to be described presently, linking D₄-receptor activation with bidirectional modulation of α -Ca²⁺/calmodulin-dependent protein kinase II (α -CaMKII), a molecule involved in synaptic plasticity, learning and memory (Malenka et al., 1999; Frankland et al., 2001). These findings illustrate D₄ receptor modulation of post-synaptic AMPA receptors localized on inhibitory PFC interneurons and pyramidal neurons (Yuen et al., 2009; Gu and Yan, 2004; Gu et al., 2006) may provide a compelling mechanism to account for D₄-receptor-mediated modulation of emotional salience and memory encoding.

Previous molecular studies performed in PFC slice preparations characterized an interesting bidirectional relationship between D₄-receptor activation during high or low neuronal activity levels, and the resulting effect on CaMKII activity. Thus, Gu and Yan (2004) reported that *in vitro* slice preparations of rodent mPFC, D₄-receptor activation strongly increases α -CaMKII activity through the stimulation of the phospholipase-C pathway and subsequent elevation of intracellular Ca²⁺ through inositol-1,4,5,-triphosphate receptors. Interestingly, this stimulatory effect of D₄-receptor activation on intracellular levels of CaMKII occurred only during low baseline levels of PFC neuronal activity. In contrast, during high levels of neuronal activity, D₄ agonist application causes a marked decrease in α -CaMKII levels, an effect that was mediated through a protein kinase A, protein-phosphatase-1 dependent mechanism, demonstrating that D₄ receptor signaling within the PFC bidirectionally modulates α -CaMKII activity as a function of baseline mPFC neuronal activity levels. Interestingly, in a follow-up study, Gu et al. (2006) reported that concomitant with D₄-receptor activation induced upregulation of CaMKII, there was an increase in post-synaptic AMPA-receptor-mediated excitatory currents and increased surface expression of the GluR1 subunit of the AMPA receptor complex, within cultured PFC pyramidal neurons. These results are particularly interesting in the context of a more recent report, discussed previously, demonstrating that D₄-receptor activation can specifically decrease AMPA-receptor expression associated with GABAergic interneurons within the PFC (Yuen et al., 2009). Indeed, these differential effects of D₄-receptor activation upon PFC pyramidal neuron populations (increased AMPA-receptor surface expression) versus inhibitory interneurons (decreased AMPA-

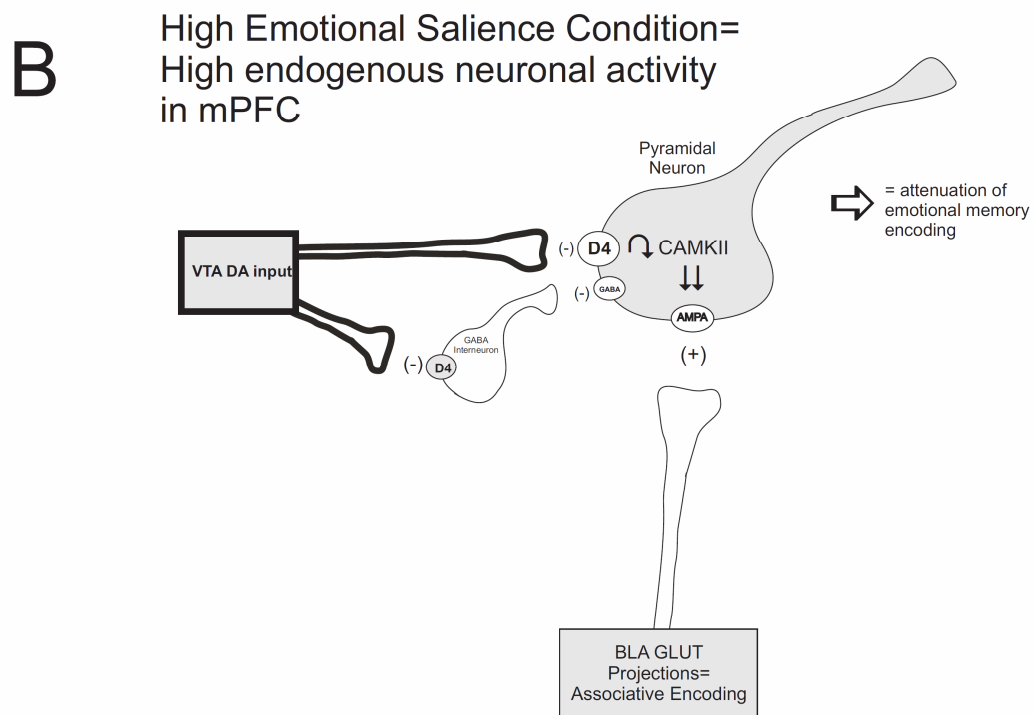
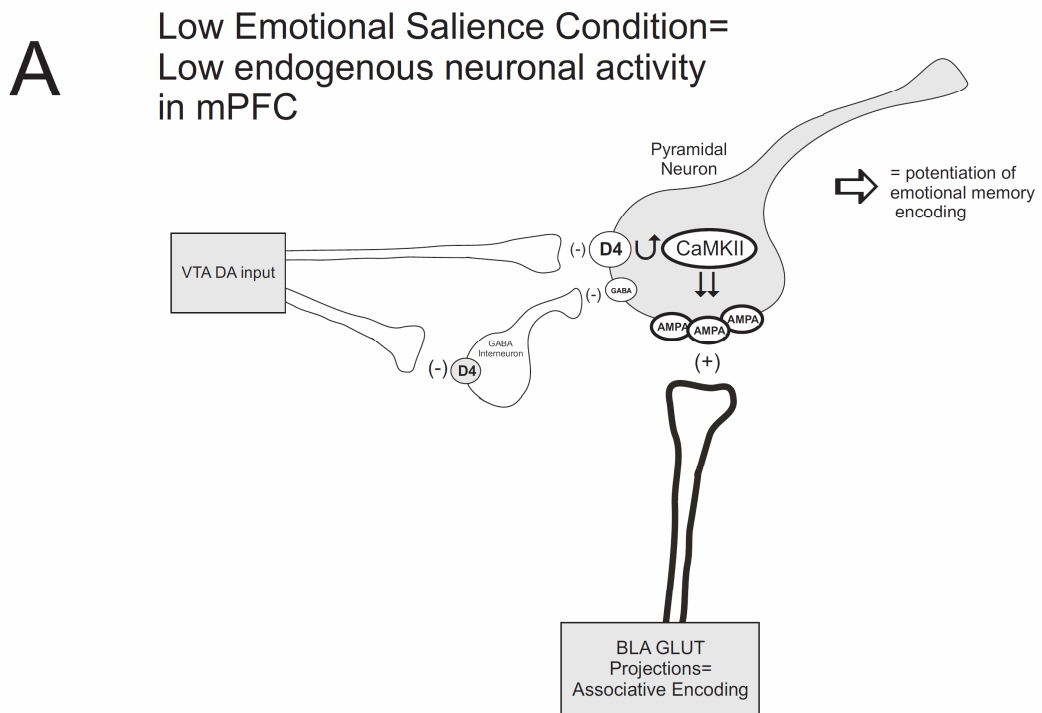
receptor surface expression) provides a compelling mechanism whereby D₄-receptor activation may shift neuronal network activity within the PFC towards decreased inhibitory influence and a concomitant potentiation of pyramidal neuron activity. Given the well established role of AMPA receptor transmission in various forms of synaptic plasticity related to learning and memory (Barria et al., 1997), such a functional interaction between D₄ receptor transmission, CaMKII activity levels, and modulation of AMPA-receptor substrates localized to PFC pyramidal neurons suggests an interesting framework within which convergent inputs from DA terminals in the VTA and glutamatergic inputs from the BLA, may converge upon PFC neurons to control the encoding of emotionally salient learning and memory.

In Figure 1.3, we present a simplified schematic demonstrating how D₄-receptor-mediated modulation of CaMKII levels and AMPA-receptor transmission in the PFC may bidirectionally control the emotional salience of sensory information as a function of baseline activity levels of PFC neuronal populations. For the sake of simplicity, we have excluded the potential roles of D₄-receptor-mediated modulation of NMDA receptors (Wang et al., 2003) and/or AMPA receptor substrates associated with PFC interneurons (Yuen et al., 2009), although these mechanisms, described previously, are equally compelling candidates. In this hypothetical model, we propose that during low levels of PFC activity (as would be predicted during the experience of normally non-emotionally salient sensory events), neuronal emotional memory encoding networks would be susceptible to disruptions in D₄ receptor signaling (as may be present in disorders such as schizophrenia or ADHD), which in turn could

alter downstream regulation of CaMKII levels and/or the expression of AMPA receptor substrates within PFC pyramidal neurons. This could theoretically lead to a potentiation in the emotional salience and associative memory magnitude of inputs converging on PFC pyramidal neuron populations, as the relative magnitude of GLUT inputs from the BLA converging upon GLUT receptor substrates on PFC pyramidal neurons may be amplified by the effects of D₄-receptor-mediated increases in CaMKII levels and the resulting increased expression of AMPA receptors on this neuronal population (Gu et al., 2006). This may lead to the formation of a maladaptive associative memory between that stimulus and its distorted emotional significance, as suggested by behavioural fear conditioning studies (Shah et al., 2004). In contrast, during high levels of PFC baseline activity (as may be predicted during a highly salient emotional experience), DAergic inputs from the VTA, and GLUT inputs from the BLA would be increased. During high neuronal activity baseline, D₄-receptor activation would be predicted to inhibit feedforward GABAergic inhibition on PFC pyramidal neurons, whereas D₄ receptor activation on pyramidal neurons themselves would lead to a decrease in CaMKII signaling, and resulting decrease in surface expression of AMPA receptors on the PFC pyramidal neurons. Such a mechanism may lead to the blockade of appropriate associative memory formation, as suggested by behavioural and electrophysiological evidence in rodents (Laviollette et al., 2005; Floresco et al., 2007; Shah et al., 2004).

Figure 1.3 An alternative framework for PFC D₄-receptor-mediated modulation of emotional processing based on molecular evidence demonstrating a role for PFC D₄ signaling in the control of CaMKII and AMPA-receptor function in PFC pyramidal neurons.

In this hypothetical model, levels of CaMKII and AMPA-receptor expression levels in PFC pyramidal neurons are controlled by D₄-receptor activation via VTA inputs. However, the effects of D₄-receptor-mediated signaling on intra-neuronal CaMKII and AMPA-receptor modulation depends upon the existing baseline level of PFC neuronal activity: (A) in the presence of sensory input containing low levels of emotional salience, D₄-receptor activation increases CaMKII levels, which in turn increases surface expression of AMPA receptors localized on PFC pyramidal neurons (Gu & Yan, 2004; Gu et al., 2006) theoretically potentiating the associative memory encoding of normally emotionally sub-threshold sensory inputs by amplifying the emotional memory encoding effects of DA and GLUT inputs from the VTA and BLA onto specific subpopulations of PFC neurons, as suggested by behavioural and molecular evidence (Gu et al., 2006). (B) In contrast, in the presence of sensory input containing high levels of emotional salience, D₄-receptor activation decreases CaMKII activity levels and reduces the expression of AMPA-receptor substrates linked to associative GLUTergic inputs from the BLA, thereby attenuating the encoding of emotionally salient associative memory by decreasing the impact of GLUT and DA inputs from the BLA and VTA, respectively, again suggested by extant behavioural and molecular evidence (Gu et al., 2006). In either case, dysregulation of endogenous D₄-receptor transmission within the PFC would be predicted to distort the emotional meaning and associative memory encoding of incoming sensory information, by either pathological amplification or dampening of the emotional meaning of this input.



The apparent functional arrangement between D₄ receptor mediated control of CaMKII within the PFC could account for many of the emotional processing abnormalities observed in complex disorders such as schizophrenia, ADHD, and addiction. Thus, hyperactive stimulation of PFC D₄ receptor populations could amplify the emotional and/or motivational salience of normally non-salient sensory inputs, for example, environmental cues previously associated with drug taking that become pathologically relevant during states of psychostimulant addiction and trigger persistent drug seeking and relapse. Alternatively, olfactory, auditory, or visual cues, whether real or imagined, may gain distorted emotional significance in disorders such as schizophrenia, possibly leading to delusional thinking or psychotic ideation as these associations become pathologically encoded within the PFC over time. In contrast, given that D₄ receptor activation appears to block encoding of emotionally salient associative memory in the presence of highly salient sensory cues (Laviolette et al., 2005) and can actively decrease levels of CaMKII within memory encoding neurons of the PFC (Gu and Yan, 2004), one potential consequence of this functional relationship would be an inability to form appropriate associative memories between sensory cues and their appropriate emotional meaning in the context of a highly salient emotional experience.

1.4 SUMMARY AND RATIONALE

The mPFC is well recognized as an important integrative centre for executive cognitive functioning and is crucial in the processing and filtering of emotionally relevant sensory information. Increasing evidence from genetic, behavioural,

electrophysiological, and molecular research endeavors points to important and unique roles for the DA D₁ versus D₄ receptor subtypes in the processing of emotionally salient information and modulation of learning and memory within the mPFC neural circuitry. The author proposes that DA transmission within PFC may exert a 'bidirectional' mode of action on PFC interneuron versus pyramidal neuron network activity with DA D₁ and D₄ receptors controlling activity of intrinsic, GABAergic circuit neurons. Such an arrangement is consistent with other studies suggesting that DA transmission is involved in regulating inhibitory influences within the PFC. The role of differential DA transmission within mPFC in controlling not only neuronal network activity within this area, but also in the different phases of learning; specifically, encoding and expression of associative emotional information within the mesocorticolimbic system - has important implications for a variety of neuropsychiatric syndromes.

A clearer understanding of the specific roles of DA D₁ and D₄ receptor transmission during emotional learning and memory may help elucidate how abnormalities in the mPFC neural circuitry may lead to aberrant emotional processing, learning, and memory in disorders such as schizophrenia, drug addiction, PTSD, and ADHD. Therefore, the overarching goal of our research project is to characterize the D₁ and D₄ receptor modulation of the functional interactions between the VTA, BLA, and the mPFC in the processing of emotionally salient sensory information and explore how perturbations in these systems may lead to inappropriate conditioned associations between sensory input and internal emotional states.

1.5 HYPOTHESIS

DA differentially acts upon D₁ and D₄ receptors within the mPFC to modulate emotional associative learning and memory processes through distinct second messenger pathways, which in turn increase, or decrease pyramidal output activity.

1.5.1 Objectives

1. Investigate the specific roles of DA D₁ versus D₄ receptor activation during the encoding and recall phases of emotional learning and memory using an olfactory fear conditioning assay in rats.
2. Investigate the potential role of D₄ receptor-mediated control of emotional memory salience through intracellular signaling via CaMKII, cAMP/protein kinase A (PKA), and protein phosphatase-1 (PP1) signaling in the mPFC.
3. Investigate the potential role of D₁ receptor-mediated control of emotional memory expression through intracellular signaling via cAMP and PKA signaling in the mPFC.

1.6 REFERENCES

- Abi-Dargham A, Mawlawi O, Lombardo I, Gil R, Martinez D, Huang Y, Hwang DR, Keilp J, Kochan L, Van Heertum R, Gorman JM, Laruelle M. Prefrontal dopamine D1 receptors and working memory in schizophrenia. *J Neurosci*. 2002 May 1;22(9):3708-19.
- Aguirre AJ, Apiquian R, Fresan A, Cruz-Fuentes C. Association analysis of exon III and exon I polymorphisms of the dopamine D₄ receptor locus in Mexican psychotic patients. *Psych Res* 2007; 153 :209-215.
- Ambrosio AM, Kennedy JL, Macciardi F, Barr C, Soares MJ, Oliviera CR, Pato CN. No evidence of association of linkage disequilibrium between polymorphisms in the 5' upstream and coding regions of the dopamine D4 receptor gene and schizophrenia in a Portuguese population. *Am j Med Genet Neuropsychiatr Genet* 2004; 125B :20-24.
- American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. 2000. Washington, D.C.
- Angrist BM, Sathanantham G, Wilk S, Gershon S. Amphetamine psychosis: behavioural and biochemical aspects. *J Psychiat Res*. 1974; 11:13-23
- Ariano MA, Wang J, Noblett KL, Larson ER, Sibley DR. Cellular distribution of the rat D₄ dopamine receptor protein in the CNS using anti-receptor antisera. *Brain Res* 1997; 752 :26-34.
- Ariano MA, Wang J, Noblett KL, Larson ER, Sibley DR. Cellular distribution of the rat D4 dopamine receptor protein in the CNS using anti-receptor antisera. *Brain Res*. 1997 Mar 28;752(1-2):26-34.
- Arnsten AF, Cai JX, Murphy BL, Goldman-Rakic PS. Dopamine D1 receptor mechanisms in the cognitive performance of young adult and aged monkeys. *Psychopharmacology (Berl)*. 1994 Oct;116(2):143-51.
- Avale ME, Falzone TL, Gelman DM, Low MJ, Grandy DK, Rubinstein M. The dopamine D₄ receptor is essential for hyperactivity and impaired behavioural inhibition in a mouse model of attention deficit-hyperactivity disorder. *Mol Psych* 2004; 9:718-726.
- Bailey JN, Breidenthal SE, Jorgenson MJ, McCracken JT, Fairbanks LA. The association of DRD₄ and novelty seeking is founding a nonhuman primate model. *Psychiatr Genet*. 2007; 17:23-27.
- Barria A, Muller D, Derkach V, Griffith LC, Soderling TR. Regulatory phosphorylation of AMPA-type glutamate receptors by CaMKII during long-term potentiation. *Science* 1997; 276:2042-2045.
- Beaulieu JM, Gainetdinov RR. The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev*. 2011 Mar;63(1):182-217

- Benjamin J, Lin L, Patterson C, Greenberg BD, Murphy DL, Hamer DH. Population and familial association between the D₄ dopamine receptor gene and measures of novelty seeking. *Nat Genetics* 1996; 12:81-84.
- Bergson C, Mrzljak L, Smiley JF, Pappy M, Levenson R, Goldman-Rakic PS. Regional, cellular, and subcellular variations in the distribution of D1 and D5 dopamine receptors in primate brain. *J Neurosci*. 1995 Dec;15(12):7821-36.
- Boeckler F, Russig H, Zhang W, Lober S, Schetz J, Hubner H, Ferger B, Gmeiner P, Feldon J. Fauc 213, a highly selective dopamine D₄ receptor full antagonist, exhibits atypical antipsychotic properties in behavioural and neurochemical models of schizophrenia. *Psychopharmacology* 2004; 175 :7-17.
- Bristow LJ, Collinson N, Cook GP, Curtis N, Freedman SB, Kulagowski JJ, Leeson PD, Patel S, Ragan CI, Ridgil M, Saywell KL, Tricklebank MD. L-745-870, a subtype selective dopamine D₄ receptor antagonist does not exhibit a neuroleptic-like profile in rodent behavioural tests. *J Pharmacol Exp Ther* 1997 : 283-1256-1263.
- Cai JX, Arnsten AF. Dose-dependent effects of the dopamine D1 receptor agonists A77636 or SKF81297 on spatial working memory in aged monkeys. *J Pharmacol Exp Ther*. 1997 Oct;283(1):183-9
- Carrion VG, Weems CF, Watson C, Eliez S, Menon V, Reiss AL. Converging evidence for abnormalities of the prefrontal cortex and evaluation of midsagittal structures in pediatric posttraumatic stress disorder: an MRI study. *Psychiatry Res*. 2009; 172:226-234.
- Ceci A, Brambilla A, Duranti P, Grauert M, Grippa N, Borsini F. Effect of antipsychotic drugs and selective dopaminergic antagonists on dopamine-induced facilitatory activity in prefrontal cortical pyramidal neurons: an *in vitro* study. *Neuroscience* 1999; 93:107-115.
- Chang FM, Kidd JR, Livak KJ, Pakstis AJ, Kidd KK. The world-wide distribution of allele frequencies at the human dopamine D₄ receptor locus. *Hum Genet*. 1996; 98 :91-101.
- Cools R, D'Esposito M. Inverted-U-shaped dopamine actions on human working memory and cognitive control. *Biol Psychiatry*. 2011 Jun 15;69(12):e113-25.
- Corrigan MH, Gallen CC, Bonura ML, Merchant KM. Effectiveness of the selective D₄ antagonist sonepiprazole in schizophrenia: a placebo controlled trial. *Biol Psych* 2004; 55:445-451.
- Crespo-Facorro B, Kim J, Andreasen NC, O'Leary DS, Magnotta V. Regional frontal abnormalities in schizophrenia: a quantitative gray matter volume and cortical surface size study. *Biol Psychiatry*. 2000 Jul 15;48(2):110-9.
- Daglish MR, Weinstein A, Malizia AL, Wilson S, Melichar JK, Britten S, Brewer C, Lingford-Hughes A, Myles JS, Grasby P, Nutt DJ. Changes in regional cerebral blood flow elicited by craving memories in abstinent opiate-dependent subjects. *Am J Psychiatry*. 2001 Oct;158(10):1680-6.

- Darbandi N, Rezayof A, Zarrindast MR. Modulation of morphine state-dependent learning by muscarinic cholinergic receptors of the ventral tegmental area. *Physiol Behav.* 2008 Jul 5;94(4):604-10.
- Defagot MC, Malchiodi EL, Villar MJ, Antonelli MC. Distribution of D₄ dopamine receptor in rat brain with sequence specific antibodies. *Brain Res Mol Brain Res* 1997; 45 :1-12.
- de Wit H, Stewart J. Reinstatement of cocaine-reinforced responding in the rat. *Psychopharmacology (Berl).* 1981;75(2):134-43.
- Ding YC, Chi HC, Grady DL, Morishima A, Kidd JR, Kidd KK, Flodman P, Spence MA, Schuck S, Swanson JM, Zhang YP, Moyzis RK. Evidence of positive selection acting at the human dopamine receptor D₄ gene locus. *Proc Natl Acad Sci.* 2002; 99 :10-12.
- Dolan SL, Bechara A, Nathan PE. Executive dysfunction as a risk marker for substance abuse : the role of ipulsive personality traits. *Behav Sci Law* 2008; 26 :799-822.
- Dougherty DD, Bonab AA, Spnecer TJ, Rauch SL, Madras BK, Fischman AJ. Dopamine transporter density in patients with attention deficit hyperactivity disorder. *Lancet* 1999; 354 :2132-2133.
- Durstewitz D, Seamans JK. The dual-state theory of prefrontal cortex dopamine function with relevance to catechol-o-methyltransferase genotypes and schizophrenia. *Biol Psychiatry.* 2008 Nov 1;64(9):739-49.
- Duwala SC, Grandy DK, Low MJ, Paulus MP, Geyer MA. Dopamine D₄ receptor knockout mice exhibit reduced exploration of novel stimuli. *J Neurosci* 1999; 19:9550-9556.
- Ebstein RP, Novivk O, Umansky R, Priel B, Osher Y, Blaine D, Bennet ER, Nemanov L, Katz M, Belmaker RH. Dopamine D₄ receptor exon III polymorphism associated with the human personality trait of novelty seeking. *Nat Genet* 1996; 12 :78-80.
- Ehrman RN, Robbins SJ, Childress AR, O'Brien CP. Conditioned responses to cocaine related stimuli in cocaine abuse patients. *Psychopharmacology* 1992; 107:523-9.
- Ernst M, Zametkin AJ, Matochik JA, Jons PH, Cohen RM. DOPA decarboxylase activity in attention deficit hyperactivity disorder in adults. A [fluorine-18] flourodopa positron emission tomographic study. *J Neurosc* 1998; 18:5901-5907.
- Falzone TL, Gelman DM, Young JI, Grandy DK, Low MJ, Rubinstein M. Absence of dopamine D₄ receptors results in enhanced reactivity to unconditioned but not conditioned fear. 2002; *Eur J Neurosci* 15:158-164.
- Farde L, Wiesel FA, Stone-Elander S, Halldin C, Nordstrom AL, Hall H, Sedvall G. D2 dopamine receptors in neuroleptic-naive schizophrenic patients. A positron mission tomography study with [11C] raclopride. *Arch Gen Psychiatry* 47 :213-219.
- Floresco SB, Magyar O, Ghods-Sharifi S, Vexelman C, Tse MT. Multiple dopamine receptor subtypes in the medial prefrontal cortex of the rat regulates set-shifting. *Neuropsychopharmacology* 2006; 31:297-309.

- Floresco SB, Tse MT. Dopaminergic regulation of inhibitory and excitatory transmission in the basolateral amygdala-prefrontal cortical pathway. *J Neurosci* 2007; 27:2045-2057.
- Franke P, Nothen MM, Wang T, Knapp M, Lichtermann D, Neidt H, Sander T, Propping P, Maier W. DRD₄ exon III VNTR polymorphism susceptibility factor for heroin dependence? Results of a case-control and a family based association approach. *Mol Psych* 2000; 5:101-104.
- Frankland PW, O'brien C, Ohon M, Kirkwood A, Silva AJ. Alpha-CAMKII-dependent plasticity in the cortex is required for permanent memory. *Nature* 2001; 411:309-313.
- Gao WJ, Goldman-Rakic PS. Selective modulation of excitatory and inhibitory microcircuits by dopamine. *Proc Natl Acad Sci U S A*. 2003 Mar 4;100(5):2836-41.
- Gaspar P, Bloch B, LeMoine C. D₁ and D₂ receptor gene expression in rat frontal cortex: cellular localization in different classes of efferent neurons. 1995; *Eur J Neurosci*. 7:1050-1063.
- Gilboa A, Shalev AY, Laor L, Lester H, Louzoun Y, Chisin R, Bonne O. Functional connectivity of the prefrontal cortex and the amygdala in posttraumatic stress disorder. *Biol Psychiatry*. 2004 Feb 1;55(3):263-72.
- Goldman-Rakic PS. Working memory and the mind. *Sci Am*. 1992 Sep;267(3):110-7.
- Goldman-Rakic PS. Working memory dysfunction in schizophrenia. *J Neuropsychiatry Clin Neurosci*. 1994 Fall;6(4):348-57.
- Goldman-Rakic PS. Cellular basis of working memory. *Neuron*. 1995 Mar;14(3):477-85.
- Goldman-Rakic PS, Muly EC 3rd, Williams GV. D(1) receptors in prefrontal cells and circuits. *Brain Res Brain Res Rev*. 2000 Mar;31(2-3):295-301.
- Goldman-Rakic PS, Castner SA, Svensson TH, Siever LJ, Williams GV. Targeting the dopamine D1 receptor in schizophrenia: insights for cognitive dysfunction. *Psychopharmacology (Berl)*. 2004 Jun;174(1):3-16.
- Goldstein RZ, Volkow ND. Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. *Am J Psychiatry* 2002; 159:1642-52.
- Golimbet VE, Alfimova MV, Gritsenko IK, Ebstein RP. Relationship between dopamine system genes and extraversion and novelty seeking. *Neurosci Behav Physio* 2007; 37 :601-606.
- Gorelova N, Seamans JK, Yang CR. Mechanisms of dopamine activation of fast-spiking interneurons that exert inhibition in rat prefrontal cortex. *J Neurophysiol*. 2002 Dec;88(6):3150-66.
- Gu Z, Yan Z. Bidirectional regulation of Ca²⁺/calmodulin-dependent protein kinase II activity by dopamine D₄ receptors in prefrontal cortex. *Mol Pharmacol* 2004; 66:948-955.

- Gu Z, Jiang Q, Yuen EY, Yan Z. Activation of dopamine D4 receptors induces synaptic translocation of Ca²⁺/calmodulin-dependent protein kinase II in cultured prefrontal cortical neurons. *Mol Pharmacol* 2006; 69:813-822.
- Gur RC, Schroeder L, Turner T, McGrath C, Chan RM, Turetsky BI, Alsup D, Maldjian J, Gur RE. Brain activation during facial emotion processing. *Neuroimage*. 2002 Jul;16(3 Pt 1):651-62.
- Hall J, Harris JM, Sprengelmeyer R, Sprengelmeyer A, Young AW, Santos IM, Johnstone EC, Lawrie SM. Social cognition and face processing in schizophrenia. *Br J Psychiatry*. 2004 Aug;185:169-70.
- Helms CM, Gubner NR, Wilhelm CJ, Mitchell SH, Grandy DK. D₄ receptor deficiency in mice has limited effects on impulsivity and novelty seeking. *Pharmacol Biochem Behav*. 2008; 90:387-393.
- Herbener ES, Song W, Khine TT, Sweeney JA. What aspects of emotional functioning are impaired in schizophrenia? *Schizophr Res*. 2008 Jan;98(1-3):239-46.
- Hill SY, Zezza N, Wipprecht G, Xu J, Neiswander K. Linkage studies of D₂ and D₄ receptor genes and alcoholism. *Am J Med Genet* 1999; 88:676-685.
- Hutchison KE, McGeary J, Smolen A, Bryan A, Swift RM. The DRD4 VNTR polymorphism moderates craving after alcohol consumption. *Health Psychol* 2002; 21:139-146. et al.
- Inoue T, Tsuchiya K, Koyama T. Effects of typical and atypical antipsychotic drugs on freezing behaviour induced by conditioned fear. *Pharmacol Biochem Behav* 1996; 55:195-201.
- Izquierdo I, Dias RD. Memory as a state dependent phenomenon: role of ACTH and epinephrine. *Behav Neural Biol* 1983; 38: 144-149.
- Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry* 1991; 148:1301-1308.
- Jentsch JD, Elsworth JD, Taylor JR, Redmond DE, Roth RH. Dysregulation of mesoprefrontal dopamine neurons induced by acute and repeated phencyclidine administration in the nonhuman primate: implications for schizophrenia. *Adv Pharmacol* 1998; 42:810-824.
- Jonsson EG, Nothen MM, Gustavsson JP, Neidt H, Brene S, Tylec A, Propping P, Sedvall GC. Lack of evidence for allelic association between personality traits and the dopamine D₄ receptor gene polymorphisms. *Am J Psych*. 1997; 154 :697-699.
- Katz JL, Chausmer AL, Elmer GI, Rubinstein M, Low MJ, Grandy DK. Cocaine-induced locomotor activity and cocaine discrimination in dopamine D₄ receptor mutant mice. *Psychopharmacology* 2003; 170:108-114.
- Kapur S, Mamo D. Half a century of antipsychotics and still a central role for dopamine D₂ receptors. *Prog. Neuropsychopharm. & Biol. Psych*. 2003; 27:1081-1090.
- Kebabian JW, Calne DB. Multiple receptors for dopamine. *Nature* 1979; 277: 93-96.

- Kim SJ, Kim YS, Kim CH, Lee HS. Lack of association between polymorphisms of the dopamine receptor D₄ and dopamine transporter genes and personality traits in a Korean population. *Yonsei Med J.* 2006; 47 :787-792.
- Khan ZU, Gutierrez A, Martin R, Penafiel A, Rivera A, De la Calle A, Differential regional and cellular distribution of dopamine D₂-like receptors : an immunocytochemical study of subtype-specific antibodies in rat and human brain. *J Comp Neurol.* 1998 ; 402 :353-371.
- Kolb LC (1989) Chronic post-traumatic stress disorder: implications of recent epidemiological and neuropsychological studies. *Psychological Med* 19:821-824.
- Kotler M, Cohen H, Segman R, Gritsenko I, Nemanov L, Lerer B, Kramer I, Zer-Zion M, Kletz I, Ebstein RP. Excess dopamine D₄ receptor exon III seven repeat allele in opioid-dependent subjects. *Mol Psych* 1997; 2:251-254.
- Koya E, Spijker S, Voorn P, Binnekade R, Schmidt ED, Schoffelmeer ANM, De Vries TJ, Smit AB (2006) Enhanced cortical and accumbal molecular reactivity associated with conditioned heroin, but not sucrose seeking behaviour. *J Neurochem*; 98: 905-915.
- Kramer MS, Last B, Getson A, Reines SA. The effects of a selective D₄ dopamine receptor antagonist (L-745-870) in acutely psychotic inpatients with schizophrenia. *Arch Gen Psych.* 1997; 54:567-572.
- Kröner S, Rosenkranz JA, Grace AA, Barrionuevo G. Dopamine modulates excitability of basolateral amygdala neurons *in vitro*. *J Neurophysiol.* 2005 Mar;93(3):1598-610.
- Kuepper R, Skinbjerg M, Abi-Dargham A. The dopamine dysfunction in schizophrenia revisited: new insights into topography and course. *Handb Exp Pharmacol.* 2012;(212):1-26
- Lahti RA, Roberts RC, Cochrane EV, Primus RJ, Gallager DW, Conley RR, Tamminga CA. Direct determination of dopamine D₄ receptors in normal and schizophrenic postmortem brain tissue : a [3H]NGD-94-1 study. *Mol Psych* 1998; 3 :528-533.
- Langleben DD, Ruparel K, Elman I, Busch-Winokur S, Pratiwadi R, Loughhead J, O'Brien CP, Childress AR. Acute effect of methadone maintenance dose on brain fMRI response to heroin-related cues. *Am J Psychol* 2008;165:390-394.
- Laviolette SR, Lipski WJ, Grace AA. A subpopulation of neurons in the medial prefrontal cortex encodes emotional learning through burst and frequency codes through a dopamine D₄ receptor-dependent basolateral amygdala input. *J Neurosci.* 2005; 25:6066-6075.
- Laviolette SR, Grace AA. (a) The roles of cannabinoid and dopamine receptor systems in neural emotional learning circuits: implications for schizophrenia and addiction. *Cell. Mol. Life Sci.* 2006; 63:1597-613.
- Laviolette SR, Grace AA (b) Cannabinoids potentiate emotional learning plasticity in neurons of the medial prefrontal cortex through basolateral amygdala inputs. *J Neurosci* 26:6458-6468.

- Laviolette SR. Dopamine modulation of emotional processing in cortical and subcortical neural circuits: evidence for a final common pathway in schizophrenia? *Schiz Bull* 2007; 33:971-981.
- Lawrie SM, Whalley HC, Job DE, Johnstone EC. Structural and functional abnormalities of the amygdala in schizophrenia. *Ann N Y Acad Sci*. 2003 Apr;985:445-60.
- Lee HJ, Lee HS, Kim YK, Kim SH, Kim L, Lee MS, Joe SH, Jung IK, Suh KY, Kim S. Allelic variants interaction of dopamine receptor D₄ polymorphism correlate with personality traits in young Korean female population. *Am J Med Genet Neuropsychiatry Genet* 2003; 118B :76-80.
- Lee HJ, Kang SG, Choi JE, Paik JW, Kim YK, Kim SH, Lee MS, Joe SH, Jung IK, Kim L. No association between dopamine D₄ receptor gene -521 C/T polymorphisms and tardive dyskinesia in schizophrenia. *Neuropsychobiology* 2007; 55 :47-51.
- LeDoux J. The amygdala. *Curr Biol*. 2007 Oct 23;17(20):R868-74.
- Lerer B, Ebstein RP, Shestatsky M, Shemesh Z, Greenberg D. Cyclic AMP signal transduction in posttraumatic stress disorder. *Am J Psych* 1987; 144:1324-1327.
- Leung PW, Lee CC, Hung SF, Ho TP, Tang CP, Kwong SL, Leung SY, Yuen ST, Lieh-Mak F, Oosterlaan J, Grady D, Harzhi A, Ding YC, Chi HC, Flodman P, Schuxk S, Spence MA, Moyzis R, Swanson J. *Am J Med Genet B Neuropsychiatr Genet*. 2005; 133B :54-56.
- Li T, Xu K, Deng H, Cai G, Liu J, Liu X, Wang R, Xiang X, Zhao X, Murray RM, Sham PC, Collier DA. Association analysis of the dopamine D₄ gene exon III VNTR and heroin abuse in Chinese subjects. *Mol Psychiatry* 1997; 2:413-416.
- Lieberman JA, Sheitman BB, Kinon BJ. Neurochemical sensitization in the pathophysiology of schizophrenia: deficits and dysfunction in neuronal regulation and plasticity. *Neuropsychopharmacology* 1997; 17:205-229.
- Litcher JB, Barr CL, Kennedy JL, Van Tol HH, Kidd KK, Livak KJ. A hypervariable segment in the human dopamine receptor D₄ gene. *Hum Mol Genet* 1993; 767-773.
- Lung FW, Chen N, Shu BC. Dopamine D₄ receptor gene and the -521C>T polymorphisms of the upstream region of the dopamine D₄ receptor gene in schizophrenia. *Psychiatr Genet* 2006; 16 :139-143.
- Luo F, Xi ZX, Wu G, Liu C, Gardner EL, Li SJ. Attenuation of brain response to heroin correlates with the reinstatement of heroin-seeking in rats by fMRI. *Neuroimage* 2004; 22: 1328-1335
- Lynn DE, Lubke G, Yang M, McCracken JT, McGough JJ, Ishii J, Loo SK, Nelson SF, Smalley SL. Temperament and character profiles and the dopamine D₄ receptor gene in ADHD. *Am J Psych* 2005; 162 :906-913.
- Malenka RC, Nicoll RA. Long-term potentiation- a decade of progress? 1999; *Science* 285:325-334.

- Martina M, Bergeron R. D₁ and D₄ dopaminergic receptor interplay mediates coincident G-protein-independent and dependent regulation of glutamate NMDA receptors in the lateral amygdala. *J Neurochem* 2008; 106:2421-2435.
- Meador-Woodruff JH, Grandy DK, Van Tol HH, Damask SP, Little KY, Civelli O, Watson SJ. Dopamine receptor gene expression in the human medial temporal lobe. *Neuropsychopharmacology*. 1994; 10 :239-248.
- Mitsuyasu H, Hirata N, Sakai Y, Shibata H, Takeda Y, Ninomiya H, Kawasaki H, Tashiro N, Fukumaki Y. Association analysis of polymorphisms in the upstream region of the human dopamine D₄ receptor gene with schizophrenia and personality traits. *J Human Genet* 2001; 46 :26-31.
- Mitsuyasu H, Kawasaki H, Ninomiya H, Kinukawa N, Yamanaka T, Tahira T, Stanton VP, Springett GM, Hayashi K, Tashiro N, Kanba S. J. Association analysis of polymorphisms in the upstream region of the human dopamine D₄ receptor gene with schizophrenia and personality traits. *Psychiatr Res* 2007; 41 :763-775.
- Moore H, West AR, Grace AA. The regulation of forebrain dopamine transmission: relevance to the pathophysiology and psychopathology of schizophrenia. *Biol Psychiatry*. 1999 Jul 1;46(1):40-55. Review.
- Mrzljak L, Bergson C, Pappy M, Huff R, Levenson R, Goldman-Rakic PS. Localization of dopamine D₄ receptors in GABAergic neurons of the primate brain. *Nature* 1996; 381 :245-248.
- Mulcrone J, Kerwin RW. No difference in the expression of the D₄ gene in post-mortem frontal cortex from controls and schizophrenics. *Neurosci Lett* 1996; 219:163-166.
- Muly EC 3rd, Szigeti K, Goldman-Rakic PS. D₁ receptor in interneurons of macaque prefrontal cortex: distribution and subcellular localization. *J Neurosci*. 1998 Dec 15;18(24):10553-65.
- Munafò MR, Yalcin B, Willis-Owen SA, Flint J. Association of the dopamine D₄ receptor gene and approach related personality traits: meta-analysis and new data. *Biol Psych* 63:197-206.
- Murray AM, Hyde TM, Knable MB, Herman MM, Bigelow LB, Carter JM, Weinberger DR, Kleinman JE. Distribution of putative D₄ dopamine receptors in postmortem striatum from patients with schizophrenia. *J Neurosci*. 1995; 15 :2186-2191.
- Nakajima M, Hattori E, Yamada K, Iwayama Y, Toyota T, Iwata Y, Tsuchiya KJ, Sugihara G, Hashimoto K, Watanabe H, Iyo M, Hoshika A, Yoshikawa T. J. Association and synergistic interaction between promoter variants of the DRD₄ gene in Japanese subjects. *Hum Genet* 2007; 52 :86-91.
- Nobel EP, Ozkaragoz TZ, Ritchie TL, Zhang X, Belin TR, Sparkes RS. D₂ and D₄ dopamine receptor polymorphisms and personality. *Am J Med Genet*. 1998; 81 :257-67.

- Nowak K, Meyza K, Nikolaev E, Hunt MJ, Kasicki S. Local blockade of NMDA receptors in the rat prefrontal cortex increases c-Fos expression in multiple subcortical regions. *Acta Neurobiol Exp (Wars)*. 2012;72(3):207-18.
- O'Malley KL, Harmon S, Tang L, Todd RD. The rat dopamine D₄ receptor: sequence, gene structure and demonstration of expression in the cardiovascular system. *New Biol* 1992; 4:137-146.
- Okuyama Y, Ishiguro H, Nankai M, Shibuya H, Watanabe A, Arinami T, Identification of a polymorphism in the promoter region of DRD₄ associated with the human novelty seeking personality trait. *Mol Psychiatry* 2000; 5 :64-69.
- Onn SP, Wang XB, Lin M, Grace AA. Dopamine D1 and D4 receptor subtypes differentially modulate recurrent excitatory synapses in prefrontal cortical pyramidal neurons. *Neuropsychopharmacology* 2005; 31:318-338.
- Paterson AD, Sunohara GA, Kennedy JL. Dopamine D₄ receptor gene: novelty or nonsense? *Neuropsychopharmacology* 1999; 21:3-16.
- Persson ML, Wasserman D, Geijer T, Frisch A, Rockah R, Michaelovsky E, Apter A, Weizman A, Jonsson Eg, Bergman H. Dopamine D₄ receptor gene polymorphism and personality traits in healthy volunteers. *Eur Arch Psych Clin Neurosci* 250:203-206.
- Phelps EA, Delgado MR, Nearing KI, LeDoux JE. Extinction learning in humans: role of the amygdala and vmPFC. *Neuron*. 2004 Sep 16;43(6):897-905.
- Primus RJ, Thurkauf A, Xu J, Yevich E, Mcinerney S, Shaw K, Tallman JF, Gallagher DW. Localization and characterization of dopamine D₄ binding sites in rat and human brain by use of the novel D₄ receptor-selective ligand [3H]NGD 94-1. *J Pharmacol Exp Ther*. 1997; 282 :1020-1027.
- Rapoport JL, Castellanos FX, Gogate N, Janson K, Kohler S, Nelson P. Imagine normal and abnormal brain development : new perspectives for child psychiatry. *Aust N Z J Psychiatry* 2001; 35 :272-281.
- Ray LA, Bryan A, Mackillop J, McGeary J, Hesterberg K, Hutchinson KE. The dopamine D₄ receptor gene exon III polymorphism, problematic alcohol use and novelty seeking: direct and mediated genetic effects. *Addict Biol* 2009; 14:238-244.
- Reynolds GP, Mason SL. Absence of detectable striatal dopamine D₄ receptors in drug-treated schizophrenia. *Eur J Pharmacol* 1995; 281:R5-6.
- Ronai Z, Guttman A, Keszler G, Sasvari-Szekely M. Capillary electrophoresis study on DNA-protein complex formation in the polymorphic 5' upstream region of the dopamine D₄ receptor. *Curr Med Chem*. 2004; 11 :1023-1029.
- Rothbaum BO, Davis M. Applying learning principles to the treatment of post-trauma reactions. *Ann N Y Acad Sci* 2003; 1008:112-21.
- Rosenkranz JA, Grace AA. The prefrontal cortex regulates lateral amygdala neuronal plasticity and responses to previously conditioned stimuli. *J Neurosci* 2003; 23:11054 -11064.

- Rubinstein M, Phillips TJ, Bunzow JR, Falzone TL, Dziewczapolski G, Zhang G, Fang Y, Larson JL, McDougall JA, Chester JA, Saez C, Pugsley TA, Gershanik O, Low MJ, Grandy DK. Mice lacking dopamine D₄ receptors are supersensitive to ethanol, cocaine and methamphetamine. *Cell* 1997; 90:991-1002.
- Rubinstein M, Carlos Cepeda C, Hurst RS, Flores-Hernandez j., Ariano MA, Falzone TL, Kozell LB, Meshul CK, Bunzow JR, Low MJ, Levine MS, Grandy DK. Dopamine D₄ receptor-deficient mice display cortical hyperexcitability. *J Neurosci* 2001; 21:3756-3763.
- Sander T, Harms H, Dufeu P, Kuhn S, Rommelspacher H, Schmidt LG. Dopamine D₄ receptor exon alleles and variation of novelty seeking alcoholics. *Am J Med Genet* 1997; 74:483-487.
- Santana N, Mengod G, Artigas F. Quantitative analysis of the expression of dopamine D₁ and D₂ receptors in pyramidal and GABAergic neurons of the rat prefrontal cortex. *Cereb Cortex*. 2009 Apr;19(4):849-60.
- Sawaguchi T, Goldman-Rakic PS. The role of D₁-dopamine receptor in working memory: local injections of dopamine antagonists into the prefrontal cortex of rhesus monkeys performing an oculomotor delayed-response task. *J Neurophysiol*. 1994 Feb;71(2):515-28.
- Schinka JA, Letsch EA, Crawford FC. DRD₄ and novelty seeking: results of meta-analyses. 2003; *Am J Med Genet*. 114:643-648.
- Self DW, Barnhart WJ, Lehman DA, Nestler EJ. Opposite modulation of cocaine-seeking behaviour by D₁- and D₂-like dopamine receptor agonists. *Science*. 1996; 271:1586-9.
- Self DW, Karanian DA, Spencer JJ. Effects of the novel D₁ dopamine receptor agonist ABT-431 on cocaine self-administration and reinstatement. *Ann N Y Acad Sci*. 2000; 909:133-44.
- Senogles SE, Amlaiky N, Berger JG, Caron MG. Biochemical properties of D₁ and D₂ dopamine receptors. *Adv. Exp. Med. Biol*. 1988, 235, 33–41.
- Seamans JK, Yang CR. The principal features and mechanisms of dopamine modulation in the prefrontal cortex. *Prog Neurobiol* 2004; 74:1–57.
- Seamans JK, Floresco SB, Phillips AG. D₁ receptor modulation of hippocampal–prefrontal cortical circuits integrating spatial memory with executive functions in the rat. *J Neurosci* 1998; 18:1613–1621.
- Seamans JK, Gorelova N, Durstewitz D, Yang CR. Bidirectional dopamine modulation of GABAergic inhibition in prefrontal cortical pyramidal neurons. *J Neurosci* 2001; 21:3628 –3638.
- Seeman P, Van Tol HH. Dopamine receptor pharmacology. *Trends Pharmacol Sci* 1994; 15:264 –270.
- Seeman P, Guan HC, Van Tol HHM. Dopamine D₄ receptors elevated in schizophrenia. *Nature* 1993: 441-445.

- Serretti A, Lilli R, Lorenzi C, Lattuada E, Smeraldi E. DRD₄ exon 3 variants associated with delusional symptomatology in major psychoses: a study on 2,011 affected subjects. *Am J Med Genet.* 2001; 105:283-290.
- Sesack SR, Bunney BS. Pharmacological characterization of the receptor mediating electrophysiological responses to dopamine in the rat medial prefrontal cortex: a microiontophoretic study. *J Pharmacol Exp Ther.* 1989 Mar;248(3):1323-33.
- Shah AA, Sjovold T, Treit D. Selective antagonism of medial prefrontal D₄ receptors decreases fear-related behaviours in rats. *Eur J Neurosci* 2004; 19:3392-3397.
- Shao C, Li Y, Jiang K, Zhang D, Xu Y, Lin L, Wang Q, Zhao M, Jin L. Dopamine D₄ receptor polymorphisms modulates cue-elicited heroin craving in Chinese. *Psychopharmacology* 2006; 186:185-190.
- Shin, L.M., Rauch, S.L., Pitman, R.K. Amygdala, medial prefrontal cortex, and hippocampal function in PTSD. *Ann. N. Y. Acad. Sci.* 2006; 1071:67-79
- Sobotka S, Diltz MD, Ringo JL. Can delay-period activity explain working memory? *J Neurophysiol.* 2005 Jan;93(1):128-36.
- Sowell ER, Thompson PM, Welcome SE, Henkenius AL, Toga AW, Peterson BS. Cortical abnormalities in children and adolescents with attention-deficit hyperactivity disorder. *Lancet* 2003; 362 :1699-1707.
- Starr M (1987) Opposing roles of dopamine D₁ and D₂ receptors in nigral gamma-[3H]aminobutyric acid release? *J Neurochem* 49: 1042-1049.
- Strobel A, Wehr A, Michel A, Brocke B. Association between the dopamine D₄ receptor exon III polymorphisms and measures of novelty seeking in a German population. *Mol Psych* 1999; 4 :378-384.
- Sumiyoshi T, Stockmeiser CA, Overholser JC, Thompson PA, Meltzer HY. Dopamine D₄ receptors and effects of guanine nucleotides on [3H]raclopride binding in postmortem caudate nucleus of subjects with schizophrenia or major depression. *Brain Res* 1995; 681 :109-116.
- Sun N, Laviolette S (2012) Inactivation of the basolateral amygdala during opiate reward learning disinhibits prelimbic cortical neurons and modulates associative memory extinction. *Psychopharmacology*. E-pub ahead of print Mar 21, 2012.
- Sun N, Chi N, Lauzon N, Bishop S, Tan H, Laviolette SR. Acquisition, extinction, and recall of opiate reward memory are signaled by dynamic neuronal activity patterns in the prefrontal cortex. *Cereb Cortex.* 2011; 21:2665-80.
- Takahashi H, Kato M, Takano H, Arakawa R, Okumura M, Otsuka T, Kodaka F, Hayashi M, Okubo Y, Ito H, Suhara T. Differential contributions of prefrontal and hippocampal dopamine D(1) and D(2) receptors in human cognitive functions. *J Neurosci.* 2008 Nov 12;28(46):12032-8.
- Takahashi H, Yamada M, Suhara T. Functional significance of central D1 receptors in cognition: beyond working memory. *J Cereb Blood Flow Metab.* 2012 Jul;32(7):1248-58.

- Tavanti M, Battaglini M, Borgogni F, Bossini L, Calossi S, Marino D, Vatti G, Pieraccini F, Federico A, Castrogiovanni P, De Stefano N. Evidence of diffuse damage in frontal and occipital cortex in the brain of patients with post-traumatic stress disorder. *Neurol Sci.* 2012; 33:59-68.
- Taylor SF, Liberzon I, Decker LR, Koeppe RA. A functional anatomic study of emotion in schizophrenia. *Schizophr Res.* 2002 Dec 1;58(2-3):159-72.
- Thanos P, Bermeo C, Rubinstein M, Suchland K, Wang G, Grandy D, Volkow N. Conditioned place preference and locomotor activity in response to methylphenidate, amphetamine and cocaine in mice lacking dopamine D₄ receptors. *J Psychopharmacology* 2009
- Tomitaka M, Tomitaka S, Otuka Y, Kim K, Matuki H, Sakamoto K, Tanaka A. Association between novelty seeking and dopamine receptor D₄ exon III polymorphism in Japanese subjects. *Am J Med Genet* 1999; 88 :469-471.
- Trantham-Davidson H, Neely LC, Lavin A, Seamans JK. Mechanisms underlying differential D1 versus D2 dopamine receptor regulation of inhibition in prefrontal cortex. *J Neurosci.* 2004 Nov 24;24(47):10652-9.
- Van Tol HHM, Bunzow JR, Guan HC, Sunahara RK, Seeman P, Niznik HB, Civelli O. Cloning of the gene for a human dopamine D₄ receptor with high affinity for the antipsychotic clozapine. *Nature* 1991; 350:610-614.
- Van Tol HHM, Caren MW, Guan HC, Ohara K, Bunzow JR, Civelli O, Kennedy J, Seeman P, Niznik HB, Jovanovic V. Multiple dopamine D₄ receptor variants in the human population. *Nature* 1992; 358:149-152.
- Vijayraghavan S, Wang M, Birnbaum SG, Williams GV, Arnsten AF. Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. *Nat Neurosci.* 2007 Mar;10(3):376-84.
- Vincent S.L., Khan Y., Benes F.M. (1993) Cellular distribution of dopamine D1 and D2 receptors in rat medial prefrontal cortex. *J. Neurosci.*; 13: 2551–2564
- Wang X, Zhong P, Gu Z, Yan Z. Regulation of NMDA receptors by dopamine D₄ signalling in prefrontal cortex. *J Neurosci* 2003; 23:9852-9861.
- Wedzony K, Chocyk A, Mackwiak M, Fijał K, Czyrak A . Cortical localization of dopamine D₄ receptors in the rat brain: an immunocytochemical study. *J Physiol Pharmacol* 2000; 51:205-221.
- Weiss J, Magert HJ, Cieslak A, Forssmann WG. Association between different psychotic disorders and the DRD₄ polymorphism, but no differences in the main ligand binding region of the DRD₄ receptor protein compared to controls. *Eur J Med Res.* 1996; 1 :439-445.
- Williams GV, Goldman-Rakic PS. Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. *Nature.* 1995 Aug 17;376(6541):572-5.
- Williams LM, Das P, Harris AW, Liddell BB, Brammer MJ, Olivieri G, Skerrett D, Phillips ML, David AS, Peduto A, Gordon E. Dysregulation of arousal and

- amygdala-prefrontal systems in paranoid schizophrenia. *Am J Psychiatry*. 2004 Mar;161(3):480-9.
- Williams GV, Castner SA. Under the curve: critical issues for elucidating D1 receptor function in working memory. *Neuroscience*. 2006 Apr 28;139(1):263-76.
- Windemuth A, Calhoun VD, Pearlson GC, Kochlerla M, Jagannathan K, Ruano G. Physiogenomic analysis of localized fMRI brain activity in schizophrenia. *Ann Biomed Eng* 2008; 36:877-888.
- Wise, RA. Dopamine, learning and motivation. *Nat Rev Neurosci*. 2004; 5:1-12.
- Whitfield-Gabrieli S, Thermenos HW, Milanovic S, Tsuang MT, Faraone SV, McCarley RW, Shenton ME, Green AI, Nieto-Castanon A, LaViolette P, Wojcik J, Gabrieli JD, Seidman LJ. Hyperactivity and hyperconnectivity of the default network in schizophrenia and in first-degree relatives of persons with schizophrenia. *Proc Natl Acad Sci U S A*. 2009 Jan 27;106(4):1279-84.
- Xiang L, Szebeni K, Szebeni A, Klimek V, Stockmeier CA, Karolewicz B, Kalbfleisch J, Ordway GA. Dopamine receptor gene expression in human amygdaloid nuclei : elevated D₄ receptor mRNA in major depression. *Brain Res* 2008; 1207 :214-224.
- Yang CR, Seamans JK. Dopamine D1 receptor actions in layers V-VI rat prefrontal cortex neurons *in vitro*: modulation of dendritic-somatic signal integration. *J Neurosci*. 1996 Mar 1;16(5):1922-35.
- Yashiro K, Philpot BD. Regulation of NMDA receptor subunit expression and its implications for LTD, LTP and metaplasticity. *Neuropharmacology* 55:1081-1094.
- Yuen EY, Yan Z. Dopamine D₄ receptors regulate AMPA receptor trafficking and glutamatergic transmission in GABAergic interneurons of prefrontal cortex. *J Neurosci* 2009; 29:550-562.
- Zahrt J, Taylor JR, Mathew RG, Arnsten AF. Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance. *J Neurosci*. 1997 Nov 1;17(21):8528-35.

Chapter 2:

- 2 Dopamine D₁ versus D₄ receptors differentially modulate the encoding of salient versus nonsalient emotional information in the medial prefrontal cortex¹

¹ This chapter has been adapted from a published journal article: **Lauzon NM**, Bishop SF, Laviolette SR. (2009) Dopamine D₁ versus D₄ receptors differentially modulate the encoding of salient versus nonsalient emotional information in the medial prefrontal cortex. *Journal of Neuroscience*; 29(15):4836-45

2.1 INTRODUCTION

The ability to assign appropriate emotional significance to incoming sensory information is essential for normal cognitive function and adaptive behaviour. These psychological processes require prefrontal cortical mechanisms that allow accurate filtering of salient versus nonsalient sensory information. Disturbances in these processes may lead to the formation of inappropriately reinforced associative memories, maladaptive behaviours, and delusional ideation, all of which are typically present in schizophrenia-related psychoses. Within the mPFC, cognitive, emotional, and motivational processes are strongly modulated by DAergic transmission and, in particular, through signaling via the DA D₁ and D₄ receptor subtypes (Seamans et al., 1998; Pezze et al., 2003; Laviolette et al., 2005; Onn et al., 2006).

DAergic modulation of mPFC neuronal networks is known to control the encoding of working memory and the temporal sequencing of behavioural output (Goldman-Rakic et al., 1989; Goldman-Rakic, 1995; Seamans et al., 1998). Functionally, DAergic transmission within the mPFC influences neuronal network activity in a biphasic manner, with D₁ receptor activation increasing the intrinsic excitability of inhibitory GABAergic interneurons and D₂-like receptor activation decreasing the activity of these interneurons, presumably leading to decreased probability of tonic inhibitory GABA release within the mPFC (Seamans et al., 2001). The D₄ subtype is expressed highly in mPFC relative to other brain regions and plays a critical role in modulating neuronal activity in both prelimbic and infralimbic mPFC regions (Ceci et al., 1999; Onn et al., 2005). Additional support for a modulatory role

of DA D₄ receptor transmission is suggested by findings that DA-mediated amplification of mPFC neuronal activity is preferentially mediated through DAergic actions on the D₄ receptor subtype (Ceci et al., 1999). Given the critical role of the mPFC in the encoding and expression of emotionally salient information and its ability to amplify DAergic effects within mPFC neuronal networks, one possibility is that DA D₄ receptor activation within the mPFC may amplify the emotional salience of sensory information and the subsequent encoding of this associative information.

A subpopulation of neurons within the mPFC is capable of encoding and expressing emotional conditioned associations via inputs from the basolateral nucleus of the amygdala (BLA) (Laviolette et al., 2005; Laviolette and Grace, 2006a, b). We have reported previously that D₄ receptor blockade, either systemically or directly within the mPFC, can prevent the acquisition (but not expression) of emotionally salient associative fear memories, as demonstrated behaviourally and at the level of the single neuron (Laviolette et al., 2005). However, it is presently not known how activation of DA D₄ versus D₁ receptor transmission within the mPFC may be involved in modulating the acquisition and/or expression (recall) of emotionally salient information or how these receptors may modulate salient versus nonsalient sensory stimuli in the context of emotional memory encoding. Accordingly, we conducted a series of behavioural pharmacological studies comparing the potential roles of DA D₄ versus D₁ receptor transmission during the acquisition or expression phases of emotional learning and memory processing, by comparing emotionally

salient versus nonsalient associative conditioning stimuli during an olfactory fear-conditioning procedure.

2.2 MATERIALS AND METHODS

2.2.1 Animals and surgery

Male Sprague Dawley rats (300–350 g) were obtained from Charles River Laboratories. All procedures were performed in accordance with the Canadian Council on Animal Care and were approved by the Council on Animal Care at the University of Western Ontario. Rats were anesthetized with a ketamine (80 mg/ml)–xylazine (6 mg/kg) mixture administered intraperitoneally and placed in a stereotaxic device. All stereotaxic coordinates were based on the atlas of Paxinos and Watson (1996). Two stainless steel guide cannulae (22 gauge) were implanted into the mPFC using the following stereotaxic coordinates (15° angle; in millimeters from bregma): anteroposterior (AP), +2.9; lateral (LAT), ±1.9; ventral (V), –3.0 from the dural surface. For bilateral intra-BLA placements, the following stereotaxic coordinates were used (0° angle): AP, –3.0; LAT, ±5.0; V, –8.0 from the dural surface (see Fig. 2.4). Jeweler's screws and dental acrylic were used to secure the cannulae.

2.2.2 Olfactory fear conditioning

Rats were taken from their home cages, received sham microinfusions into the mPFC, and were habituated for 30 min in a ventilated conditioning chamber with an electric grid floor inside a sound-attenuated room. Olfactory fear conditioning took place in one of two distinct environments, counterbalanced within groups: “shock”

environment A was a 30 × 30 inch Plexiglas box with black stripes on a white background and a metallic grid shock floor, whereas shock environment B was a 30 × 30 inch Plexiglas box with black dots on a white background with a grid shock floor. Testing 24 h later took place in one of two alternate environments, in which animals had not previously received electric shock, counterbalanced within groups: test environment A had walls with black dots and a gray Plexiglas floor, whereas test environment B had walls with black and white stripes and a gray Plexiglas floor. On day 1 (habituation phase), animals were habituated to a random combination of shock environment A or B and test environment A or B in a counterbalanced order for 30 min in each environment. On day 2 (conditioning phase), animals were returned to the conditioning room and placed in the previously assigned shock environment. During the conditioning phase, one of the odours (almond or peppermint) was presented to the animal for 19 s, and a footshock was then delivered (0.4 or 0.8 mA) for 1 s [positive conditioned stimulus (CS+)]. The two different levels of footshock (0.4 and 0.8 mA) correspond to subthreshold and suprathreshold levels of emotional stimuli, as reported previously (Laviolette and Grace, 2006a). One hundred twenty seconds later, the alternate odour was presented for 20 s [negative conditioned stimulus (CS-)] in the absence of footshock. This cycle was repeated five times. On the following day (test phase), rats were returned to the test room and placed in the previously assigned test environment. Before odour presentation, the rat was allowed to explore the environment for 1 min, during which time baseline levels of freezing and exploratory behaviour were observed. Odours (CS+ or CS-) were then presented for 5 min each to the animal in a counterbalanced order, and the amount of time freezing was

recorded. Freezing was defined as complete immobility with the exception of respiratory-related movement. We also analyzed exploratory behaviour in response to presentations of CS+ or CS- odours, as described previously (Rosenkranz and Grace, 2003). Exploratory behaviour was scored as follows, with a score assigned for every minute of each of the 5 min during the CS+ or CS- odour presentations: 0, no locomotion; 1, ambulation across one side of the testing chamber; 2, ambulation across two sides; 3, exploration of the full perimeter of the testing chamber; 4, exploration of the center and entire perimeter of the test chamber. To analyze DA receptor modulation on acquisition of emotionally salient information, microinfusions were given directly before the conditioning procedure. To examine the effects of DA receptor manipulation on the expression phase of learned emotional memory drug, microinfusions were given directly before testing (24 h after fear conditioning). Experimental procedures for examining acquisition (encoding) versus expression (recall) of associative fear conditioning are summarized schematically in Figure 2.1C.

2.2.3 Drug administration

For mPFC and BLA microinfusions, stainless steel guide cannulae (22 gauge) were implanted bilaterally into the mPFC or BLA, and drugs were administered through a 28 gauge microinfusion injector (Plastics One). All drugs were dissolved in physiological saline, with pH adjusted to 7.4. Bilateral intra-mPFC microinjection of saline vehicle, the highly selective D₄ agonist PD 168077 (dose range, 2.5–50 ng/0.5 µl; Tocris), the D₁-like receptor agonist SKF 38393 (dose range, 100–1000 ng/0.5 µl; Sigma), the D₂-like agonist quinpirole (dose range, 100–1000 ng/0.5 µl; Sigma), or

the D₄ antagonist L-741,741 (1000 ng; Tocris) and the full D₁ receptor agonist SKF 81297 (dose range, 1–100 ng/0.5 µl; Tocris) were microinfused immediately before the olfactory conditioning procedure (acquisition phase challenge) or immediately before the testing phase (recall challenge). For studies examining the role of BLA inputs, bilateral intra-BLA microinjections of either saline vehicle or muscimol (500 ng/0.5 µl) were performed immediately before intra-mPFC PD 168077 (50 mg/0.5 µl) microinjections.

2.2.4 Footshock sensitivity analysis

To determine whether our intra-mPFC pharmacological manipulations induced alterations in sensitivity to footshock, we rigorously monitored behavioural responses to these manipulations before and after drug administration. For intra-mPFC experiments, we performed separate control experiments, wherein animals received either bilateral intra-mPFC saline (control) or the D₄ agonist PD 186077 (50 ng/0.5 µl) and were placed in a clear Plexiglas environment with a footshock grid floor. Based on previously published methodology (Laviolette and Grace, 2006), we presented either a behaviourally subthreshold footshock level (0.4 mA, 1 s) or a behaviourally suprathreshold footshock level (0.8 mA) over five times and measured sensitivity to footshock over four separate variables: (1) percentage of time spent freezing in 20 s postfootshock intervals; (2) number of jumps in response to footshock; (3) amount of defecation (in pieces) during footshock session; and (4) number of times the animal reared during the sensitivity trial. All of these behavioural indices of footshock sensitivity have been reported as reliable indicators of fear

reactivity to the presentation of a footshock stimulus (Antoniadis and McDonald, 1999).

2.2.5 Histology

After completion of the experiment, the animals were perfused, and brains were removed and stored in formalin with 25% sucrose solution for at least 24 h. Brains were then sectioned into 40 μm coronal slices, mounted, and stained using cresyl violet to allow for histological analysis of the site of injection. The majority of mPFC placements were localized within the boundaries of the prelimbic cortical area (PLC).

2.2.6 Data analysis

Data were analyzed with one-, two-, or three-way ANOVA where appropriate or Student's *t* tests. *Post hoc* analyses were performed with Newman–Keuls tests.

2.3 RESULTS

2.3.1 Intra-mPFC histological analysis

Histological analysis revealed microinfusion injector cannulae placements to be bilaterally localized within the anatomical boundaries of the mPFC region, as determined by the atlas of Paxinos and Watson (1996). In Figure 2.1A, we present a microphotograph showing a typical, representative injector placement within the mPFC. In Figure 2.1B, we present a schematic illustration showing representative intra-mPFC bilateral cannulae placements along the rostrocaudal axis of the mPFC.

2.3.2 Intra-mPFC DA receptor activation potentiates associative learning to emotionally nonsalient conditioning stimuli

For this and all subsequent behavioural experiments, we present a schematic illustration in Figure 2.2A, showing our fear-conditioning procedure in terms of conditioning timelines and methods for challenging either the acquisition (encoding phase) or the expression (recall phase) of emotional associative learning. To establish salient versus nonsalient levels of footshock for use in our fear-conditioning procedures, we selected two levels of footshock (0.4 and 0.8 mA) for use in our olfactory fear-conditioning procedures, based on a previously published report (Laviolette and Grace, 2006). Preliminary studies determined that whereas the lower level of footshock (0.4 mA; $n = 5$) (Fig. 2.2B) produced no significant associative memory encoding (animals demonstrated no conditioned freezing behaviour in response to CS+ relative to CS- presentations; $t_{(4)} = 0.15$; $p > 0.05$), a higher level of footshock (0.8 mA; $n = 6$) (Fig. 2.2B) produced strong associative fear conditioning, demonstrated by significantly greater levels of conditioned freezing in response to CS+ relative to CS- presentations ($t_{(5)} = 5.84$; $p < 0.01$). Similarly, whereas the lower level of footshock (0.4 mA; $n = 5$) produced no associative suppression of exploratory behaviour in response to CS+ versus CS- presentations (Fig. 2C) ($t_{(4)} = 1.78$; $p > 0.05$), a higher level of footshock (0.8 mA; $n = 6$) (Fig. 2.2B) produced conditioned suppression of exploratory behaviour specifically in response to CS+ relative to CS- presentations (Fig. 2.2C) ($t_{(5)} = 2.2$; $p < 0.05$).

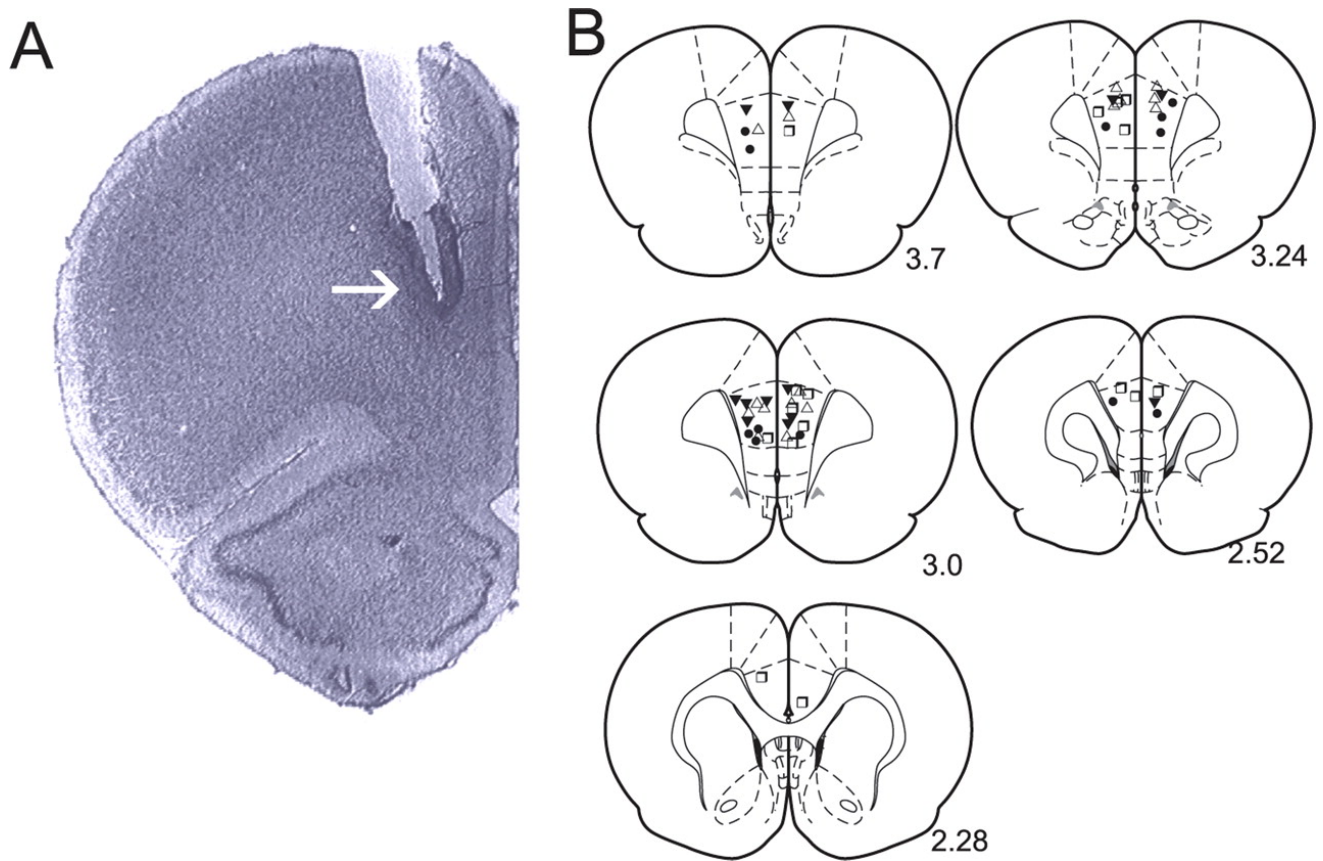


Figure 2.1 Histological analysis of intra-mPFC microinjection sites.

(A) Microphotograph of a representative injector placement within the mPFC. (B) Schematic illustration showing representative bilateral placements of microinjection cannulae. For illustrative clarity, only a subset of experimental groups is presented. Symbols represent separate experimental groups: \triangle , PD 168077, 50 ng/0.5 μ l versus acquisition of subthreshold stimuli; \square , SKF 38393, 1000 ng/0.5 μ l versus acquisition of subthreshold stimuli; \blacktriangledown , PD 168077, 50 ng/0.5 μ l versus acquisition of suprathreshold stimuli; \bullet , SKF 38393, 1000 ng/0.5 μ l versus expression of suprathreshold stimuli.

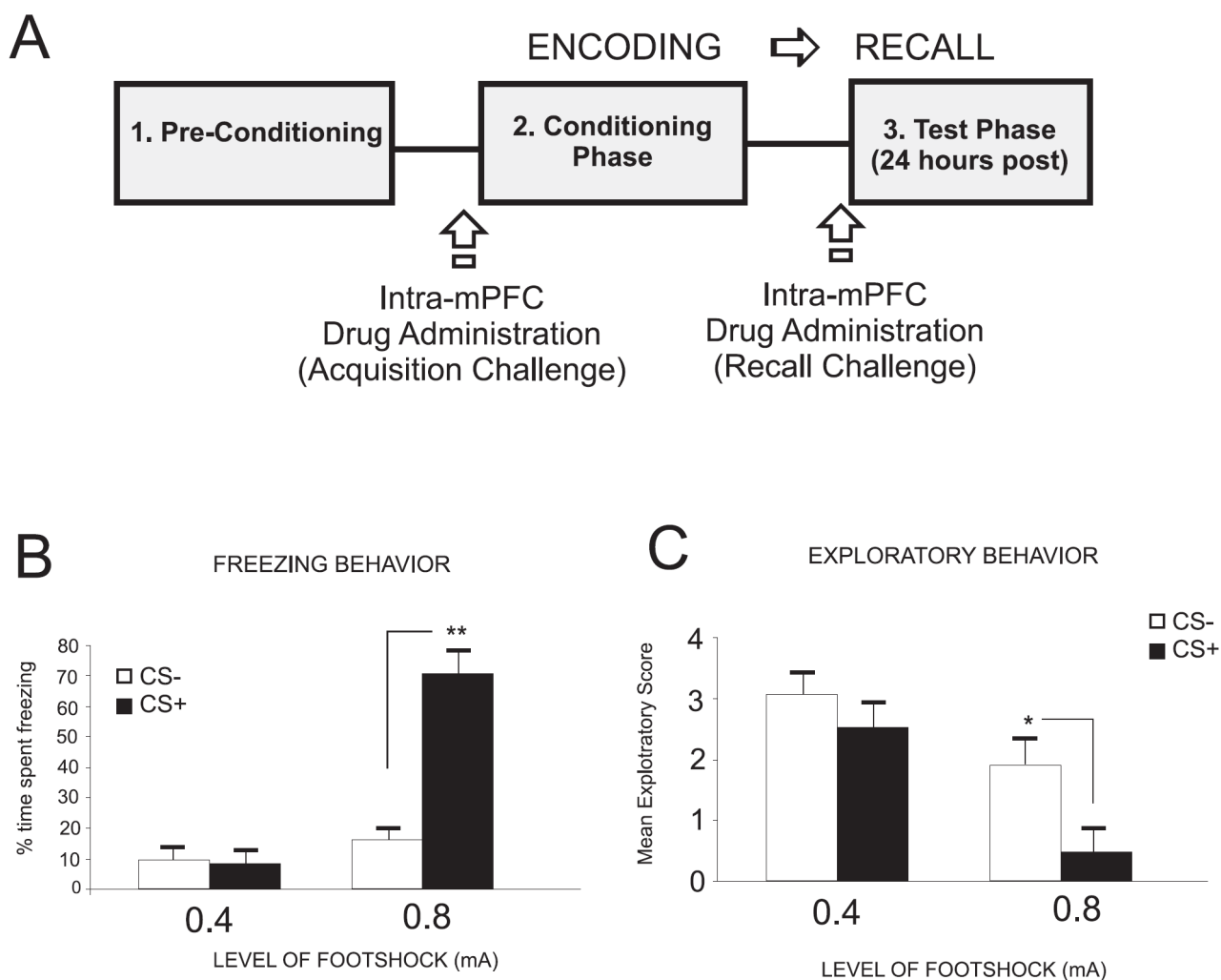


Figure 2.2 Experimental protocol summary and footshock sensitivity level assay.

(A) Schematic representation showing experimental associative fear conditioning assay and timeline for examining acquisition (encoding) versus expression (recall) phases of associative olfactory fear memory. (B, C) Behavioural sensitivity assay demonstrating that although subthreshold (0.4 mA) footshock stimuli produce neither conditioned freezing behaviour (B) nor conditioned suppression of exploratory behaviour (C), presentation of a suprathreshold (0.8 mA) footshock level produces significant levels of freezing (B) and suppression of exploratory behaviour (C). Error bars represent SEM for this and all subsequent figures. For this and all subsequent figures, * $p < 0.05$; ** $p < 0.01$.

Thus, similar to previous reports (Lavolette et al., 2005; Lavolette and Grace, 2006a), these levels of footshock were selected for subsequent experiments to represent “salient” versus “nonsalient” emotional conditioning stimuli. Using our associative olfactory fear-conditioning procedure (see Materials and Methods) (Lavolette et al., 2005; Lavolette and Grace, 2006a) with an emotionally nonsalient (0.4 mA) level of footshock (which produces no associative fear conditioning in control animals) (Lavolette and Grace, 2006a), we next performed bilateral intra-mPFC microinfusions of either saline or the specific DA D₄ agonist PD 168077 (2.5, 25, or 50 ng/0.5 µl) immediately before fear conditioning, to determine the effects of D₄ receptor activation during the acquisition phase (encoding) of this associative information. ANOVA revealed a significant interaction between group and treatment ($F_{(3,53)} = 11.2; p < 0.001$) on times spent freezing to either CS+ or CS- cue presentations at testing. *Post hoc* analysis illustrated a dose-dependent potentiation in associative fear-conditioning expression at the two higher doses of PD 168077, 25 ng ($n = 7; p < 0.05$) and 50 ng ($n = 8; p < 0.01$), demonstrated by a significantly greater time spent freezing to the CS+ compared with CS- (Fig. 2.3A). In contrast, *post hoc* analysis revealed no significant difference between freezing time to the CS+ compared with CS- for the saline ($n = 5; p > 0.05$) control group or animals receiving the lowest dose of PD 168077 (2.5 ng/0.5 µl; $n = 7; p > 0.05$).

To demonstrate the receptor specificity of our D₄ agonist effect, we performed a separate control experiment ($n = 7$) wherein rats received co-infusion of the highest effective dose of PD 168077 (50 ng/0.5 µl) with a competitive antagonist of the D₄

receptor, L-741,741 (1000 ng/0.5 μ l), before subthreshold olfactory fear conditioning. Statistical analysis revealed that D₄ antagonist co-administration blocked the potentiating effect of PD 168077 because rats showed no significant difference between percentage of time spent freezing ($t_{(6)} = 1.22$; $p > 0.05$) in response to the CS+ relative to the CS- (Fig. 2.3A). Analysis of spontaneous exploratory behaviour during presentations of either CS+ or CS- (Fig. 2.3B) revealed a significant interaction between group and treatment ($F_{(3,63)} = 2.57$; $p < 0.01$) with *post hoc* analysis revealing that rats treated with the highest dose PD 169077 (50 ng/0.5 μ l; $n = 8$) or 25 ng/0.5 μ l ($n = 6$) displayed significantly decreased exploratory behaviour in response to the CS+ relative to the CS- presentations ($p < 0.01$ and $p < 0.05$, respectively). In addition, *post hoc* analysis revealed there was no significant difference in exploratory behaviour observed in response to the CS+ relative to the CS- with the lower doses of PD 168077, 2.5 ng/0.05 μ l ($n = 7$; $p > 0.05$) and 25 ng/0.5 μ l ($n = 7$; $p > 0.05$), as well as the saline control group ($n = 5$; $p > 0.05$) (Fig. 2.3B). In addition, in the pharmacological control group receiving co-administration of PD 168077 (50 ng/0.5 μ l) with the D₄ antagonist L-741,741 (1000 ng/0.5 μ l) demonstrated no significant difference between exploratory behaviour in response to the CS+ and CS- presentations ($t_{(6)} = 1.05$; $p > 0.05$) (Fig. 2.3B).

To further characterize the specific role of the DA D₄ subtype relative to other D₂-like receptors within the mPFC in our observed emotional associative learning potentiation effects, we ran an additional control experiment using a nonspecific agonist of D₂-like receptors, quinpirole, with relative K_i values of 4.8, ~24, ~30, and

~1900 nm at the D₂, D₃, D₄, and D₁ DA receptor subtypes, respectively (Seeman and Van Tol, 1994; Levant et al., 1996), for comparison with the effects of the specific D₄ receptor agonist PD 168077 (Fig. 2.3A). We performed bilateral intra-mPFC microinfusions of quinpirole, immediately before conditioning, at two different concentrations: a lower concentration (100 ng/0.5 μ l), which should display greater selectivity for the D₂ receptor subtype, and a higher concentration (1000 ng/0.5 μ l), which would not provide specificity at the D₂ receptor but, rather, likely interact with all D₂-like subtypes, including the D₄ receptors. ANOVA revealed a significant effect of treatment on time spent freezing (Fig. 2.3C) ($F_{(2, 33)} = 5.6$; $p < 0.01$). *Post hoc* analysis revealed no significant difference in the time spent freezing to the CS+ relative to the CS- in rats receiving a lower, D₂ receptor-specific dose of quinpirole ($n = 6$; $p > 0.05$). In contrast, *post hoc* analysis revealed that intra-mPFC microinfusions of quinpirole at the higher (nonspecific) dose (1000 ng/0.5 μ l) resulted in a potentiation of subthreshold fear stimuli as demonstrated by a significant increase in the time spent freezing to the CS+ relative to the CS- ($n = 6$; $p < 0.01$) (Fig. 2.3C).

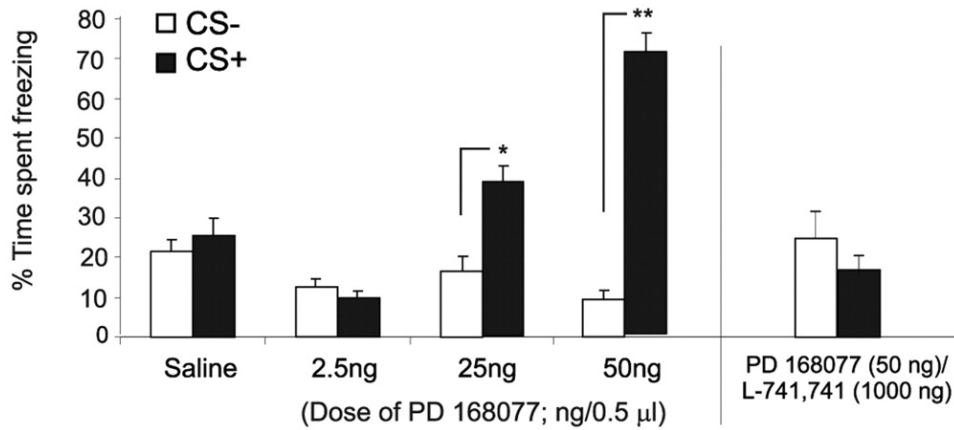
Whereas it is not possible to directly compare *in vitro* binding kinetics with that which would be observed *in vivo*, these results demonstrate the DA activation within the mPFC specifically potentiates emotional associative learning via actions on a highly sensitive D₄ receptor population (showing emotional learning potentiation at doses as low as 25 ng/0.5 ml (Fig. 2.3A) rather than via nonspecific effects on all D₂-like receptor subtypes within the mPFC.

Figure 2.3 Bilateral intra-mPFC DA D₄ and D₂ receptor manipulations versus subthreshold levels of fear-conditioning stimuli.

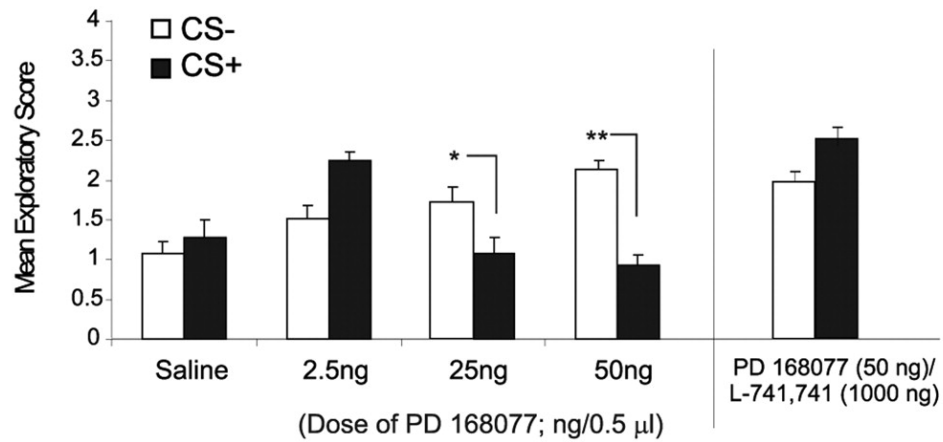
(A) Bilateral intra-mPFC microinfusions of the DA D₄ receptor agonist PD 168077 (2.5, 25, and 50 ng/0.5 μ l) dose-dependently potentiated the acquisition of subthreshold (0.4 mA footshock) olfactory fear conditioning. Both saline controls and rats receiving a lower dose of PD 168077 (2.5 ng/0.5 μ l) display no significant difference in time freezing to the CS+ relative to the CS- presentations. In contrast, rats receiving higher doses of PD 168077 (25 ng and 50 ng/0.5 μ l) display significantly greater levels of freezing specifically in response to presentation of the CS+ relative to CS-. In a separate control experiment, this D₄ receptor-induced potentiation was blocked when the D₄ receptor antagonist L-741,741 (1000 ng/0.5 μ l) was coadministered with PD 168077 (50 ng/0.5 μ l), as rats display no significant difference in conditioned freezing behaviour in response to the CS+ relative to CS- presentations. (B) Measurement of exploratory behaviour in response to the CS+ and CS- odours revealed saline control rats and rats receiving the lower two doses of PD 168077 (2.5 and 25 ng/0.5 μ l) showed no significant difference in exploratory scores. However, with the 50 ng/0.5 μ l PD 168077 dose, there was a significant decrease in spontaneous exploratory behaviour after CS+ relative to CS- presentation. Similarly, intra-mPFC coadministration with the D₄ receptor antagonist L-741,741 (1000 ng/0.5 μ l) with PD 168077 (50 ng/0.5 μ l) blocked conditioned suppression of exploratory behaviour, demonstrated by no significant difference between exploratory behaviour in response to CS+ and CS- presentations. (C) Bilateral intra-mPFC microinfusions of the D₂-like receptor agonist quinpirole failed to potentiate subthreshold fear-conditioning acquisition at a lower dose of 100 ng/0.5 μ l, demonstrated by no significant freezing response to CS+ versus CS- presentations. However, bilateral intra-mPFC microinfusions of quinpirole at a higher (nonspecific) dose (1000 ng/0.5 μ l) resulted in a potentiation of subthreshold fear stimuli as demonstrated by a significant increase in the time spent freezing to the CS+ relative to the CS-.

A

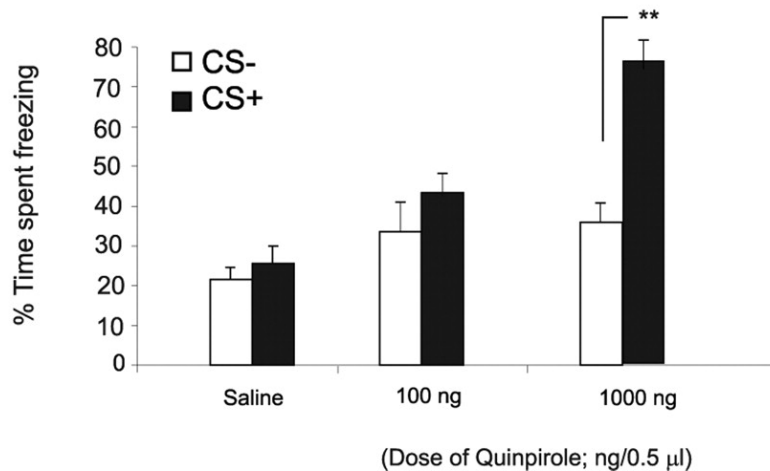
SUB-THRESHOLD FEAR CONDITIONING (0.4 mA)

**B**

SUB-THRESHOLD FEAR CONDITIONING (0.4 mA)

**C**

SUB-THRESHOLD FEAR CONDITIONING (0.4 mA)



These behavioural results are consistent with anatomical and electrophysiological evidence showing higher relative concentrations of the D₄ subtype and greater neuronal sensitivity within the mPFC to DA D₄ receptor activation, relative to other DA receptor subtypes (Mrzljak et al., 1996; Ceci et al., 1999; Wedzony et al., 2000; Onn et al., 2005).

2.3.3 Intra-mPFC DA D₄ receptor activation blocks the encoding but not the expression of associative learning to emotionally salient conditioning stimuli

We next examined the possible effects of mPFC DA D₄ activation on the encoding of suprathreshold fear-conditioning stimuli, using a higher, emotionally salient dose of footshock (0.8 mA). We first examined the acquisition phase of emotional learning by administering an effective dose of the D₄ agonist (50 ng/0.5 µl) immediately before conditioning. A control group ($n = 7$) receiving intra-mPFC saline before fear conditioning to the suprathreshold footshock level showed significant levels of conditioned fear at testing, demonstrated by a significant increase both in freezing behaviour ($n = 7$; $t_{(6)} = 4.29$; $p < 0.01$) (Fig. 2.4A) and a decrease in exploratory behaviour ($n = 7$; $t_{(6)} = 3.45$; $p < 0.05$) (Fig. 2.4B) in response to the CS+ presentation relative to the CS-. In contrast, rats receiving bilateral intra-mPFC microinfusions of the effective dose of PD 168077 ($n = 8$; 50 ng/0.5 µl) demonstrated a complete block in the acquisition of emotionally salient fear stimuli as demonstrated by no significant difference in response to CS+ versus CS- presentations in either freezing behaviour ($t_{(7)} = 1.14$; $p > 0.05$) (Fig. 2.4A) or exploratory behaviour ($t_{(7)} = 1.15$; $p > 0.05$) (Fig.

2.4B). Thus, activation of mPFC D₄ receptors blocked the encoding of emotional associative learning to a suprathreshold, emotionally salient footshock stimulus.

We next examined the expression phase of emotional learning by performing bilateral intra-mPFC microinfusions of the D₄ agonist ($n = 8$; 50 ng/0.5 μ l) immediately before the testing (recall phase; 24 h after conditioning). Analysis revealed intra-mPFC PD 168077 (50 ng/0.5 μ l) did not block the expression of previously learned emotionally salient information as demonstrated by a significant increase in the percentage of time spent freezing to the CS+ relative to the CS- ($t_{(7)} = 3.03$; $p < 0.05$) (Fig. 2.4A), as well as a significant decrease in the exploratory behaviour observed in response to the CS+ relative to the CS- presentations ($t_{(7)} = 3.46$; $p < 0.05$) (Fig. 2.4B). Thus, activation of intra-mPFC DA D₄ receptors specifically blocks the encoding of emotionally salient associative learning, while having no effect on the expression (recall) of this information.

2.3.4 Footshock sensitivity analysis

Although no existing evidence supports a modulatory role for mPFC DA D₄ receptor transmission in the processing of nociceptive information, an alternative explanation for our observed conditioning effects on potentiation of subthreshold (0.4 mA) emotional fear conditioning or blockade of the acquisition of suprathreshold (0.8 mA) footshock presentations may be a D₄ receptor-mediated increase or decrease in peripheral nociceptive sensitivity to footshock presentations. To rule out this possibility, we analyzed behavioural sensitivity to our low footshock level (0.4 mA), comparing rats receiving microinfusions of either saline ($n = 5$) or the effective dose

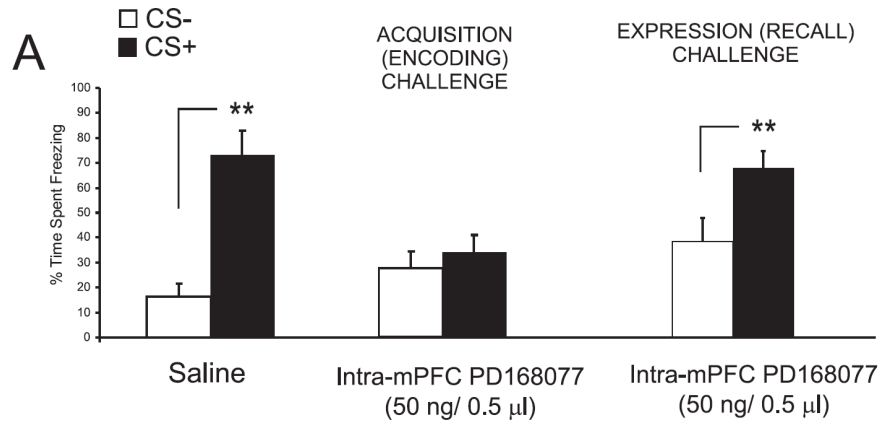
of the D₄ agonist PD 168077 ($n = 5$; 50 ng/0.5 μ l). In addition, we analyzed behavioural sensitivity to our higher, suprathreshold level of footshock (0.8 mA) comparing rats receiving microinfusions of either saline ($n = 6$) or the effective dose of the D₄ agonist PD 168077 ($n = 6$; 50 ng/0.5 μ l).

We compared physiological and behavioural reactions to footshock presentations as described previously (see Materials and Methods) (Laviolette and Grace, 2006a). For control groups receiving the subthreshold footshock presentations (0.4 mA), comparing saline control versus intra-mPFC PD 168077 pretreated groups revealed no significant differences in footshock-induced freezing behaviour ($t_{(4)} = 0.28$; $p > 0.5$) (Fig. 2.4C). Furthermore, no significant differences were observed in the number of rears between groups in response to footshock ($t_{(4)} = 1.29$; $p > 0.05$) (Fig. 2.4D), in the quantity of footshock-related jumping between groups ($t_{(4)} = 0.20$; $p > 0.5$) (Fig. 2.4E), and for defecation between groups during the footshock presentation session ($t_{(4)} = 0.23$; $p > 0.05$) (Fig. 2.4F). For control groups receiving the suprathreshold footshock presentations (0.8 mA), comparing saline control versus intra-mPFC PD 168077 pretreated groups revealed no significant differences in footshock-induced freezing behaviour ($t_{(5)} = 0.88$; $p > 0.5$) (Fig. 2.4C). Furthermore, no significant differences were observed in the number of rears between groups in response to footshock ($t_{(5)} = 0.92$; $p > 0.05$) (Fig. 2.4D), in the quantity of footshock-related jumping between groups ($t_{(5)} = 0.23$; $p > 0.5$) (Fig. 2.4E), and in the amount of defecation between groups during the footshock presentation session ($t_{(5)} = 0.18$; $p > 0.05$) (Fig. 2.4F).

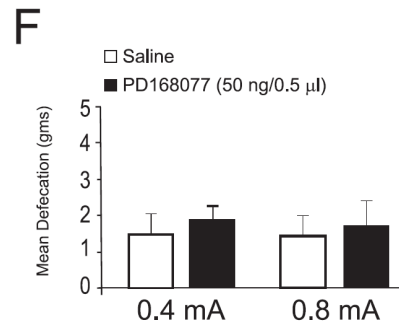
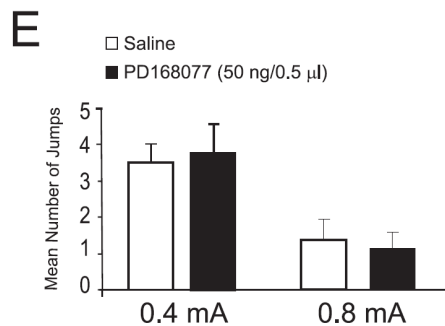
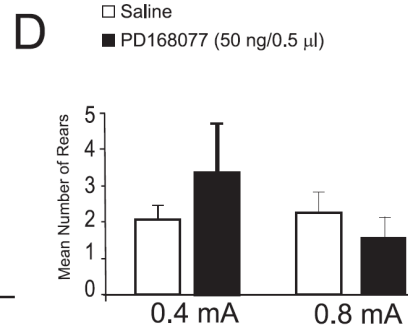
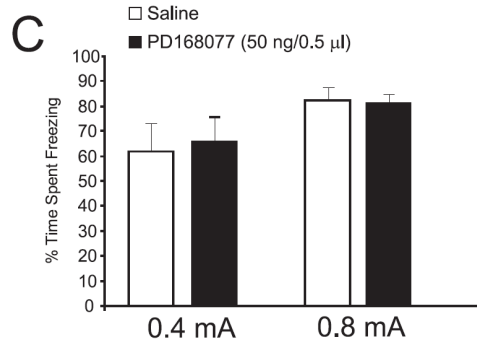
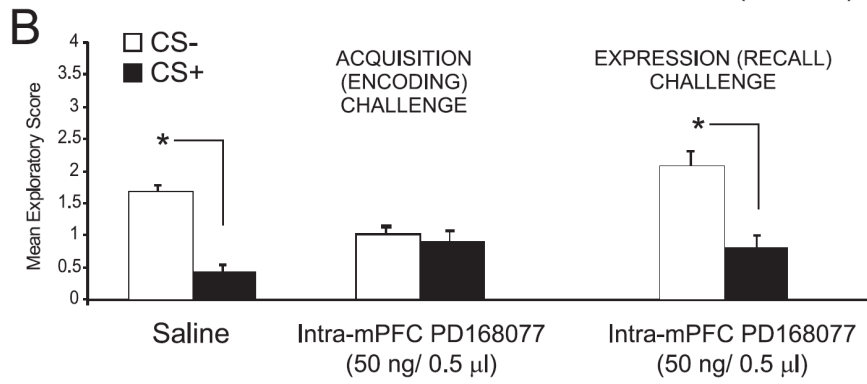
Figure 2.4 Intra-mPFC D₄ agonist effects on suprathreshold olfactory fear conditioning and footshock sensitivity analyses.

(A) Saline control rats showed significant freezing behaviour to CS+ versus CS- presentations after conditioning with suprathreshold footshock (0.8 mA) levels. In contrast, preconditioning microinfusion of intra-mPFC PD 168077 (50 ng/0.5 µl) blocked the acquisition of suprathreshold olfactory fear conditioning as demonstrated by no significant difference in the percentage of time spent freezing to the CS+ relative to the CS- at testing. However, intra-mPFC PD 168077 (50 ng/0.5 µl) microinfusions immediately before testing did not block the expression (recall) of suprathreshold olfactory fear conditioning. (B) Analysis of exploratory activity after CS+ or CS- presentation revealed that whereas saline control rats showed significant conditioned suppression of exploratory behaviour in response to CS+ versus CS- presentations, this effect was blocked in rats receiving intra-mPFC PD 168077 (50 ng/0.5 µl) immediately before conditioning. In contrast, intra-mPFC PD 168077 (50 ng/0.5 µl) administered immediately before testing did not block the expression (recall) of suprathreshold olfactory fear conditioning observed as a significant decrease in exploratory behaviour in response to CS+ presentations. (C) Footshock sensitivity testing (see Materials and Methods) revealed no significant differences between control groups receiving a subthreshold footshock (0.4 mA) versus intra-mPFC saline control ($n = 5$) or intra-mPFC PD 168077 (50 ng/µl; $n = 5$) or between control groups receiving a suprathreshold level of footshock (0.8 mA) versus intra-mPFC saline control ($n = 6$) or intra-mPFC PD 168077 (50 ng/µl, $n = 6$) in the percentage of time spent freezing in response to footshock presentations. (D) Similarly, no significant differences were observed in the mean number of rears between groups in response to either level of footshock. (E) No significant differences were observed between the mean numbers of jumps between groups in response to either level of footshock. (F) No significant differences were observed in the mean amount of defecation between groups in response to either level of footshock.

SUPRA-THRESHOLD FEAR CONDITIONING (0.8 mA)



SUPRA-THRESHOLD FEAR CONDITIONING (0.8 mA)



These behavioural and physiological indices are well established correlates of footshock-related sensitivity (Antoniadis and McDonald, 1999; Laviolette and Grace, 2006a) and demonstrate that activation of DA D₄ receptors with the highest effective dose of intra-mPFC PD 168077 (50 ng/0.5 µl) does not produce any appreciable alterations in physiological/peripheral sensitivity to subthreshold (0.4 mA) or suprathreshold (0.8 mA) footshock presentations. In addition, because the present studies used a context-independent form of associative emotional memory conditioning, our observed effects are not likely related to any effects of DA D₁ or D₄ modulation of spatial memory processing, as emotional associative memory encoding was linked specifically to olfactory cues, independent of spatial/environmental context, further underscoring the importance of DA D₁ and D₄ transmission during the encoding, modulation, and expression of associative learning and memory, specifically in the context of emotionally salient experience.

2.3.5 D₄ receptor mediated potentiation of emotional learning in the mPFC is dependent on the BLA

We have reported previously that a subpopulation of neurons within the mPFC that receive a direct, functional input from the BLA, encode, and express emotional associative learning (Laviolette et al., 2005; Laviolette and Grace, 2006a). Indeed, pharmacological inactivation of the BLA before single neuron associative conditioning in the mPFC blocks the encoding of this information (Laviolette et al., 2005; Laviolette and Grace, 2006a). To examine the potential role of the BLA in our observed potentiation of emotional learning via D₄ receptor activation within the

mPFC, we examined the effects of pharmacological inactivation of the BLA before administration of intra-mPFC PD68077 (50 ng/0.5 μ l) using a subthreshold level of footshock as the conditioning cue (0.4 mA). Bilateral microinfusions of the GABA_A receptor agonist muscimol ($n = 7$; 500 ng/0.5 μ l) or saline ($n = 7$) were performed in the BLA, immediately before intra-mPFC D₄ agonist microinfusions. Histological analysis confirmed the placement of intra-amygdala cannulae within the anatomical boundaries of the BLA (Fig. 2.5A–C). Analysis revealed that intra-BLA muscimol pretreatment (500 ng/0.5 μ l) significantly blocked the effects of intra-mPFC microinfusions of PD 168077 (50 ng/0.5 μ l) as demonstrated by no significant difference in the percentages of time spent freezing ($n = 7$; $t_{(6)} = 0.31$; $p > 0.05$) (Fig. 2.5D) or exploratory behaviour ($n = 7$; $t_{(6)} = 0.31$; $p > 0.05$) (Fig. 2.5E) in response to the CS+ versus CS– presentations, compared with saline control groups. Thus, the ability of mPFC D₄ receptor activation to potentiate subthreshold emotional associative learning is dependent on the BLA, suggesting an important role for BLA>mPFC inputs in the processing of emotional learning and memory within the mPFC, as reported previously (Laviolette et al., 2005; Laviolette and Grace, 2006a).

2.3.6 Effects of intra-mPFC DA D₁ receptor activation on the encoding of nonsalient versus salient emotional stimuli during associative fear conditioning

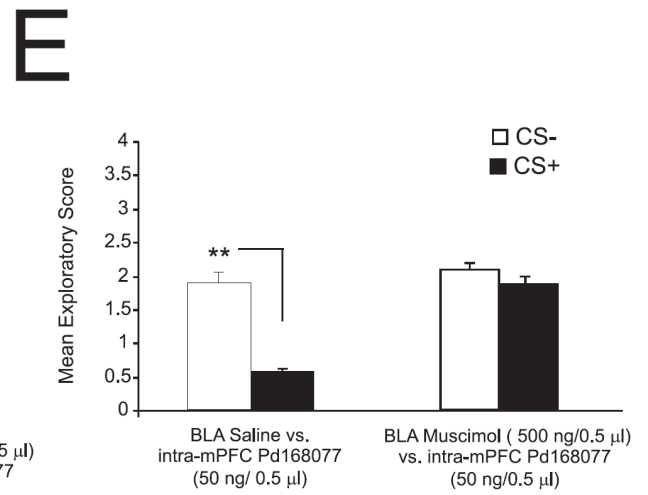
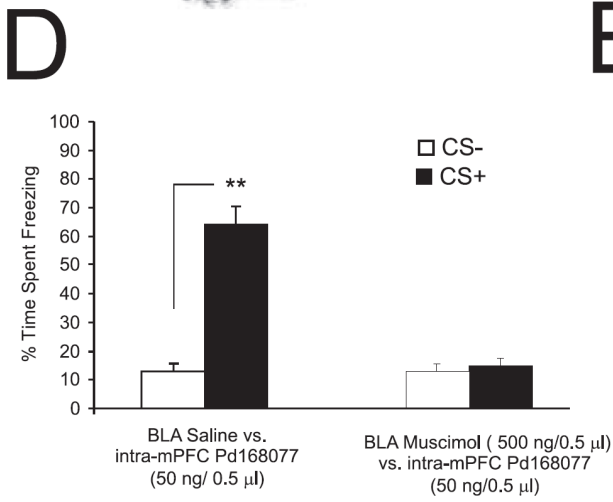
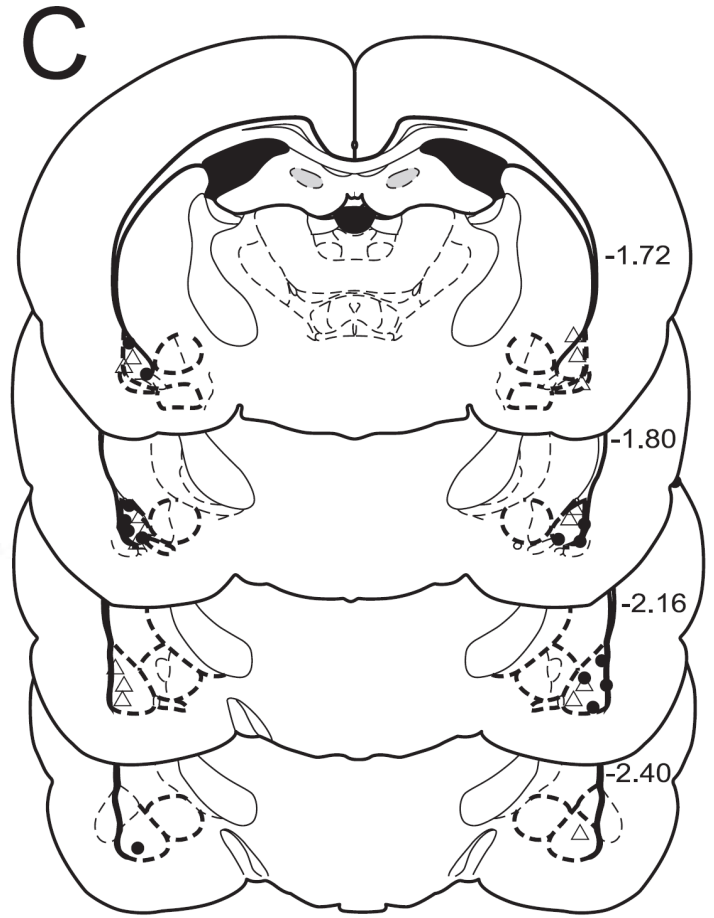
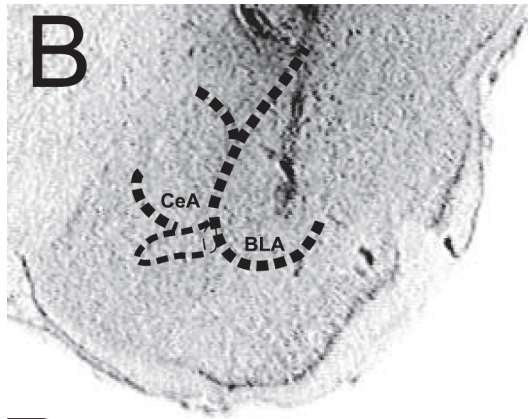
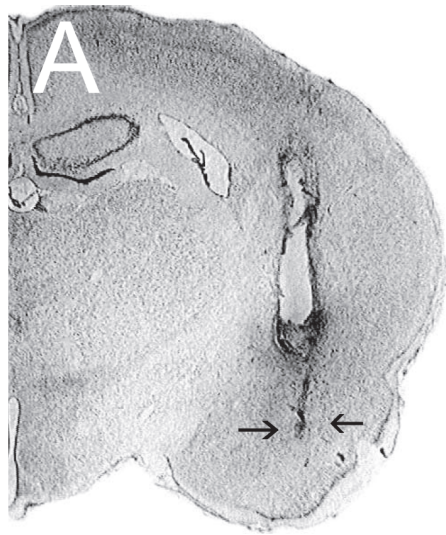
We next examined the potential role of mPFC DA D₁ receptor transmission during the encoding of emotionally nonsalient sensory information, by performing bilateral microinfusions of the DA D₁-like agonists (SKF 38393; 100 and 1000 ng/0.5 μ l) or SKF 81297 (100 ng/0.5 μ l) before conditioning rats with our subthreshold level of

footshock (0.4 mA). Initial pilot studies revealed that doses of intra-mPFC SKF 81297 higher than 100 ng/0.5 μ l produced seizures and/or aversive reactions in some animals; hence, 100 ng was the highest dose of this compound used for this and all subsequent experiments. For experiments using SKF 38393, ANOVA revealed no significant effect of treatment or group ($F_{(2,33)} = 0.68$; $p > 0.05$) on the acquisition of subthreshold fear conditioning as demonstrated by no significant difference in the time spent freezing ($n = 6$, 100 ng/0.5 μ l; $n = 6$, 1000 ng/0.5 μ l; $p > 0.05$) (Fig. 2.6A) or exploratory behaviour ($F_{(2,33)} = 0.97$; $p > 0.05$; $n = 6$, 100 ng/0.5 μ l; $n = 6$, 1000 ng/0.5 μ l; $p > 0.05$) (Fig. 2.6B) in response to the CS+ compared with CS- presentations.

Given that intra-mPFC D₁ receptor activation had no observable effect on fear conditioning to subthreshold (emotionally nonsalient) levels of footshock, we next investigated the possible role of D₁ receptor activation on the acquisition of suprathreshold (emotionally salient levels of footshock; 0.8 mA). Rats received either bilateral microinfusions of intra-mPFC SKF 38393 ($n = 8$; 1000 ng/0.5 μ l) or saline, immediately before olfactory fear conditioning with the suprathreshold footshock stimulus. ANOVA revealed a significant main effect of treatment on the percentage of time spent freezing in response to CS+ versus CS- presentations ($F_{(1,25)} = 83.2$; $p < 0.0001$) with *post hoc* analysis revealing that both SKF 38393 and saline pretreated groups displayed significantly greater amounts of freezing to the CS+, relative to CS- (Fig. 2.6C) ($p < 0.01$)

Figure 2.5 Histological analysis of intra-BLA cannulae placements and effects of BLA inactivation on D₄-mediated emotional associative learning potentiation.

(A) A microphotograph of a coronal section of the BLA showing a representative microinjection site (arrows). (B) Microphotograph of the BLA at higher magnification with a superimposed outline of the anatomical boundaries of the BLA relative to the adjacent central nucleus of the amygdala (CeA). (C) Representative schematic diagram showing representative intra-BLA microinjection sites: Δ , BLA saline control placements; \bullet , intra-BLA muscimol (500 ng/0.5 μ l) injector locations. (D) Intra-BLA saline administration before intra-mPFC PD 168077 (50 ng/0.5 μ l) had no significant effect on the ability of mPFC D₄ receptor activation to potentiate subthreshold (0.4 mA) olfactory fear-conditioning freezing behaviour, as rats displayed significant levels of freezing after CS+ presentations. Intra-BLA muscimol administration (500 ng/0.5 μ l) before intra-mPFC PD 168077 (50 ng/0.5 μ l) significantly blocked the ability of mPFC D₄ receptor activation to potentiate subthreshold olfactory fear conditioning, as rats displayed no differences in freezing behaviour to CS+ versus CS- presentations. (E) Similar to the effects observed with freezing behaviour, intra-BLA saline did not affect the ability of intra-mPFC D₄ activation to potentiate emotional associative learning, as rats showed significantly greater conditioned suppression of exploratory behaviour in response to CS+ relative to CS- presentations. In contrast, this associative response was blocked in rats receiving intra-BLA muscimol before intra-mPFC PD 168077 (50 ng/0.5 μ l).



Analysis of spontaneous exploratory behaviour revealed a significant group by treatment interaction on mean exploratory behaviour scores after CS+ versus CS- presentations ($F_{(1,25)} = 3.8$; $p < 0.01$), with *post hoc* analysis revealing that both SKF 38393 and saline pretreated groups displayed significantly attenuated exploratory behaviour in response to CS+ versus CS- presentations (Fig. 2.6D) ($p < 0.01$).

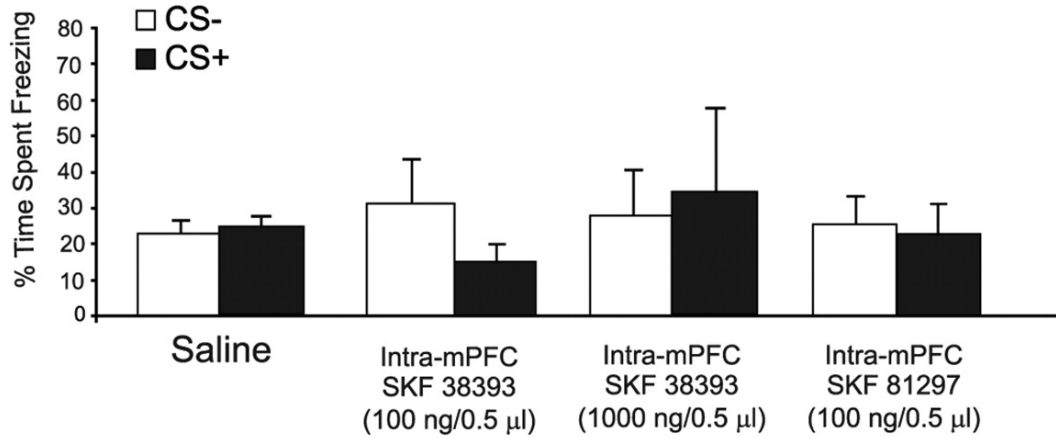
2.3.7 Effects of intra-mPFC DA D₁ receptor activation on the expression (recall) of emotionally salient associative fear conditioning

Given that intra-mPFC D₁ receptor activation produced no observable effects on the encoding of emotional associative learning using a nonsalient or salient footshock stimuli (Fig. 2.6), we next examined the potential role of mPFC D₁ receptor transmission on the expression (recall) phase of emotional associative learning, using a suprathreshold, emotionally salient level of footshock (see Materials and Methods) as the conditioning stimulus. We administered either intra-mPFC SKF 38393 (100 and 1000 ng/0.5 μ l) or the full D₁ receptor agonist SKF 81297 (1–100 ng/0.5 μ l) directly before the testing phase of olfactory fear conditioning (24 h after conditioning). For experiments using SKF 38393, ANOVA revealed an interaction between treatment and group ($F_{(3, 50)} = 5.81$; $p < 0.01$). *Post hoc* analysis revealed that rats pretreated with either saline ($n = 8$) or a lower dose of SKF 38393 (10 ng/0.5 μ l) demonstrated significant fear conditioning at testing, with greater amounts of time spent freezing to CS+ relative to CS- presentations ($p < 0.01$).

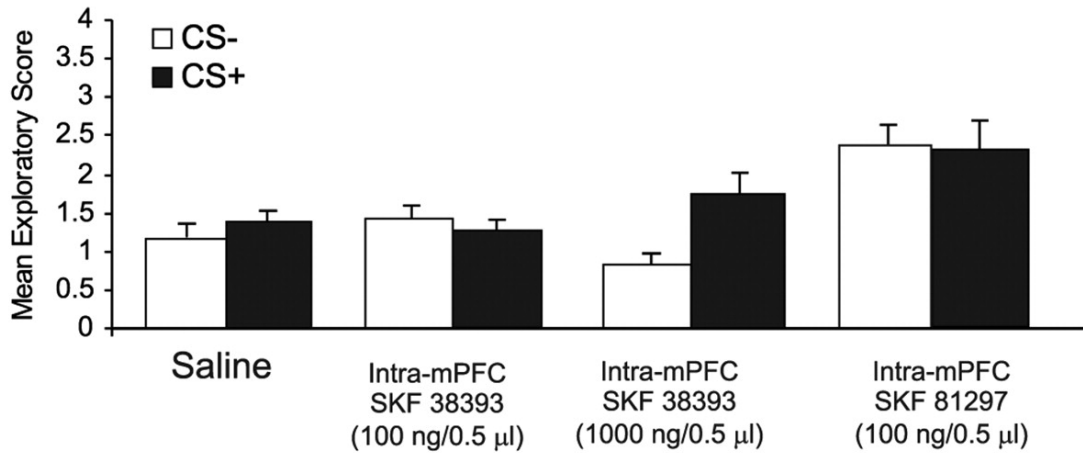
Figure 2.6 Behavioural effects of bilateral intra-mPFC DA D₁ receptor activation on the acquisition of salient or nonsalient associative fear conditioning memory.

(A) Intra-mPFC DA D₁ agonist SKF 38393 (100 and 1000 ng/0.5 µl; $n = 7$ and $n = 8$, respectively) had no effect on the percentage of time spent freezing to CS+ versus CS- presentations after olfactory fear conditioning with subthreshold footshock levels (0.4 mA). Bilateral intra-mPFC administration of another D₁ receptor agonist, SKF 81297 (100 ng/0.5 µl), similarly had no effect on conditioning to a subthreshold level of footshock. (B) Intra-mPFC SKF 38393 (100 and 1000 ng/0.5 µl) had no effect on mean exploratory scores after CS+ versus CS- presentations after olfactory fear conditioning with subthreshold footshock stimuli. Bilateral intra-mPFC administration of the D₁ receptor agonist SKF 81297 (100 ng/0.5 µl) similarly had no effect on exploratory scores after CS+ or CS- presentations when conditioned with this subthreshold footshock level. (C) Intra-mPFC saline control and intra-mPFC SKF 38393 (1000 ng/0.5 µl) groups displayed a significant increase in freezing in response to CS+ versus CS- presentations after fear conditioning with suprathreshold footshock levels (0.8 mA). (D) In addition, intra-mPFC saline control and intra-mPFC SKF 38393 (1000 ng/0.5 µl) groups displayed a significant decrease in exploratory behaviour in response to CS+ versus CS- presentations after fear conditioning with suprathreshold levels of footshock.

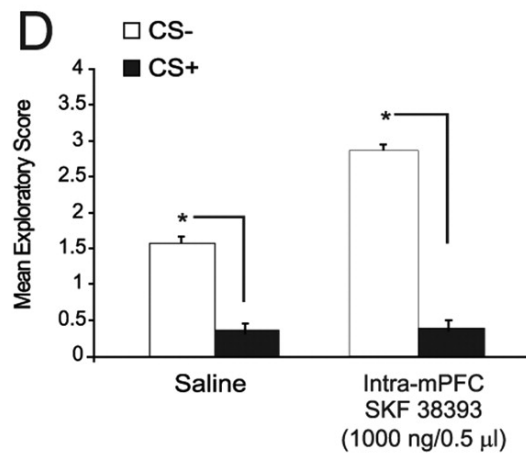
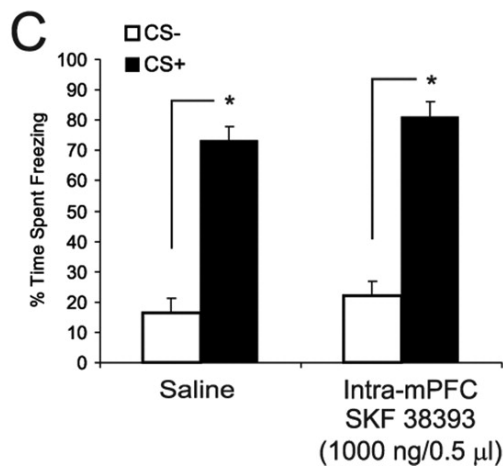
A SUB-THRESHOLD FEAR CONDITIONING (0.4 mA)



B SUB-THRESHOLD FEAR CONDITIONING (0.4 mA)



SUPRA-THRESHOLD FEAR CONDITIONING (0.8 mA)

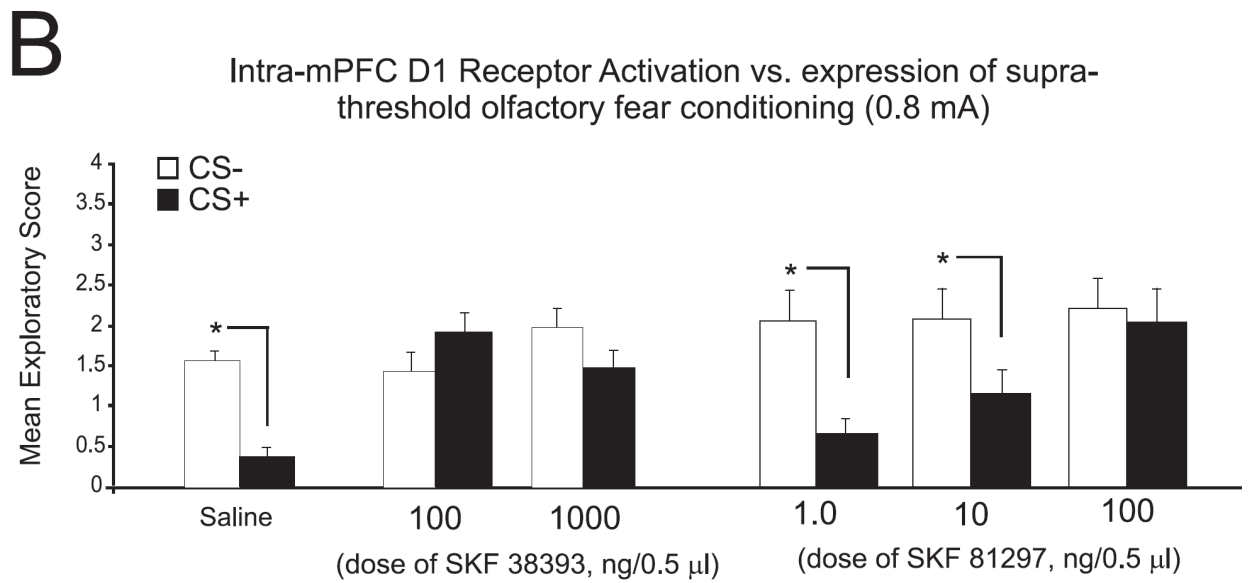
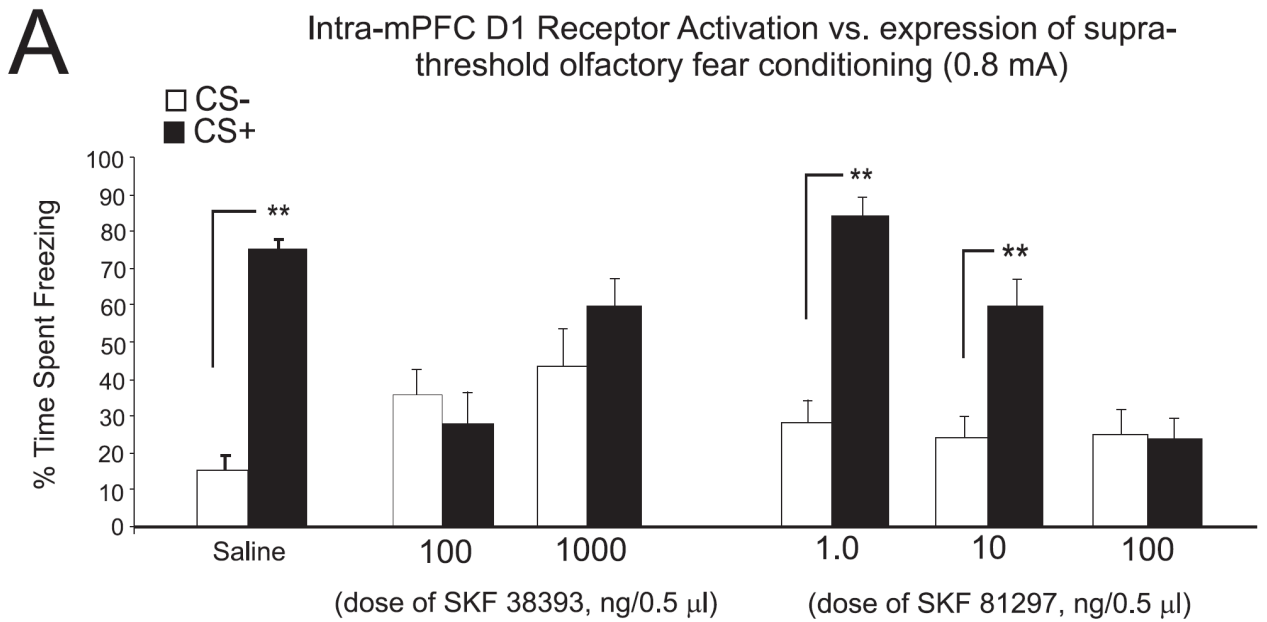


In contrast, higher doses of intra-mPFC SKF 38393 produced a complete block of associative emotional memory recall, demonstrated by no significant difference in times spent freezing to CS+ versus CS- presentations (100 ng/0.5 μ l, $n = 7$; 1000 ng/0.5 μ l, $n = 8$; $p > 0.05$) (Fig. 2.7A). Analysis of exploratory behaviour revealed a significant interaction between group and treatment ($F_{(3,57)} = 3.71$; $p < 0.01$) with *post hoc* analysis revealing that groups treated with saline ($n = 8$) or a lower dose of SKF 38393 ($n = 8$; 10 ng/0.5 μ l) displayed significant conditioned suppression of exploratory behaviour in response to CS+ versus CS- presentations ($p < 0.05$), whereas this effect was blocked in groups receiving the higher doses of intra-mPFC SKF 38393 (100 ng/0.5 μ l, $n = 7$; 1000 ng/0.5 μ l, $n = 8$; $p > 0.05$) (Fig. 2.7B) in response to CS+ versus CS- presentations. For experiments using SKF 81297 (1–100 ng/0.5 μ l), ANOVA revealed an interaction between treatment and group ($F_{(3, 59)} = 6.4$; $p < 0.001$). *Post hoc* analysis revealed that rats pretreated with either saline ($n = 6$) or the lower doses of SKF 81297 (1 or 10 ng/0.5 μ l; $n = 8$ for both groups) demonstrated significant fear conditioning at testing, with greater amounts of time spent freezing to CS+ relative to CS- presentations ($p < 0.01$). In contrast, higher doses of intra-mPFC SKF 81297 (100 ng/0.5 μ l) produced a complete block of associative emotional memory recall, demonstrated by no significant difference in times spent freezing to CS+ versus CS- presentations ($n = 8$; $p > 0.05$) (Fig. 2.7A). For groups receiving intra-mPFC SKF 38393, analysis of exploratory behaviour revealed a significant interaction between group and treatment ($F_{(3,57)} = 3.71$; $p < 0.01$) with *post hoc* analysis revealing that groups treated with saline ($n = 8$) or a lower dose of SKF 38393 ($n = 8$; 10 ng/0.5 μ l) displayed significant conditioned

suppression of exploratory behaviour in response to CS+ versus CS- presentations ($p < 0.05$), whereas this effect was blocked in groups receiving the higher doses of intra-mPFC SKF 38393 (100 ng/0.5 μ l, $n = 7$; 1000 ng/0.5 μ l, $n = 8$; $p > 0.05$) (Fig. 2.7B) in response to CS+ versus CS- presentations. For groups receiving intra-mPFC SKF 81297, analysis of exploratory behaviour revealed a significant main effect of treatment ($F_{(3,59)} = 19.5$; $p < 0.0001$) with *post hoc* analysis revealing that groups treated with saline ($n = 6$) or the lower doses of SKF 81297 (1 or 10 ng/0.5 μ l; $n = 8$) displayed significant conditioned suppression of exploratory behaviour in response to CS+ versus CS- presentations ($p < 0.05$), whereas this effect was blocked in groups receiving the higher doses of intra-mPFC SKF 81297 (100 ng/0.5 μ l; $n = 8$) (Fig. 2.7B) in response to CS+ versus CS- presentations. Thus, in contrast to our observations with intra-mPFC DA D₄ receptor modulation, pharmacological activation of mPFC D₁ receptors produced no effect on the encoding of salient or nonsalient fear-conditioning stimuli during the acquisition phase of associative learning (Fig. 2.6) but dose-dependently blocks the expression (recall) of previously encoded emotional associative memories (Fig. 2.7).

Figure 2.7 Behavioural effects of bilateral intra-mPFC DA D₁ receptor activation on the expression (recall) of suprathreshold associative fear-conditioning memory.

(A) Rats receiving intra-mPFC saline displayed normal expression (recall) of suprathreshold (0.8 mA footshock) fear conditioning when administered saline immediately before testing. In contrast, administration of a D₁ agonist (SKF 38393; 100–1000 ng/0.5 µl) immediately before testing completely blocked the expression (recall) of previously acquired emotional learning, with rats displaying no significant difference in freezing behaviour in response to CS+ relative to CS– presentations. Similarly, bilateral intra-mPFC administration of the full D₁ receptor agonist SKF 81297 (1–100 ng/0.5 µl) produced a dose-dependent attenuation of emotional memory recall at the highest dose of 100 ng, but not at subthreshold doses (1–10 ng/0.5 µl). (B) Similar effects were observed in conditioned suppression of exploratory behaviour scores after CS+ versus CS– presentations, with intra-mPFC D₁ receptor activation blocking conditioned suppression of exploratory behaviour after pretesting microinfusions of SKF 38393 (100–1000 ng/0.5 µl), relative to saline controls. Bilateral intra-mPFC administration of another D₁ receptor agonist, SKF 81297 (1–100 ng/0.5 µl), also produced a dose-dependent attenuation of emotional memory recall reflected in conditioned suppression of exploratory behaviour, at the highest dose of 100 ng but not at subthreshold doses of 1–10 ng/0.5 µl.



2.4 DISCUSSION

Evidence from behavioural, electrophysiological, and neuroanatomical studies suggests a complex role for DA transmission within the mPFC during the processing of emotional and cognitive information. Studies examining executive processes such as set-shifting and cognitive flexibility suggest that optimal levels of D₁ or D₂-like receptor activity are essential for normal cognition and memory function. For example, supranormal stimulation of either D₁ or D₄ receptors within the mPFC impairs spatial working memory and/or performance on set-shifting tasks in rodents (Zahrt et al., 1997; Floresco et al., 2006). Anomalies in D₄ receptor transmission have been reported also in schizophrenia (Seeman et al., 1993; Stefanis et al., 1998). Nevertheless, little is known about the relative roles of mPFC DA D₁ versus D₄ receptor transmission specifically in the context of emotional learning and memory processing.

2.4.1 DA D₄ receptors modulate the encoding of salient and nonsalient emotional information in the mPFC

Given the ability of D₄ receptor activation to amplify DA-mediated neuronal activity within the mPFC (Ceci et al., 1999), we hypothesized that activation of mPFC D₄ receptors may potentiate the emotional salience of normally subthreshold sensory stimuli, leading to heightened emotional memory encoding. Our results demonstrate that activation of mPFC D₄ receptors potentiates the emotional salience of subthreshold fear-conditioning stimuli during acquisition but not expression (recall) phases of associative learning. However, once associative memories are encoded, D₄

manipulations no longer appear capable of modulating these memories, consistent with findings in single mPFC neurons (Laviolette et al., 2005). Whereas future studies are required to examine the precise neuronal basis of this effect, recent *in vivo* electrophysiological evidence demonstrates that D₄ receptor agonists can decrease feedforward inhibition on mPFC neurons receiving input from the BLA (Floresco and Tse, 2007), suggesting the possibility that our observed potentiation of emotional associative memory may be attributable to increasing associative information input from the BLA to the mPFC, in effect “priming” neurons in the mPFC to receive emotionally salient associative information from the BLA. We have reported previously that blockade of the D₄ receptor within the mPFC can block the encoding of emotionally salient olfactory associative learning (Laviolette et al., 2005). This may suggest that the encoding of emotionally salient memories requires an optimal level of DA D₄ receptor stimulation as either overstimulation or blockade of intra-mPFC D₄ receptor transmission can inhibit the encoding of this information.

The observed emotional memory potentiating effects of D₄ receptor activation are intriguing given previous evidence linking D₄ receptor activation with bidirectional modulation of α -Ca²⁺/calmodulin-dependent protein kinase II (α -CaMKII), a molecule involved in synaptic plasticity, learning, and memory (Malenka and Nicoll, 1999; Frankland et al., 2001). Gu and Yan (2004) have reported that within *in vitro* slice preparations of rodent mPFC, D₄ receptor activation strongly increases α -CaMKII activity during low baseline levels of neuronal activity. In contrast, during high levels of neuronal activity, D₄ agonist application causes a

marked decrease in α -CaMKII levels, demonstrating that D₄ receptor signaling within the mPFC bidirectionally modulates α -CaMKII activity as a function of mPFC neuronal activity levels. Thus, one possibility is that our observed potentiation of normally nonsalient emotional stimuli (Fig. 2.3) may be related to the ability of D₄ receptor activation to increase endogenous levels of α -CaMKII during periods of low mPFC neuronal activity (as may be predicted during exposure to subthreshold footshock). Conversely, the ability of mPFC D₄ receptor activation to block emotional memory formation to suprathreshold, emotionally salient footshock stimuli (Fig. 2.4 A,B) may be related to D₄ receptor-mediated attenuation of endogenous α -CaMKII levels, specifically during periods of high neuronal activity within the mPFC (as would be predicted after exposure to higher footshock levels) (Gu and Yan, 2004; Gu et al., 2006).

The present results demonstrate a critical role for prefrontal cortical DA D₄ receptor signaling during the encoding phase of emotional associative memory and suggest that perturbations in this system may result in inappropriate associative encoding of sensory stimuli via D₄ receptor-mediated modulation of emotional salience variables. Given that similar psychopathological symptoms are present in syndromes such as schizophrenia, autism, and posttraumatic stress disorder, the present results may implicate abnormalities in prefrontal cortical DA D₄ transmission in the emotional processing and memory disturbances observed in such disorders.

Previous studies suggest a functional link between the BLA and mPFC both in terms of modulating DA-mediated mPFC neuronal network activity and in regulating

associative encoding within mPFC neurons (Laviolette et al., 2005, 2006a; Floresco et al., 2007). We found that BLA inactivation prevented mPFC D₄ agonist-mediated potentiation of memory for nonsalient fear stimuli, suggesting that mPFC D₄ modulation of emotional learning depends on functional amygdala input. Although the present studies do not differentiate between ascending versus descending pathways within the BLA↔mPFC circuit, given that mPFC neurons that respond to BLA inputs are involved in emotional memory encoding in mPFC neurons, BLA inactivation may prevent the flow of first-order emotional salience information from the amygdala to the mPFC, thereby blocking any potentiating effects of D₄ receptor activation within the mPFC, as suggested by previous studies (Laviolette et al., 2005; Laviolette and Grace, 2006a).

2.4.2 DA D₁ receptors modulate the expression (recall), but not the encoding, of emotionally salient associative information in mPFC

At the behavioural systems level, systemic or intra-mPFC activation of D₁ receptors impairs working memory performance (Zahrt et al., 1997) and delayed response performance (Cai and Arnsten, 1990; Arnsten et al., 1994). Although few studies have examined directly the role of mPFC D₁ receptor transmission during the encoding and/or recall of emotionally salient associative memories, current theories of mPFC DA function have suggested that D₁ receptor transmission may be crucial for the preservation and recall of memories over temporal delay periods (Durstewitz and Seamans, 2008). For example, Seamans et al. (1998) reported that blockade of mPFC D₁ receptors blocked the ability of rodents to use previously acquired spatial memory

during a delayed performance radial arm task, while having no effect on non-delayed memory recall. These studies suggest that executive memory functions within mPFC neuronal networks may require an optimal level of DA D₁ signaling, specifically during periods of memory recall, rather than during associative memory encoding. Such a role for mPFC D₁ signaling is consistent with our findings that whereas D₁ receptor activation had no influence on the acquisition (encoding phase) of emotional memory, activation of D₁ receptors potentially blocked the ability to recall emotional associative memory during the expression (test phase) of this learning. Our results suggest that D₁ receptor-mediated inhibition of mPFC pyramidal neuron activity may be a mechanism whereby recall of emotionally salient information can be suppressed through DAergic modulation of mPFC neuronal associative memory networks.

In terms of DA D₁ receptor-mediated control of mPFC neuronal network activity, behavioural and electrophysiological evidence implicates DA D₁ receptor transmission in the modulation of inhibitory, GABAergic interneurons. Specifically, activation of mPFC D₁ receptors is reported to increase feedforward GABAergic inhibition on mPFC pyramidal neurons (Seamans et al., 2001; Seamans and Yang, 2004). However, because single neurons within the mPFC are capable of actively encoding emotional associative learning during the acquisition phase (Laviolette et al., 2005; Laviolette and Grace, 2006a), one prediction would be that activation of mPFC D₁ receptors, which is known to increase feedforward inhibition on mPFC pyramidal neurons (Seamans et al., 2001; Gorelova et al., 2002), also would block associative memory encoding during the acquisition phase of learning. Instead, we

observed no blockade of emotional associative learning acquisition after intra-mPFC D₁ receptor activation (Fig. 2.6), further demonstrating a functional dissociation in mPFC DA D₁ function during the acquisition (encoding) versus recall phases of emotional learning and memory.

2.5 CONCLUSION

Our results demonstrate distinct functional roles for DA D₁ versus D₄ receptor subtypes during the encoding versus recall phases of emotional associative memory within the mPFC and demonstrate a functional role for DA D₄ transmission in the modulation of the emotional salience value of sensory stimuli during associative learning and memory processing. Although the present experiments focused primarily on the prelimbic (PLC) division of the mPFC, during the acquisition and expression (recall) phases of emotional associative memory, considerable evidence points to a functional dissociation between the prelimbic and infralimbic divisions of the mPFC during the acquisition versus extinction of emotional memory (Quirk and Mueller, 2008; Santini et al., 2008), such that neurons within the PLC appear critical for emotional memory acquisition whereas infralimbic neurons appear critical for extinction-related learning. The present results, although not directly examining the role of the infralimbic cortex in DA-related emotional memory acquisition and recall, also demonstrate an important role for the PLC in the encoding phase of emotional associative memory.

Future studies are required to examine the potential roles of DA D₁ versus D₄ receptor transmission during the extinction phase of emotional memory processing. A clearer understanding of the roles of DA D₁ versus D₄ receptor transmission within the mPFC during the processing of emotionally salient associative learning and memory may help elucidate how abnormalities in mPFC DA transmission may lead to disturbed emotional processing and learning present in disorders such as schizophrenia and autism.

2.6 REFERENCES

- Antoniadis EA, McDonald RJ (1999) Discriminative fear conditioning to context expressed by multiple measures of fear in the rat. *Behav Brain Res* 101:1–13.
- Arnsten AFT, Cai JX, Murphy BL, Goldman-Rakic PS (1994) Dopamine D1 receptor mechanisms in the cognitive performance of young adult and aged monkeys. *Psychopharmacology* 116:143–151.
- Cai JX, Arnsten AFT (1990) Dose-dependent effects of the dopamine D1 receptor agonists A77636 or SKF81297 on spatial working memory in aged monkeys. *J Pharmacol Exp Ther* 282:1–7.
- Ceci A, Brambilla A, Duranti P, Grauert M, Grippa N, Borsini F (1999) Effect of antipsychotic and selective dopaminergic antagonists on dopamine-induced facilitatory activity in prefrontal cortical pyramidal neurons: an *in vitro* study. *Neuroscience* 93:107–115.
- Durstewitz D, Seamans JK (2008) The dual-state theory of prefrontal cortex dopamine function with relevance to catechol-O-methyltransferase genotypes and schizophrenia. *Biol Psychiatry* 64:739–749.
- Floresco SB, Tse MT (2007) Dopaminergic regulation of inhibitory and excitatory transmission in the basolateral amygdala-prefrontal cortical pathway. *J Neurosci* 27:2045–2057.
- Floresco SB, Magyar O, Ghods-Sharifi S, Vexelman C, Tse MTL (2006) Multiple dopamine receptor subtypes in the medial prefrontal cortex of the rat regulate set-shifting. *Neuropsychopharmacology* 31:297–309.
- Frankland PW, O'Brien C, Ohon C, Kirkwood A, Silva AJ (2001) Alpha-CamKII-dependent plasticity in the cortex is required for permanent memory. *Nature* 411:309–313.
- Goldman-Rakic PS (1995) Cellular basis of working memory. *Neuron* 14:477–485.
- Goldman-Rakic PS, Leranthi C, Williams SM, Mons N, Geffard M (1989) Dopamine synaptic complex with pyramidal neurons in primate cerebral cortex. *Proc Natl Acad Sci U S A* 86:9015–9019.
- Gorelova N, Seamans JK, Yang C (2002) Mechanisms of dopamine activation of fast-spiking interneurons that exert inhibition in rat prefrontal cortex. *J Neurophysiol* 88:3150–3166.
- Gu Z, Yan Z (2004) Bidirectional regulation of Ca²⁺/calmodulin-dependent protein kinase II activity by dopamine D4 receptors in prefrontal cortex. *Mol Pharmacol* 66:948–955.
- Gu Z, Jiang Q, Yuen EY, Yan Z (2006) Activation of dopamine D4 receptors induces synaptic translocation of CaMKII in cultured prefrontal cortical neurons. *Mol Pharmacol* 69:813–822.

- Laviolette SR, Grace AA (2006a) Cannabinoids potentiate emotional learning plasticity in neurons of the medial prefrontal cortex through basolateral amygdala inputs. *J Neurosci* 26:6458–6468.
- Laviolette SR, Grace AA (2006b) The roles of cannabinoid and dopamine receptor systems in neural emotional learning circuits: implications for schizophrenia and addiction. *Cell Mol Life Sci* 63:1597–1613.
- Laviolette SR, Lipski WJ, Grace AA (2005) A subpopulation of neurons in the medial prefrontal cortex encodes emotional learning with burst and frequency codes through a dopamine D4 receptor-dependent basolateral amygdala input. *J Neurosci* 25:6066–6075.
- Levant B, Moehlecamp JD, Morgan KA, Leonard NL, Cheng CC (1996) Modulation of [3H] quinpirole binding in brain by monoamine oxidase inhibitors: evidence for a potential novel binding site. *J Pharmacol Exp Ther* 278:145–153.
- Malenka RC, Nicoll RA (1999) Long-term potentiation: a decade of progress? *Science* 285:325–334.
- Mrzljak L, Bergson C, Pappy M, Huff R, Levenson R, Goldman-Rakic PS (1996) Localization of dopamine D4 receptors in GABAergic neurons of the primate brain. *Nature* 381:245–248.
- Onn SP, Wang XB, Grace AA (2006) Dopamine D1 and D4 receptor subtypes differentially modulate recurrent excitatory synapses in prefrontal cortical pyramidal neurons. *Neuropsychopharmacology* 31:318–338.
- Paxinos G, Watson C (1996) *The rat brain in stereotaxic coordinates*, Ed 2. San Diego: Academic.
- Pezze MA, Bast T, Feldon J (2003) Significance of dopamine transmission in the rat medial prefrontal cortex for conditioned fear. *Cereb Cortex* 13:371–380.
- Quirk GJ, Mueller D (2008) Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmacology* 33:56–72.
- Rosenkranz JA, Grace AA (2003) The prefrontal cortex regulates lateral amygdala neuronal plasticity and responses to previously conditioned stimuli. *J Neurosci* 23:11054–11064.
- Santini E, Quirk GJ, Porter JT (2008) Fear conditioning and extinction differentially modify the intrinsic excitability of infralimbic neurons. *J Neurosci* 28:4028–4036.
- Seamans JK, Yang CR (2004) The principal features and mechanisms of dopamine modulation in the prefrontal cortex. *Prog Neurobiol* 74:1–57.
- Seamans JK, Floresco SB, Phillips AG (1998) D1 receptor modulation of hippocampal–prefrontal cortical circuits integrating spatial memory with executive functions in the rat. *J Neurosci* 18:1613–1621.
- Seamans JK, Gorelova N, Durstewitz D, Yang CR (2001) Bidirectional dopamine modulation of GABAergic inhibition in prefrontal cortical pyramidal neurons. *J Neurosci* 21:3628–3638.

- Seeman P, Van Tol HH (1994) Dopamine receptor pharmacology. *Trends Pharmacol Sci* 15:264–270.
- Seeman P, Guan HC, Van Tol HH (1993) Dopamine D4 receptors are elevated in schizophrenia. *Nature* 365:441–445.
- Stefanis NC, Bresnick JN, Kerwin RW, Schofield WN, McAllister G (1998) Elevation of D4 dopamine receptor mRNA in postmortem schizophrenic brain. *Brain Res Mol Brain Res* 53:112–119.
- Wedzony K, Chocyk A, Mackowiak M, Fijal K, Czyrak A (2000) Cortical localization of dopamine D4 receptors in the rat brain: immunocytochemical study. *J Physiol Pharmacol* 51:205–221.
- Zahrt J, Taylor JR, Mathew RG, Arnsten AF. Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance. *J Neurosci.* 1997 Nov 1;17(21):8528-35.

Chapter 3:

3 Dopamine D₄ Receptor Transmission in the Prefrontal Cortex Controls the Salience of Emotional Memory via Modulation of Calcium Calmodulin–Dependent Kinase II

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3.1 INTRODUCTION

Functional disturbances in frontal cortical DA transmission and CaMKII signaling have been implicated in the pathophysiology of neuropsychiatric disorders such as schizophrenia and ADHD (Frankland et al. 2008; Lauzon and Laviolette 2010). Indeed, DAergic receptor-mediated modulation of the α and β isoforms of CaMKII has been shown to be functionally important in the context of animal models of psychosis (Greenstein et al. 2007; Yamasaki et al. 2008; Novak and Seeman 2010). However, the mechanisms by which DA and CaMKII signaling pathways may interact at the systems level to modulate emotional processing are not currently understood. The DA D_4 receptor subtype is highly expressed in medial prefrontal cortex (mPFC) (Ariano et al. 1997; De Almeida and Mengod 2010) and plays a crucial modulatory role during the acquisition (encoding) of fear memory (Laviolette et al. 2005). Using an olfactory fear conditioning procedure, we have demonstrated that activation of D_4 receptors within the prelimbic cortex (PLC) division of the mPFC potentiates the emotional salience of subthreshold fear conditioning stimuli during acquisition but not expression (recall) phases of emotional learning (Lauzon et al. 2009). In addition, activation of PLC D_4 receptors blocks the acquisition of emotionally salient suprathreshold fear memories, demonstrating a fundamental role for D_4 receptor signaling during the acquisition of emotional memory (Lauzon et al. 2009; Lauzon and Laviolette 2010).

Previous work by Gu et al. (2004, 2006) have shown that activation of cortical D_4 receptors dynamically and bidirectionally modulate levels of α -CaMKII, a

molecule essential for regulating several postsynaptic targets involved in learning and memory (Malenka and Nicoll 1999; Frankland et al. 2001; Gu and Yan 2004; Gu et al. 2006). Specifically, they reported that D₄ receptor activation strongly increases α -CaMKII activity, resulting from stimulation of phospholipase-C which in turn increases intracellular Ca²⁺ through inositol-1,4,5-triphosphate. Interestingly, the D₄ receptor-mediated elevation in α -CaMKII activity was only observed during spontaneously low levels of neuronal activity within the mPFC. In contrast, during high neuronal activation in the mPFC, activation of D₄ receptors led to a decrease in α -CaMKII activity, a process mediated via decreased PKA, which in turn increased protein phosphatase-1 (PP1) levels, ultimately resulting in dephosphorylation and reduced activity of α -CaMKII.

We hypothesized that intra-PLC D₄ receptor signaling may control the salience of emotional memory by coregulating CaMKII levels. Specifically, using an olfactory fear conditioning assay in rats, we tested whether intra-PLC D₄ receptor activation may amplify the emotional salience of normally nonsalient fear stimuli via upregulation of CaMKII levels. Furthermore, we examined whether D₄ receptor-mediated blockade of suprathreshold emotional fear memory may depend upon indirect modulation of CaMKII signaling via actions on the PP1 and the cAMP/PKA signaling pathways. We report that blockade of CaMKII signaling by either direct inhibition of CaMKII or blockade of CaMKII autophosphorylation prevents the ability of intra-PLC D₄ receptor activation to potentiate emotional memory. In contrast, activation of cAMP/PKA signaling or inhibition of PP1 rescues D₄ receptor-

mediated blockade of suprathreshold emotional fear memory acquisition, demonstrating bidirectional control of fear memory acquisition and salience via distinct signaling pathways within the PLC.

3.2 MATERIALS AND METHODS

3.2.1 Animals and surgery

All procedures were performed in accordance with the Canadian Council on Animal Care and approved by the University of Western Ontario's Council on Animal Care. Male Sprague Dawley rats (300–350 gm; Charles River) were anesthetized with a ketamine (80 mg/ml)–xylazine (6 mg/kg) mixture intraperitoneally and placed in a stereotaxic device. Stereotaxic coordinates were based upon the atlas of Paxinos and Watson (1996). Two stainless steel guide cannulae (22 gauge) were implanted into the PLC division of the mPFC using the following stereotaxic coordinates (15° angle; in mm from bregma): anteroposterior (AP) +2.9, lateral (LAT) ±1.9, ventral (V) –3.0 from the dural surface (see Fig. 3.1B). Jeweler's screws and dental acrylic were used to secure the cannulae.

3.2.2 Olfactory fear conditioning

Procedures for olfactory fear conditioning have been described previously (Chapter 2). Olfactory fear conditioning took place in 1 of 2 distinct environments, counterbalanced within groups: environment A was a 30" × 30" Plexiglas box with black stripes on a white background, whereas environment B had black dots on a white background with a grid shock floor. Testing 24 h later took place in 1 of 2

alternate environments, in which animals had not previously received electric shock, counterbalanced within groups. On day 1 (habituation phase), animals were habituated to a random combination of shock environment A or B and test environment A or B in a counterbalanced order for 30 min in each environment. On day 2 (conditioning phase), animals were returned to the conditioning room and placed in the previously assigned shock environment. During conditioning, one of the odours (almond or peppermint) was presented to the animal for 19 s and a footshock was delivered (0.4 mA or 0.8 mA) for 1 s. These 2 different levels of footshock (0.4 mA and 0.8 mA) correspond to subthreshold and suprathreshold levels of fear conditioning stimuli, as reported previously (Laviolette and Grace 2006). One hundred and twenty seconds later, the alternate odour was presented for 20 s (CS⁻) in the absence of footshock. This cycle was repeated 5 times. On the following day (test phase), rats were returned to the test room and placed in the previously assigned test environment. Before odour presentation, the rat was allowed to explore the environment for 1 min and baseline levels of freezing and exploratory behaviour were observed. Odours (CS⁺ or CS⁻) were presented for 5 min each to the animal in a counterbalanced order, and the amount of time freezing was recorded. Freezing behaviour was defined as complete immobility with the exception of respiratory-related movement. We also analyzed exploratory behaviour in response to presentations of CS⁺ or CS⁻ odours, as described previously (Lauzon et al. 2009). Exploratory behaviour was scored as follows, with a score assigned for every min of each of the 5 min during the CS⁺ or CS⁻ odour presentations: 0, no locomotion; 1, ambulation across 1 side of the testing chamber; 2, ambulation across 2 sides; 3,

exploration of the full perimeter of the testing chamber; and 4, exploration of the center and entire perimeter of the test chamber. Experimental procedures and timeline for examining olfactory fear memory acquisition are summarized schematically in Figure 3.1A.

3.2.3 Drug administration

All drugs were dissolved in physiological saline, with pH adjusted to 7.4. Bilateral intra-mPFC microinjection of either saline vehicle, the selective D₄ agonist PD 168077 (Tocris) (50 ng/0.5 μ L), the selective CaMKII inhibitors; Autocamtide-2-related inhibitory peptide, AIP (Tocris, 5–50 ng/0.5 μ L) and KN-62 (Tocris, 2.5–250 ng/0.5 μ L), the cAMP analog and protein kinase activator 8-CPT-cAMP (BioMol, 50–500 ng/0.5 μ L), or the selective PP1 inhibitor tautomycin (Tocris, 10–200 ng/0.5 μ L) were microinfused immediately prior to the olfactory conditioning procedure.

3.2.4 Histology

Following experimental completion rats were perfused, brains were removed, and stored in a formalin/25% sucrose solution for 24 h. Brains were sectioned into 40 μ m coronal slices, mounted, and stained using cresyl violet to allow for histological analysis of the site of injection. The majority of mPFC placements were localized within the boundaries of the prelimbic cortical area (PLC). Rats found to have misplaced guide cannulae were excluded from behavioural analysis.

3.2.5 Data analysis

Data were analyzed with one-, two-, or three-way analysis of variance (ANOVA) where appropriate or Student's *t*-tests. *Post hoc* analyses were performed with Fisher's least significant difference and Newman–Keuls tests.

3.3 RESULTS

3.3.1 Histological analysis

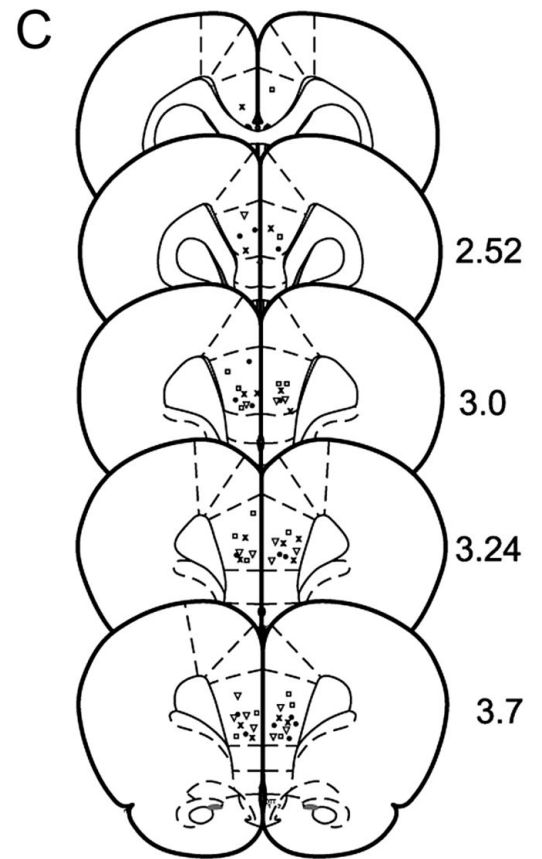
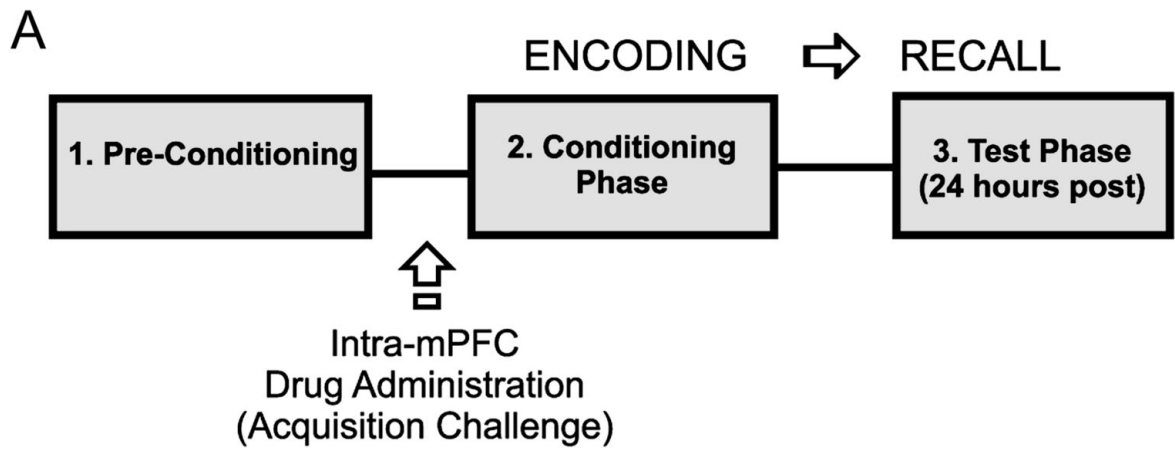
Histological analysis revealed microinfusion injector cannulae placements to be bilaterally localized within the anatomical boundaries of PLC region of the mPFC, as determined by the atlas of Paxinos and Watson (1996). In Figure 3.1B, we present a microphotograph showing a typical representative injector placement within the PLC. In Figure 3.1C, we present a schematic illustration showing representative intra-PLC bilateral cannulae placements along the rostrocaudal axis of the PLC.

3.3.2 Intra-PLC inhibition of CaMKII blocks DA D₄-mediated potentiation of subthreshold fear stimuli

We first examined the effects CaMKII inhibition on D₄-mediated potentiation of subthreshold fear stimuli by simultaneously activating DA D₄ receptors and inhibiting CaMKII in the mPFC during the subthreshold (0.4 mA) fear conditioning procedure. We performed bilateral intra-mPFC microinfusions of either saline, a specific DA D₄ agonist; PD 168077 (50 ng/0.5 μL) alone, and coadministered the selective CaMKII inhibitor; KN-62 (2.5, 25, or 250 ng/0.5 μL) or AIP (5 or 50 ng/0.5 μL) immediately prior to fear conditioning training. KN-62 inhibits CaMKII activity by binding directly to the calmodulin-binding site of the enzyme (Sumi et al. 1991; Hidaka and Yokokura 1996).

Figure 3.1 Histological analysis of intra-mPFC microinjection sites and experimental protocol summary.

(A) Microphotograph of a representative injector placement within the mPFC (white arrow represents injector tip location). (B) Schematic representation showing experimental associative fear conditioning assay and timeline for examining the acquisition (encoding) of associative olfactory fear memory. (C) Schematic illustration showing representative bilateral placements of microinjection cannulae. For illustrative clarity, only a subset of experimental groups is presented. Symbols represent separate experimental groups; ∇ , D₄ agonist PD168077; 50ng/0.5 μ l vs. acquisition of sub-threshold emotional stimuli; \times , D₄ agonist PD168077 50ng/0.5 μ l vs. acquisition of supra-threshold emotional stimuli; \square , Saline vs. acquisition of sub-threshold emotional stimuli; \bullet , Saline vs. acquisition of supra-threshold emotional stimuli.



In contrast, AIP decreases CaMKII activity by binding to the autophosphorylation site, independently of Ca^{2+} /calmodulin activity (Ishida et al. 1995; Yang et al. 2004). Examining the behavioural effects of coadministration with KN-62, ANOVA revealed a significant interaction between group and treatment ($F_{(5,89)} = 7.43, p < 0.0001$) on times spent freezing to either CS+ or CS- cue presentations at testing. *Post hoc* analysis illustrated a potentiation in associative fear conditioning expression by both PD 168077 50 ng/0.5 μL alone ($n = 7, p < 0.01$) and when coadministered with the lowest dose of KN-62, 2.5 ng/0.5 μL ($n = 8, p < 0.01$) demonstrated by a significantly greater time spent freezing to the CS+ compared with the CS- (Fig. 3.2A). In contrast, *post hoc* analysis revealed no significant difference between freezing time to the CS+ compared with the CS- for the saline ($n = 8, p > 0.05$) control group or animals receiving coadministration of PD 168077 with the higher doses of KN-62, 25 ng/0.5 μL ($n = 8; p > 0.05$), and 250 ng/0.5 μL ($n = 8, p > 0.05$) (Fig. 3.2A). To determine if intra-mPFC KN 62 produced any learning/inhibition on acquisition of fear memory, we included a control experiment wherein rats received the highest effective dose of KN-62 (250 ng/0.5 μL) alone during suprathreshold fear conditioning. *Post hoc* analysis revealed that KN-62, 250 ng/0.5 μL ($n = 8, p < 0.01$) alone did not influence the acquisition of suprathreshold fear memory, demonstrated by a significantly greater time spent freezing to the CS+ compared with the CS- (Fig. 3.2A, right side). Similarly, ANOVA of spontaneous exploratory behaviour during presentations of either CS+ or CS- revealed a significant interaction between group and treatment ($F_{(5,89)} = 4.98, p = 0.001$). *Post hoc* analysis revealed rats treated with either PD 168077 50 ng/0.5 μL alone ($n = 7, p$

< 0.01) or PD 168077 50 ng/0.5 μ L coadministered with the lowest dose of KN-62, 2.5 ng/0.5 μ L ($n = 8$; $p < 0.01$) displayed significantly decreased exploratory behaviour in response to the CS+ relative to the CS- (Fig. 3.2B). In contrast, *post hoc* analysis revealed no significant difference in exploratory behaviour observed in response to the CS+ compared with the CS- for the saline ($n = 8$, $p > 0.05$) control group or animals receiving coadministration of PD 168077 (50 ng/0.5 μ L) with the higher doses of KN-62, 25 ng/0.5 μ L ($n = 8$, $p > 0.05$), and 250 ng/0.5 μ L ($n = 8$, $p > 0.05$) (Fig. 3.2B). To further investigate the effect of CaMKII inhibition on acquisition of associative learning, we included a control experiment ($n = 8$) wherein rats received the highest dose of KN-62 (250 ng/0.5 μ L) alone during the subthreshold fear conditioning procedure. *Post hoc* analysis revealed that KN-62 (250 ng/0.5 μ L, $n = 8$, $p < 0.01$) alone produced a potentiation in associative learning demonstrated by significantly decreased exploratory behaviour in response to the CS+ relative to the CS- (Fig. 3.2B, right side).

3.3.3 Blockade of intra-mPFC CaMKII autophosphorylation blocks D₄ receptor-mediated emotional memory potentiation

Next, we examined the possible effects of simultaneously activating DA D₄ receptors and inhibiting CaMKII in the mPFC during the subthreshold (0.4 mA) fear conditioning procedure. We performed bilateral intra-mPFC microinfusions of either saline, a specific DA D₄ agonist; PD 168077 (50 ng/0.5 μ L) alone, or coadministered with specific CaMKII inhibitor; AIP (5 or 50 ng/0.5 μ L) immediately prior to fear conditioning, to determine the effects of CaMKII inhibition on D₄ receptor-mediated

potentiation of emotional learning during the acquisition phase (encoding) of this associative information. ANOVA revealed a significant interaction between group and treatment ($F_{(3,51)} = 8.11, p < 0.01$) on time spent freezing to either CS+ or CS- cue presentations at testing. *Post hoc* analysis illustrated a potentiation in associative fear conditioning expression by both PD 168077 50 ng/0.5 μ L alone ($n = 7, p < 0.01$) and when coadministered with the lowest dose of AIP, 5 ng/0.5 μ L ($n = 8, p < 0.01$) demonstrated by a significantly greater time spent freezing to the CS+ compared with the CS- (Fig. 3.3A). In contrast, *post hoc* analysis revealed no significant difference between freezing time to the CS+ compared to the CS- for the saline ($n = 8, p > 0.05$) control group or animals receiving coadministration of PD 168077 with the highest dose of AIP, 50 ng/0.5 μ L ($n = 8, p > 0.05$) (Fig. 3.3A).

ANOVA of exploratory behaviour scores during CS+/CS- presentations revealed a significant interaction between group and treatment ($F_{(3,51)} = 5.16, p \leq 0.01$). *Post hoc* analysis revealed rats treated with either PD 168077 50 ng/0.5 μ L alone ($n = 7, p < 0.01$) or PD 168077 50 ng/0.5 μ L coadministered with the lowest dose of AIP, 5 ng/0.5 μ L ($n = 8; p < 0.01$) displayed significantly decreased exploratory behaviour in response to the CS+ relative to the CS- (Fig. 3.3B). In contrast, *post hoc* analysis revealed there was no significant difference in exploratory behaviour observed in response to the CS+ compared to the CS- for the saline ($n = 8, p > 0.05$) control group or animals receiving coadministration of PD 168077 5 ng/0.5 μ L with the highest dose of AIP, 50 ng/0.5 μ L ($n = 8, p > 0.05$) (Fig. 3.3A).

Figure 3.2 Effects of intra-mPFC DA D₄ receptor and CaMKII inhibition during fear-conditioning.

(A) Saline controls displayed no significant difference in time spent freezing in response to CS+ vs. CS- presentations following subthreshold (0.4 mA; see Materials and Methods) fear conditioning. In contrast, intra-PLC PD 168077 (50 ng/0.5 μ L) significantly potentiated the acquisition of subthreshold fear memory. Coadministration with the CaMKII inhibitor KN-62 (2.5–250 ng/0.5 μ L dose dependently attenuates the effects of D₄ receptor activation. In contrast, intra-PLC KN-62 (250 ng/0.5 μ L) alone did not have a significant effect on the acquisition of suprathreshold (0.8 mA) fear memory.

(B) Analysis of exploratory scores revealed that saline controls showed no CS+-related exploratory behaviour suppression. In contrast, PD 168077 (50 ng/0.5 μ L) significantly potentiated CS+-related suppression of exploratory behaviour, which was dose dependently blocked by coadministration of KN-62 (2.5–250 ng/0.5 μ L). Bars represent mean \pm standard error of the mean for this and all subsequent figures.

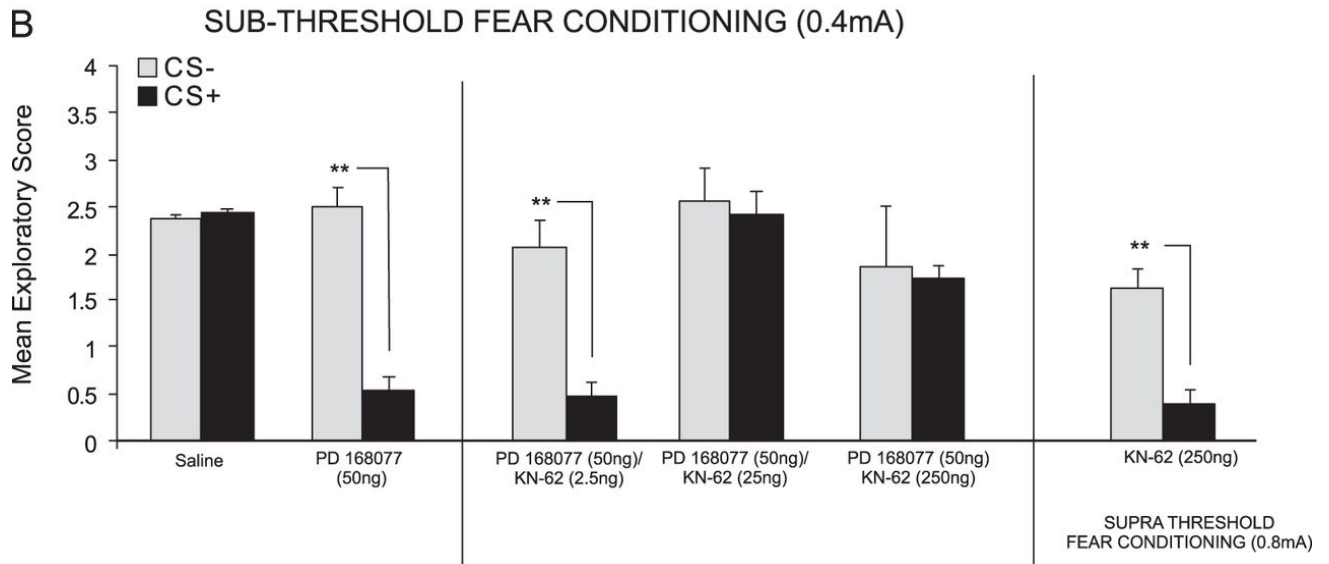
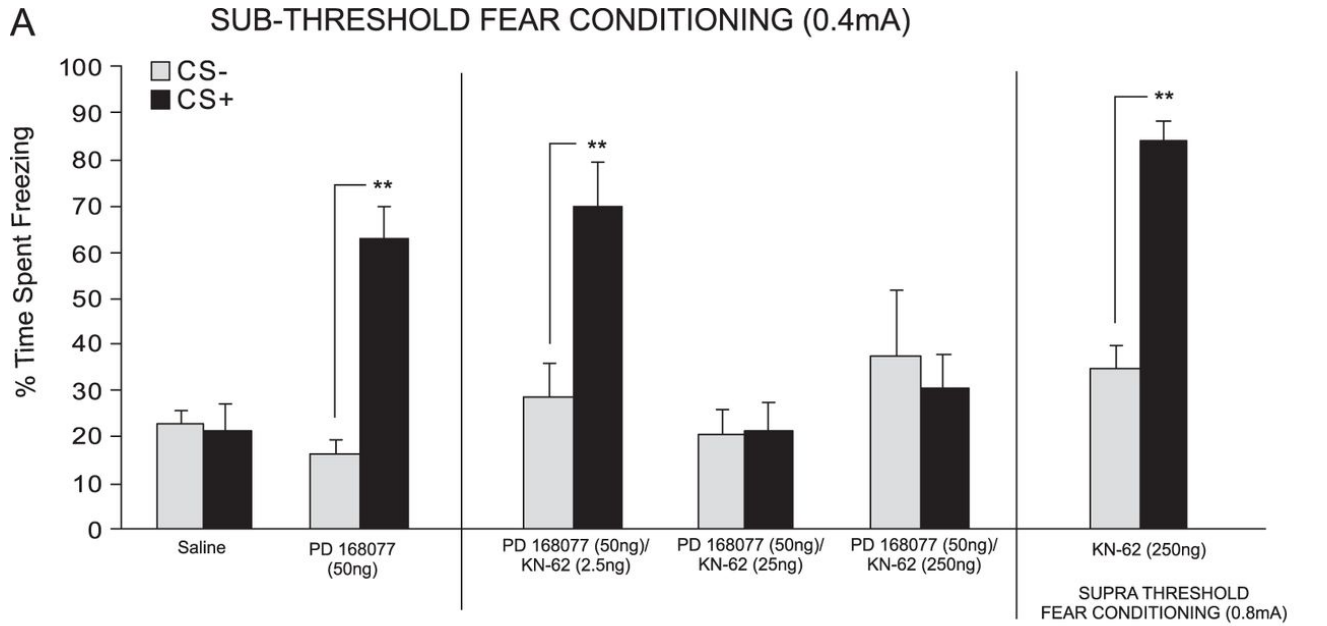
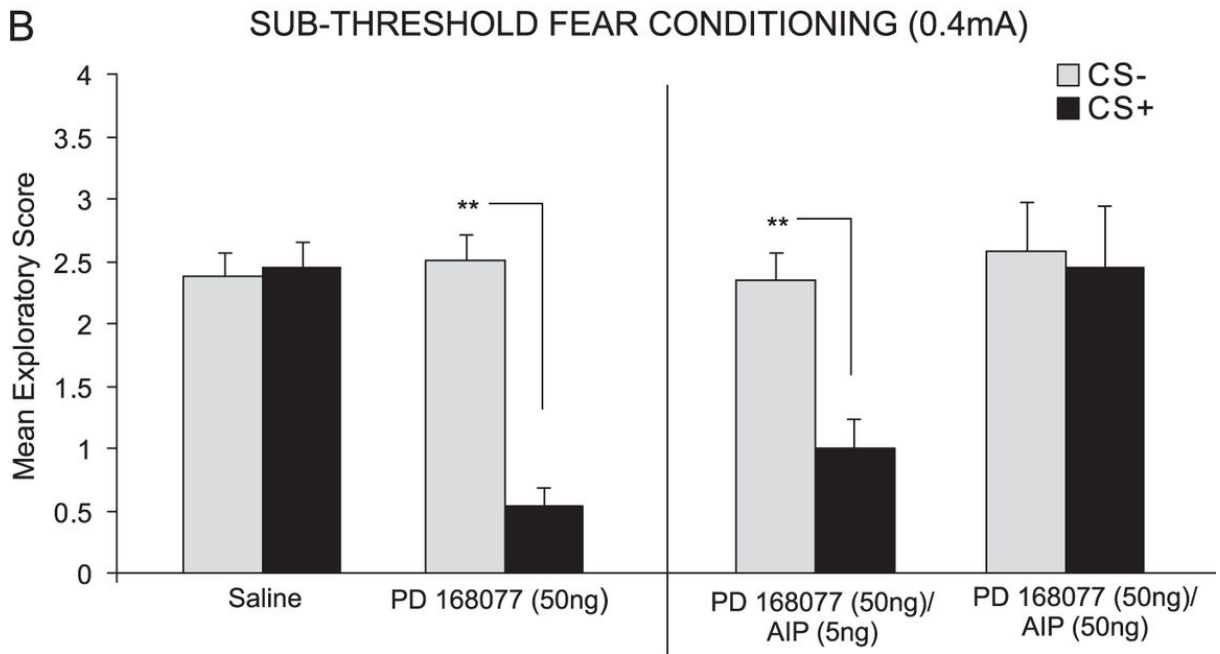
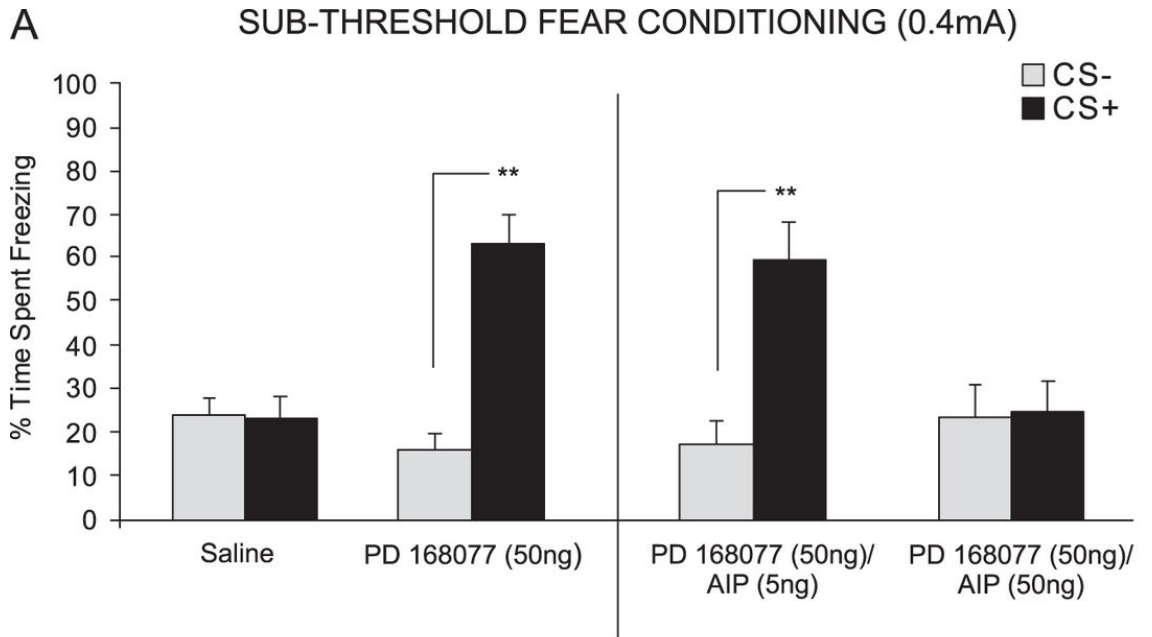


Figure 3.3 Effects of intra-PLC D₄ receptor activation and blockade of CaMKII autophosphorylation on fear-conditioning.

(A) Saline controls showed no significant CS+-related freezing behaviour. In contrast, PD 168077 (50 ng/0.5 μ L) significantly potentiated the acquisition of subthreshold olfactory fear memory, demonstrated by significantly greater CS+ freezing behaviour. This effect was dose dependently blocked by coadministration of the CaMKII autophosphorylation inhibitor AIP (5–50 ng/0.5 μ L). (B) Analysis of fear memory-related exploratory scores revealed that saline control rats showed no associative suppression of spontaneous exploratory behaviour. In contrast, PD 168077 (50 ng/0.5 μ L) significantly potentiated the acquisition of subthreshold (0.4 mA footshock) fear memory, demonstrated by significant suppression of CS+-related exploratory scores. This effect was dose dependently blocked by coadministration of AIP (5–50 ng/0.5 μ L).



3.3.4 Intra-mPFC inhibition of PP1 rescues DA D₄-mediated blockade of emotionally salient fear stimuli

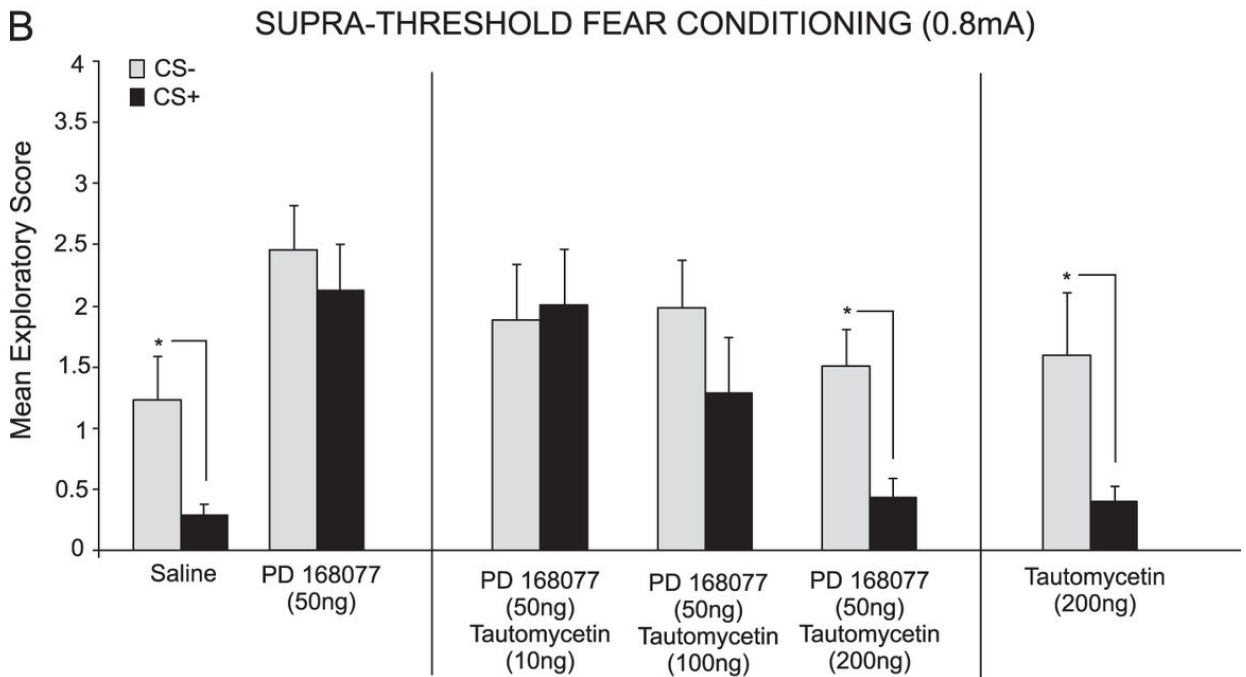
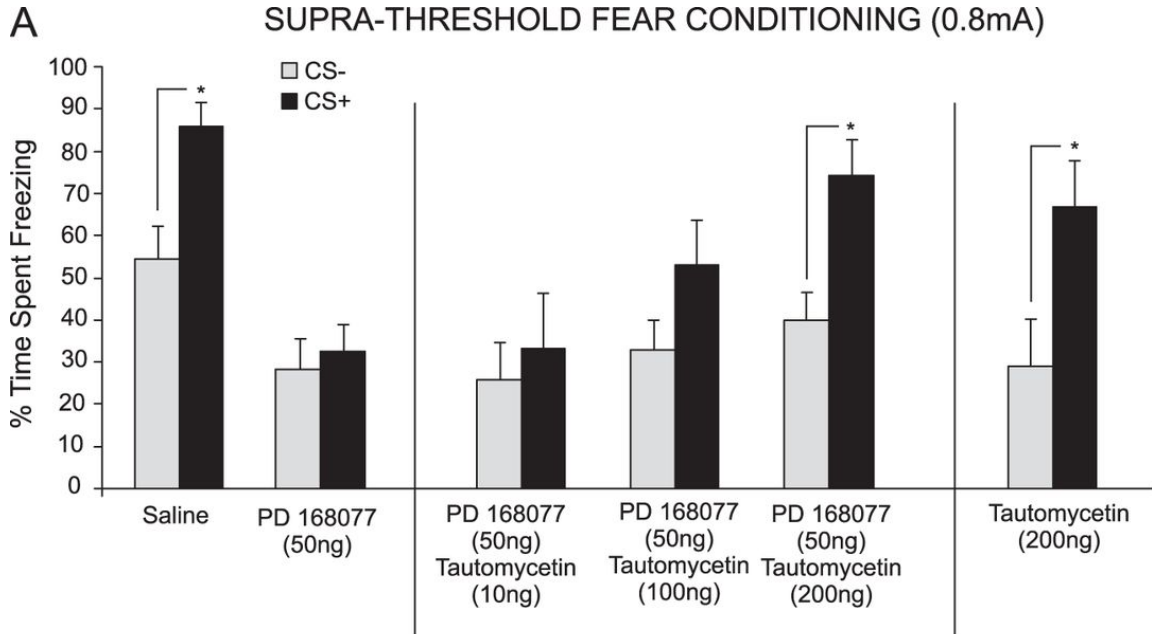
We next examined the possible effects of simultaneously activating DA D₄ receptors and inhibiting PP1 in the mPFC during suprathreshold (0.8 mA) fear conditioning. We performed bilateral intra-mPFC microinfusions of either saline, a specific DA D₄ agonist; PD 168077 (50 ng/0.5 μL) alone, or coadministered with a specific PP1 inhibitor; tautomycetin (10, 100, or 200 ng/0.5 μL) immediately prior to fear conditioning, to determine the effects of PP1 inhibition on D₄ receptor-mediated block of suprathreshold fear memory acquisition. ANOVA revealed a significant main effect of treatment ($F_{(4,68)} = 6.80, p < 0.0001$) on time spent freezing to either CS+ or CS- cue presentations at testing. *Post hoc* analysis illustrated an attenuation of associative fear conditioning acquisition by both PD 168077 50 ng/0.5 μL alone ($n = 7, p > 0.05$) and when coadministered with the lower doses of tautomycetin, 10 ng/0.5 μL ($n = 8, p > 0.05$) and 100 ng/0.5 μL ($n = 8, p > 0.05$) demonstrated by no significant difference in the time spent freezing to the CS+ compared to the CS- (Fig. 3.4A). In contrast, *post hoc* analysis revealed a significant difference between freezing time to the CS+ compared to the CS- for the saline ($n = 7, p < 0.05$) control group and animals receiving coadministration of PD 168077 50 ng/0.5 μL with the highest dose of tautomycetin, 200 ng/0.5 μL ($n = 8, p < 0.05$) (Fig. 3.4A).

To further investigate the effect of PP1 inhibition on acquisition of associative learning, we included a control experiment ($n = 7$) wherein rats received the highest dose of tautomycetin (200 ng/0.5 μL) alone during suprathreshold fear conditioning. *Post hoc* analysis revealed that tautomycetin 200 ng/0.5 μL ($t_{(6)} = 2.18, p < 0.05$)

alone did not block suprathreshold acquisition in associative learning demonstrated by a significantly greater time spent freezing to the CS+ compared to the CS- (Fig. 3.4A, right side). ANOVA of exploratory behaviour scores during CS+/CS- presentations revealed a significant main effect of treatment ($F_{(4,68)} = 6.27, p < 0.001$). *Post hoc* analysis revealed rats treated with either PD 168077 50 ng/0.5 μ L alone ($n = 7, p > 0.05$) or PD 168077 50 ng/0.5 μ L coadministered with the lower doses of tautomycetin, 10 ng/0.5 μ L ($n = 8, p > 0.05$) and tautomycetin 100 ng/0.5 μ L ($n = 8, p > 0.05$) displayed no significant difference exploratory behaviour in response to the CS+ relative to the CS- (Fig. 3.4B). In contrast, *post hoc* analysis revealed a significant difference in exploratory behaviour in response to the CS+ compared to the CS- for the saline ($n = 8, p < 0.05$) control group and animals receiving coadministration of PD 168077 50 ng/0.5 μ L with the highest dose of tautomycetin, 200 ng/0.5 μ L ($n = 8, p < 0.05$) (Fig. 3.4B). To further investigate the effect of PPI inhibition on acquisition of associative learning, we included a control experiment ($n = 7$) wherein rats received the highest dose of tautomycetin (200 ng/0.5 μ L) alone during the suprathreshold fear conditioning procedure. Statistical analysis revealed that tautomycetin (200 ng/0.5 μ L) ($t_{(6)} = 2.29, p < 0.05$) alone did not block suprathreshold acquisition in associative learning demonstrated by a significantly decreased exploratory behaviour in response to the CS+ relative to the CS- (Fig. 4B, right side).

Figure 3.4 Inhibition of PP1 rescues DA D₄ receptor-mediated blockade of suprathreshold fear memory acquisition.

(A) Saline controls showed significant freezing to CS+ presentations after conditioning with a suprathreshold footshock (0.8 mA). In contrast, intra-PLC PD 168077 (50 ng/0.5 μ L) blocked the acquisition of suprathreshold fear memory. This effect was dose dependently reversed by coadministration of tautomycetin (10–200 ng/0.5 μ L). In a separate control experiment, intra-PLC tautomycetin (200 ng/0.5 μ L) alone did not influence the acquisition of suprathreshold fear memory. (B) Saline controls showed significant suppression of exploratory behaviour in response to CS+ presentations. In contrast, intra-PLC PD 168077 (50 ng/0.5 μ L) blocked this effect. D₄-mediated blockade of suprathreshold fear memory acquisition was dose dependently reversed by tautomycetin (10–200 ng/0.5 μ L). In a separate control experiment, intra-PLC tautomycetin (200 ng/0.5 μ L) alone did not affect the acquisition of suprathreshold fear memory.

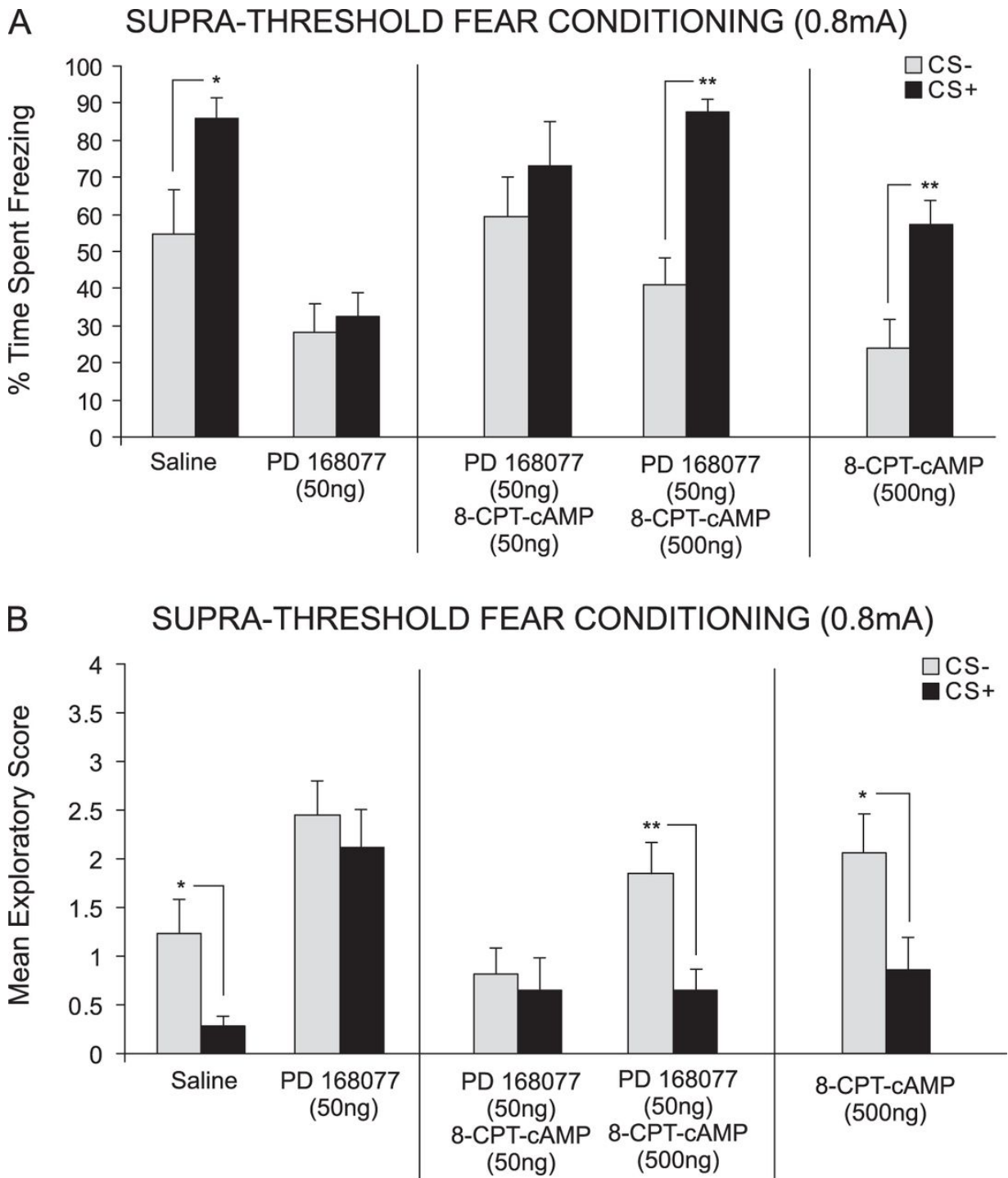


3.3.5 Activation of protein kinase A rescues DA D₄-mediated blockade of suprathreshold fear memory acquisition

We next performed bilateral intra-PLC microinfusions of either saline, PD 168077 (50 ng/0.5 μ L) alone, coadministration with the PKA activator; 8-CPT-cAMP (50 ng or 500 ng/0.5 μ L), or 8-CPT-cAMP alone (control, 500 ng/0.5 μ L) to examine the effects of intra-PLC PKA activation on D₄ receptor-mediated block of suprathreshold fear memory acquisition. ANOVA revealed a significant effect of treatment ($F_{(3,61)} = 9.23, p < 0.0001$) on time spent freezing to either CS+ or CS- cue presentations at testing. *Post hoc* analysis illustrated an attenuation of associative fear conditioning acquisition by both PD 168077 50 ng/0.5 μ L alone ($n = 7, p > 0.05$) and when coadministered with the lowest dose of 8-CPT-cAMP, 50 ng/0.5 μ L ($n = 8, p > 0.05$) demonstrated by no significant difference in the time spent freezing to the CS+ compared to the CS- (Fig. 5A). In contrast, *post hoc* analysis revealed a significant difference between freezing time to the CS+ compared to the CS- for the saline ($n = 7, p < 0.05$) control group and animals receiving coadministration of PD 168077 50 ng/0.5 μ L with the highest dose of 8-CPT-cAMP, 500 ng/0.5 μ L ($n = 8, p < 0.05$) (Fig. 3.5A). To further investigate the effect of PKA activation on acquisition of associative learning, we included a control experiment ($n = 8$) wherein rats received the highest dose of 8-CPT-cAMP, (500 ng/0.5 μ L) alone during suprathreshold fear conditioning. Statistical analysis revealed that 8-CPT-cAMP 500 ng/0.5 μ L ($t_{(7)} = 3.34, p \leq 0.05$) alone did not block suprathreshold fear memory acquisition demonstrated by significantly greater times spent freezing to the CS+ relative to the CS- ($t_{(7)} = 3.34, p \leq 0.05$; Fig. 3.5A, right side).

Figure 3.5 Activation of cAMP/PKA signaling rescues D₄ receptor-mediated blockade of suprathreshold fear memory.

(A) Saline controls showed significant CS+-related freezing after conditioning with suprathreshold footshock (0.8 mA). However, intra-PLC PD 168077 (50 ng/0.5 μ L) blocked acquisition of suprathreshold fear memory. This effect was dose dependently reversed by coadministration of the cAMP/PKA activator 8-CPT-cAMP (50–500 ng/0.5 μ L). In a separate control experiment, intra-PLC 8-CPT-cAMP (500 ng/0.5 μ L) alone did not influence the acquisition of suprathreshold fear memory. (B) Similarly, exploratory behaviour analyses demonstrated significant CS+-related attenuation in exploratory behaviour in saline controls. In contrast, intra-PLC PD 168077 (50 ng/0.5 μ L) administration blocked this effect. D₄ receptor-mediated blockade of suprathreshold fear memory acquisition was dose dependently reversed by coadministration of 8-CPT-cAMP (50–500 ng/0.5 μ L). Intra-PLC 8-CPT-cAMP (500 ng/0.5 μ L) alone did not have any significant effect on fear-related exploratory scores.



ANOVA of spontaneous exploratory behaviour during presentations of either CS+ or CS- revealed a significant effect of treatment ($F_{(3,61)} = 11.18, p < 0.0001$). *Post hoc* analysis revealed rats treated with either PD 168077 50 ng/0.5 μ L alone ($n = 7; p > 0.05$) or PD 168077 50 ng/0.5 μ L coadministered with the lowest dose of 8-CPT-cAMP 50 ng/0.5 μ L ($n = 8, p > 0.05$) displayed no significant difference exploratory behaviour in response to the CS+ relative to the CS- (Fig. 3.5B). In contrast, *post hoc* analysis revealed there was a significant difference in exploratory behaviour observed in response to the CS+ compared to the CS- for the saline ($n = 8, p < 0.05$) control group and animals receiving coadministration of PD 168077 50 ng with the highest dose of 8-CPT-cAMP 500 ng/0.5 μ L ($n = 8, p < 0.01$) (Fig. 5B). To investigate the effect of PKA activation on acquisition of associative learning, we included a control experiment ($n = 8$) wherein rats received the highest dose of 8-CPT-cAMP (500 ng/0.5 μ L) alone during the suprathreshold fear conditioning procedure. *Post hoc* analysis revealed that 8-CPT-cAMP 500 ng ($t_{(7)} = 2.30, p < 0.037$) alone did not block suprathreshold acquisition in associative learning demonstrated by a significantly decreased exploratory behaviour in response to the CS+ relative to the CS- (Fig. 3.5B, right side).

3.4 DISCUSSION

DA D_4 receptor transmission within the mPFC is an important modulator of emotional memory acquisition, in single neurons and at the behavioural level. Blockade of DA D_4 receptor transmission, either systemically or directly within the PLC division of the mPFC prevents the acquisition, but not the expression, of

associative fear memory (Laviolette et al. 2005; Lauzon et al. 2009). In addition, pharmacological activation of DA D₄ receptors in the PLC dose dependently potentiates the emotional salience of normally nonsalient subthreshold fear conditioning stimuli (footshock) and blocks the acquisition of emotionally salient (suprathreshold) associative fear memories (Lauzon et al. 2009). Thus, DA D₄ receptor transmission within the mPFC can bidirectionally modulate fear memory acquisition by regulating the emotional salience of these memories; potentiating nonsalient emotional memory while blunting the acquisition of highly salient emotional memories. However, the downstream molecular events involved in D₄-mediated modulation of emotional memory salience have not previously been identified.

Within the mPFC, CaMKII signaling is bidirectionally modulated by D₄ receptor-specific activation (Gu and Yan 2004; Gu et al. 2006). Using *in vitro* culture preparations of mPFC neurons, Gu and Yan (2004) demonstrated that during low levels of mPFC neuronal activity, D₄ receptor activation strongly increased α -CaMKII activity. In contrast, during high levels of neuronal activation, D₄ receptor activation strongly attenuated α -CaMKII activity. Taken together, these studies provide a functional molecular framework whereby D₄ receptor activation may modulate memory-related CaMKII signaling within the PFC, depending upon baseline levels of neuronal activity within mPFC networks. Given previous evidence that emotionally salient events can strongly activate prefrontal cortical networks (Surguladze et al. 2011; Dickie et al. 2011; Dresler et al. 2011), we hypothesized that the D₄-mediated

bidirectional modulation of α -CaMKII may be related to the ability of D₄ receptor activation to modulate the behavioural acquisition of emotional associative memories, depending upon the baseline emotional salience (sub vs. suprathreshold footshock levels) of the fear conditioning stimulus. Specifically, we proposed that the observed potentiation of normally subthreshold emotional stimuli may be related to the ability of D₄ receptor activation to increase endogenous levels of CaMKII during low levels of neuronal activity (corresponding to exposure to subthreshold nonsalient footshock stimuli). Such an increase in mPFC CaMKII levels was predicted to potentiate the strength of associative fear memory.

3.4.1 CaMKII bidirectionally regulates fear memory salience via DA D₄ transmission in the mPFC

Our results demonstrate that intra-PLC inhibition of CaMKII signaling, dose dependently blocked the ability of D₄ receptor activation to potentiate normally subthreshold olfactory fear memories. This effect was observed with both a direct inhibitor of CaMKII signaling via KN-62, which blocks the calmodulin-binding site; or with AIP, which blocks autophosphorylation of CaMKII, independently of Ca²⁺/calmodulin activity. Given the ability of D₄ receptor activation to increase CaMKII during low neuronal activation these results suggest that CaMKII activity downstream of the D₄ receptors is a probable molecular mechanism underlying D₄-mediated potentiation of subthreshold fear stimuli. It is important to note that neither of the CaMKII inhibitors used in the present study are specific enough to differentiate between the β and the α isoforms of CaMKII, and future studies will be required to

more precisely characterize the precise CaMKII substrate linked to D₄ receptor-mediated modulation of emotional memory, *in vivo*. Interestingly, administration of CaMKII inhibitors alone failed to modulate fear memory acquisition, suggesting that intra-mPFC blockade of CaMKII in and of itself is not sufficient to prevent the acquisition of fear memory or produce nonspecific impairments in learning or memory. Rather, consistent with *in vitro* evidence, intra-mPFC CaMKII signaling appears to depend upon co-activation of D₄ receptor substrates for the modulation of emotional memory salience.

To investigate the bidirectional nature of the relationship between D₄ receptor activation and α -CaMKII, we administered a PKA activator during D₄-mediated block of acquisition of emotionally salient fear stimuli. During high levels of mPFC neuronal activity, D₄ activation initiates an inhibitory downstream cascade resulting in increased PKA activity, which in turn increases PP1-mediated dephosphorylation of α -CaMKII (Gu and Yan 2004). We hypothesized that this inhibition of CaMKII activity may be responsible for our observed blockade of suprathreshold fear memory acquisition. Currently, there is no direct or specific pharmacological method to activate CaMKII *in vivo*. Thus, we targeted indirect components of D₄ receptor-mediated control of CaMKII *in vivo* by either pharmacologically increasing PKA activity or inhibiting PP1 activity. As predicted by previous *in vitro* studies, our behavioural results demonstrate that pharmacological activation of PKA or inhibition of PP1 can dose dependently rescue D₄-mediated blockade of suprathreshold fear memory acquisition. Taken together with the KN-62 and AIP findings, regulation of

CaMKII activity provides strong evidence of a specific molecular mechanism through which the D₄ receptors exert their bidirectional effect during the acquisition phase of emotional learning.

Functionally, D₄ receptor-mediated modulation of α -CaMKII within the PLC may elucidate a molecular mechanism underlying abnormalities in emotional processing, learning, and memory observed in neuropsychiatric disorders such as schizophrenia, PTSD, ADHD, and addiction (Lauzon and Laviolette 2010). Various lines of evidence suggest that an optimal level of D₄ receptor signaling within the mPFC is required for adaptive emotional learning, memory, and the development of appropriate adaptive behavioural and psychological responses. Specifically, whereas hyperstimulation of D₄ receptor transmission within the PLC may abnormally amplify the emotional salience of subthreshold sensory experiences; hyperstimulation of these same D₄ receptor substrates in the context of emotionally salient experiences may pathologically blunt the emotional salience of these memories leading to suboptimal emotional processing and memory encoding, as previously suggested (Lauzon et al. 2009; Lauzon and Laviolette 2010). Our findings demonstrate that D₄ receptor transmission exerts bidirectional modulation of emotional memory salience through interactions with CaMKII signaling pathways.

A functional role for intra-PLC CaMKII signaling during emotional memory formation is consistent with previous evidence implicating CaMKII as a critical mediator of associative memory formation in PFC networks (Frankland et al. 2001). Indeed, CaMKII is highly expressed in the mammalian forebrain and plays a critical

role in modulating various postsynaptic elements important for synaptic plasticity, learning, and memory (Malenka and Nicoll 1999; Frankland et al. 2001). Interestingly, the genetic locus of CaMKII is found on 5q32, a genomic region that has been associated with schizophrenia in several clinical studies (Paunio et al. 2001; Sklar et al. 2004). In addition, postmortem studies have revealed that CaMKII- β and α -isoforms are abnormally expressed in prefrontal cortical areas in individuals with schizophrenia and linked to schizophrenia-related behavioural abnormalities in rodent models (Novak et al. 2006; Novak and Seeman 2010; Seeman et al. 1993). The present findings thus add to a growing body of evidence linking CaMKII signaling to the aberrant emotional regulation present in schizophrenia-related psychoses, providing a potential DA receptor-specific substrate through which CaMKII signaling may control emotional salience processing and memory formation.

Recent *in vitro* work by Yuen and Yan (2011) has demonstrated that D₄ receptor-mediated modulation of CaMKII activity is tightly linked to AMPA receptor synaptic activity within the mPFC. Thus, during low mPFC neuronal activity levels, D₄-mediated activation of CaMKII results in a potentiation in AMPA receptor synaptic efficacy whereas during high neuronal activity levels, D₄ receptor activity decreases CaMKII levels concomitant with decreased AMPA receptor signaling. Given the essential role of AMPA-mediated glutamatergic signaling in learning and memory-related synaptic plasticity, this may provide another potential mechanism by which D₄ signaling within the PLC may control emotional memory formation and processing via CaMKII modulation. The present results demonstrate an important role

for D₄ receptor–mediated modulation of CaMKII, cAMP/PKA, and PP1 directly within the mPFC, controlling the salience of emotional memory acquisition. Interestingly, previous evidence from clinical trials has largely demonstrated a lack of therapeutic efficacy for D₄ receptor specific antagonists in the treatment of schizophrenia-related psychopathology (Kramer et al. 1997; Corrigan et al. 2004). Nevertheless, the highly effective atypical antipsychotic, clozapine, demonstrates high affinity for the D₄ receptor subtype relative to other D₂-like receptors (Govindaiah et al. 2010). The present results suggest that modulation of downstream molecular targets of intra-PLC D₄ receptor substrates, such as CaMKII, may serve as potential pharmacotherapeutic targets in the context of aberrant D₄ receptor signaling in the context of dysregulated emotional processing and memory encoding.

3.5 REFERENCES

- Ariano MA, Wang J, Noblett KL, Larson ER, Sibley DR. 1997. Cellular distribution of the rat D4 dopamine receptor protein in the CNS using anti-receptor antisera. *Brain Res.* 752:26--34.
- Corrigan MH, Gallen CC, Bonura ML, Merchant KM. 2004. Effectiveness of the selective D4 antagonist sonepiprazole in schizophrenia: a placebo controlled trial. *Biol Psychiatry.* 55:445--451.
- De Almeida J, Mengod G. 2010. D2 and D4 dopamine receptor mRNA distribution in pyramidal neurons and GABAergic subpopulations in monkey prefrontal cortex: implications for schizophrenia treatment. *Neuroscience.* 170:1133--1139.
- Dickie EW, Brunet A, Akerib V, Armony JL. 2011. Neural correlates of recovery from post-traumatic stress disorder: a longitudinal fMRI investigation of memory encoding. *Neuropsychologia.* 49:1771--1778.
- Dresler T, Hahn T, Plichta MM, Ernst LH, Tupak SV, Ehli AC, Warrings B, Deckert K, Fallgater AJ. 2011. Neural correlates of spontaneous panic attacks. *J Neural Transm.* 118:263--269.
- Frankland PW, O'brien C, Ohon M, Kirkwood A, Silva AJ. 2001. Alpha-CAMKII-dependent plasticity in the cortex is required for permanent memory. *Nature.* 411:309--313.
- Frankland PW, Sakaguchi M, Arruda-Carvalho M. 2008. Starting at the endophenotype: a role for alpha-CaMKII in schizophrenia? *Mol Brain.* 6:1--3.
- Govindaiah G, Wang T, Gillette MU, Crandall SR, Cox CL. 2010. Regulation of inhibitory synapses by presynaptic D4 dopamine receptors in thalamus. *J Neurophysiol.* 104:2757--2765.
- Greenstein R, Novak G, Seeman P. 2007. Amphetamine sensitization elevates CaMKIIb mRNA. *Synapse.* 61:827--834. Gu Z, Jiang Q, Yuen EY, Yan Z. 2006. Activation of dopamine D4 receptors induces synaptic translocation of Ca²⁺/calmodulin-dependent protein kinase II in cultured prefrontal cortical neurons. *Mol Pharmacol.* 69:813--822.
- Gu Z, Yan Z. 2004. Bidirectional regulation of Ca²⁺/calmodulin-dependent protein kinase II activity by dopamine D4 receptors in prefrontal cortex. *Mol Pharmacol.* 66:948--955.
- Hidaka H, Yokokura H. 1996. Molecular and cellular pharmacology of a calcium/calmodulin-dependent protein kinase II (CaM kinase II) inhibitor, KN-62, and proposal of CaM kinase phosphorylation cascades. *Adv Pharmacol.* 36:193--219.
- Ishida A, Kameshita I, Okuno S, Kitani T, Fujisawa H. 1995. A novel highly specific and potent inhibitor of calmodulin-dependent protein kinase II. *Biochem Biophys Res Commun.* 212:806--812.

- Kramer MS, Last B, Getson A, Reines SA. 1997. The effects of a selective D4 dopamine receptor antagonist (L-745-870) in acutely psychotic inpatients with schizophrenia. *Arch Gen Psychiatry*. 54:567--572.
- Lauzon NM, Bishop SF, Laviolette SR. 2009. Dopamine D1 versus D4 receptors differentially modulate the encoding of salient versus nonsalient emotional information in the medial prefrontal cortex. *J Neurosci*. 29:4836--4845.
- Lauzon NM, Laviolette SR. 2010. Dopamine D4-receptor modulation of cortical neuronal network activity and emotional processing: implications for neuropsychiatric disorders. *Behav Brain Res*. 208:12--22.
- Laviolette SR, Grace AA. 2006. Cannabinoids potentiate emotional learning plasticity in neurons of the medial prefrontal cortex through basolateral amygdala inputs. *J Neurosci*. 26:6458--6468.
- Laviolette SR, Lipski WJ, Grace AA. 2005. A subpopulation of neurons in the medial prefrontal cortex encodes emotional learning with burst and frequency codes through a dopamine D4 receptor-dependent basolateral amygdala input. *J Neurosci*. 25:6066--6075.
- Malenka RC, Nicoll RA. 1999. Long-term potentiation—a decade of progress? *Science*. 285:325--334.
- Novak G, Seeman P. 2010. Hyperactive mice show elevated D2 high receptors, a model for schizophrenia: calcium/calmodulin-dependent kinase II alpha knockouts. *Synapse*. 64:794--800.
- Novak G, Seeman P, Tallerico T. 2006. Increased expression of calcium/ calmodulin-dependent protein kinase IIb in frontal cortex in schizophrenia and depression. *Synapse*. 59:61--68.
- Paunio T, Ekelund J, Varilo T, Parker A, Hovatta I, Turunen JS, Rinard K, Foti A, Terwilliger JD, Juvonen H, et al. 2001. Genome-wide scan in a nationwide study sample of schizophrenia families in Finland reveals susceptibility loci on chromosome 2q and 5q. *Hum Mol Genet*. 10:3037--3048.
- Paxinos G, Watson C. 1996. *The rat brain in stereotaxic coordinates*. 2nd ed. San Diego (CA): Academic.
- Seeman P, Guan HC, Van Tol HHM. 1993. Dopamine D4 receptors elevated in schizophrenia. *Nature*. 365:441--445.
- Sklar P, Pato MT, Kirby A, Petryshen TL, Medeiros H, Carvalho C, Macedo A, Dourado A, Coelho I, Valente J, et al. 2004. Genome-wide scan in Portuguese island families identifies 5q31-5q35 as a susceptibility locus for schizophrenia and psychosis. *Mol Psychiatry*. 9:213--218.
- Sumi M, Kiuch K, Ishikawa T, Ishii A, Hagiwara M, Nagatsu T, Hidaka H. 1991. The newly synthesized selective Ca²⁺/calmodulin-dependent protein kinase II inhibitor KN 93 reduces dopamine contents in PC12h cells. *Biochem Biophys Res Commun*. 181:968--975.
- Surguladze SA, Chu EM, Marshall N, Evans A, Anilkumar AP, Timehin C, McDonale C, Ecker C, Philips ML, David AS. 2011. Emotion processing in schizophrenia:

fMRI study of patients treated with risperidone long-acting injections or conventional depot medication.

- Yamasaki N, Maekawa M, Kobayashi K, Kajii Y, Maeda J, Soma M, Takao K, Tanda K, Ohira K, Toyama K. Alpha-CaMKII deficiency causes immature dentate gyrus, a novel candidate J Psychopharmacol. 25:722--733. 2008.
- Yang HW, Hu XD, Zhang HM, Xin WJ, Li MT, Zhang T, Zhou LJ, Liu XG. Roles of CaMKII, PKA and PKC in the induction and maintenance of LTP of C-fiber-evoked field potentials in rat dorsal spinal horn phenotype of psychiatric disorders 2004. Mol Brain. 6:1—21.
- Yuen EY, Yan Z. 2011. Cellular mechanisms for dopamine D4 receptor-induced homeostatic regulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. J Biol Chem. 286:24957--24965.

Chapter 4:

4 Supra-normal Stimulation of Dopamine D₁ Receptors in the Prelimbic Cortex Blocks Spontaneous Recall of both Aversive and Rewarding Associative Memories Through a Cyclic-AMP-dependent Signaling Pathway¹

¹ This Chapter has been adapted from a published journal article: **Lauzon NM**, Bechard M, Ahmad T, Laviolette SR. (2013) Supra-normal stimulation of dopamine D₁ receptors in the prefrontal cortex blocks spontaneous recall of both aversive and rewarding associative memories through a cyclic-AMP dependent signaling pathway. *Neuropharmacology*; 67:104-14

4.1 INTRODUCTION

The processing of associative memories related to either rewarding or aversive experiences is critically dependent upon DA transmission within the mPFC (Bishop et al., 2010; Sun et al., 2011; Lauzon and Laviolette, 2010; Laviolette and Grace, 2006; Pezze et al., 2003). Disturbances in DAergic transmission within these cortical memory networks substrates may potentially lead to maladaptive emotional memory formation and/or recall, characteristic of various neuropsychiatric conditions, such as schizophrenia, addiction or PTSD (Lauzon and Laviolette, 2010; Laviolette and Grace, 2006). Indeed, PTSD and addiction are characterized by recurrent and persistent activation of memories linked to emotionally salient experiences, either traumatically aversive or drug-related rewarding experiences. In either case, an inability to suppress spontaneous memory recall related to these experiences may be an underlying neuropsychological feature triggering anxiety in the case of PTSD, or relapse-related behaviours in the context of addiction.

A considerable amount of research has demonstrated that receptor-specific DAergic transmission within the mPFC may strongly modulate emotional memory formation at both the level of the single neuron and the behavioural systems levels (Laviolette et al., 2005; Lauzon et al., 2009). We have previously reported dissociable functional roles for DA D_2 vs. D_1 -like receptor transmission within the prelimbic (PLC) division of the mPFC, in the context of fear memory acquisition and recall. Thus, direct activation of the DA D_4 receptor subtype strongly potentiates the emotional salience of normally non-salient fear conditioning cues during the

acquisition (but not recall) memory phase of an olfactory fear memory (Lauzon et al., 2009; 2011). In contrast, intra-PLC D₁ receptor activation has no role in modulating emotional salience during memory acquisition, but dose-dependently blocks the acute recall of previously learned fear memories (Lauzon et al., 2009) suggesting a specific role for intra-PLC D₁ receptor transmission during memory recall. These results are consistent with reports demonstrating that supranormal stimulation of D₁ receptor-specific substrates in the PFC impairs memory performance in both rodents and non-human primates (Zahrt et al., 1997; Arnsten et al., 1994).

Neuronal populations within the mPFC have been shown to encode associative memories related to either rewarding or aversive conditioning events. For example, single neurons in the mPFC demonstrate associative firing activity during the acquisition and recall of memories related to the rewarding effects of opiates (Sun et al., 2010; 2012) or the aversive stimulus properties of fear-related cues (Lavolette et al., 2005; Lavolette and Grace, 2006). However, the mechanisms by which DA receptor activation within the PLC may modulate the recall of emotionally salient associative memories are not currently understood. The goal of the present study was to further elucidate the functional effects of intra-PLC D₁ receptor modulation on memory recall by examining the temporal characteristics of D₁-receptor mediated block of both aversive and rewarding associative memory recall, and the downstream molecular substrates linked to these behavioural effects. Accordingly, we conducted a series of behavioural pharmacological studies examining the role of intra-PLC D₁ receptor transmission during the expression (recall) phase of emotional learning and

memory processing, employing both an olfactory fear conditioning procedure and a morphine reward conditioned place preference (CPP) procedure. We report that supranormal stimulation of intra-PLC D₁, but not D₂ receptor transmission, induces a transient block of associative memory recall for both rewarding and aversive conditioning events, through a common cAMP-dependent molecular signaling substrate.

4.2 MATERIALS AND METHODS

4.2.1 Animals and surgery

Male Sprague Dawley rats (300-350 gm) were obtained from Charles River Laboratories. All procedures were performed in accordance with the Canadian Council on Animal Care and were approved by the University of Western Ontario's Council on Animal Care. Rats were anesthetized with a ketamine (80 mg/ ml)-xylazine (6 mg/ kg) mixture administered intraperitoneally (i.p.), and placed in a stereotaxic device. All stereotaxic coordinates were based upon the atlas of Paxinos and Watson (1996). Two stainless steel guide cannulae (22 gauge) were implanted into the PLC using the following stereotaxic coordinates (15° angle; in mm from bregma): anteroposterior (AP) +2.9, lateral (LAT) ±1.9, ventral V -3.0 from the dural surface, Jeweler's screws and dental acrylic were used to secure the cannulae.

4.2.2 Olfactory fear conditioning

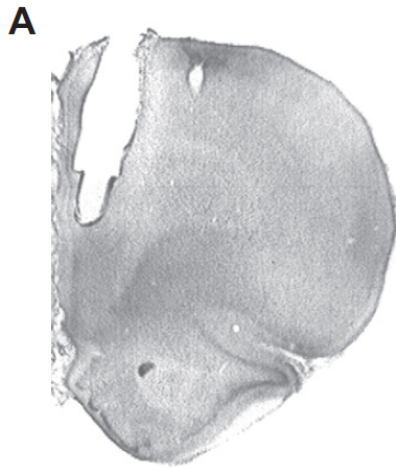
Procedures for olfactory fear conditioning have been described previously (Lauzon et al., 2009; 2011) Rats were taken from their home cages, received sham

microinfusions into the PLC, and habituated for 30 min in a ventilated conditioning chamber with an electric grid floor inside a sound-attenuated room. Olfactory fear conditioning took place in one of two distinct environments, counterbalanced within groups: “shock” environment A was a 30” x 30” Plexiglas box with black stripes on a white background and a metallic grid shock floor, whereas shock environment B was a 30” x 30” Plexiglas box with black dots on a white background with a grid shock floor. Testing 24 h later took place in one of two alternate environments, in which animals had not previously received electric shock, counterbalanced within groups: test environment A had walls with black dots and a grey Plexiglas floor, whereas test environment B had walls with black and white stripes and a grey Plexiglas floor. On day 1 (habituation phase), animals were habituated to a random combination of shock environment A or B and test environment A or B in a counterbalanced order for 30 min in each environment. On day 2 (conditioning phase), animals were returned to the conditioning room and placed in the previously assigned shock environment. During the conditioning phase, one of the odours (almond or peppermint) was presented to the animal for 19 s, and a footshock was then delivered (0.8mA) for 1 s. This level of footshock (0.8mA) produces robust conditioned fear memories, as reported previously (Lavolette and Grace, 2006a). 120 s later, the alternate odour was presented for 20 s (CS-) in the absence of footshock. This cycle was repeated five times. On the following day (test phase), rats were returned to the test room and placed in the previously assigned test environment. Before odour presentation, the rat was allowed to explore the environment for 1 min, during which time baseline levels of freezing and exploratory behaviour were observed. Odours (CS+ or CS-) were then

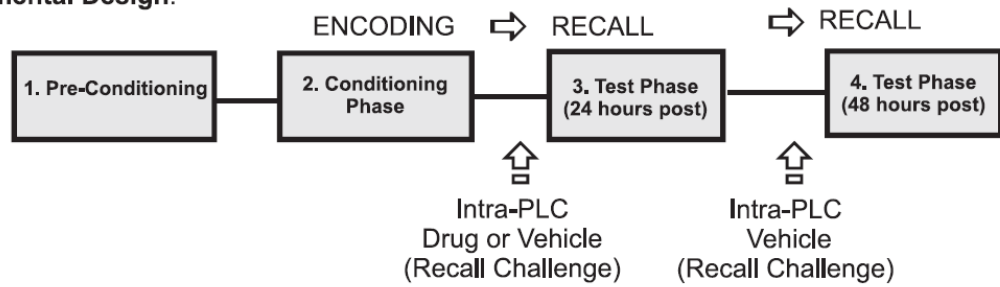
presented for 5 min each to the animal in a counterbalanced order, and the amount of time freezing was recorded. Freezing was defined as complete immobility with the exception of respiratory-related movement. We also analyzed exploratory behaviour in response to presentations of CS+ or CS- odours, as described previously (Rosenkranz and Grace, 2003). Exploratory behaviour was scored as follows, with a score assigned for every min of each of the 5 min during the CS+ or CS- odour presentations: 0, no locomotion; 1, ambulation across one side of the testing chamber; 2, ambulation across two sides; 3, exploration of the full perimeter of the testing chamber; and 4, exploration of the center and entire perimeter of the test chamber. To examine the effects of DA D₁ receptor manipulation on the “recall” phase of learned emotional memory, microinfusions were given directly before testing (24 hours after fear conditioning). To examine the effects of DA D₁ receptor manipulation on the “reconsolidation” phase of learned emotional memory, microinfusions were given directly after the first recall test (24 hrs post-conditioning), and rats were then re-tested 48 hrs to determine if post-recall experimental manipulations led to alterations in the subsequent recall of the same memory. Experimental procedures for fear conditioning experiments we present a schematic illustration in Fig. 4.1C showing our procedure in terms of timelines and methods of drug administration for challenging either the expression (recall phase) or reconsolidation phase of our olfactory fear conditioning procedure.

Figure 4.1 Histological analysis of intra-PLC microinjection sites and experimental protocol.

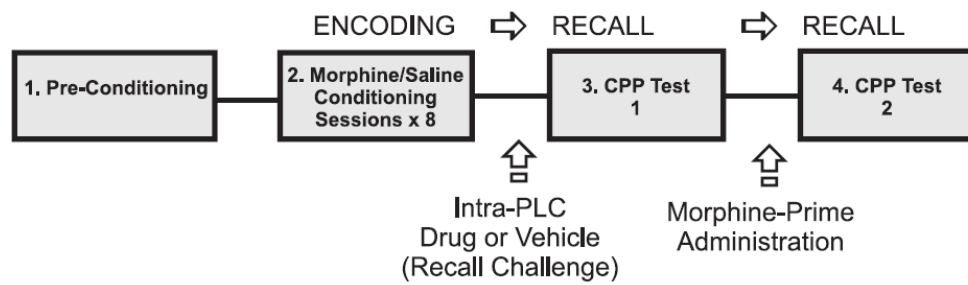
(A) Microphotograph of a representative injector placement within the mPFC. (B) Schematic illustration showing representative bilateral placements of microinjection cannulae. For illustrative clarity, only a subset of experimental groups is presented. Symbols represent separate experimental groups; Δ , SKF81297 100 ng/0.5 μ l vs. expression of olfactory fear conditioning; \square , SKF81297 100 ng/0.5 μ l co-administered with Rp-cAMP 500 ng/0.5 μ l vs. expression of olfactory fear conditioning; \blacktriangledown , SKF81297 100 ng/0.5 μ l vs. morphine CPP; \bullet , SKF81297 100 ng/0.5 μ l co-administered with Rp-cAMP 500 ng/0.5 μ l vs. morphine CPP. (C) Schematic representation showing experimental associative fear conditioning assay and timeline for examining acquisition (encoding), expression (expression), and reconsolidation phases of associative olfactory fear memory. (D) Schematic representation of morphine CPP experimental test procedure and schedule.



C
Olfactory Fear Conditioning
Experimental Design:



D
Morphine Conditioned Place Preference
Experimental Design:



4.2.3 Place conditioning

All rats were conditioned using an unbiased, fully counterbalanced CPP procedure, as described previously (Laviolette and van der Kooy, 2002; Laviolette and van der Kooy, 2003). Conditioning took place in one of two environments, which differed in color, texture, and scent. One environment was white with a wire mesh floor, covered in wood chips. The other environment was black with a smooth Plexiglas floor, wiped down with a 2% acetic acid solution before each conditioning session. Rats display no baseline preference for either of these two environments (Laviolette and van der Kooy, 2003). During pre-conditioning (24 h prior to conditioning), rats were placed in gray Plexiglas boxes for 30 min to minimize stress during future conditioning. Rats received intraperitoneal (i.p.) injections of either morphine (5 mg/kg) or saline vehicle in an alternating order for eight days. This conditioning dose of morphine produces robust CPP that is behaviourally observable at least 14 days post-conditioning (unpublished observations). Immediately after injections, rats were placed in one of the two conditioning environments for 30 min. Rats received four drug-environment and four saline-environment conditioning sessions. At testing, rats received bilateral intra-PLC microinfusions of either saline vehicle, the D₂ receptor agonist quinpirole (100–1000 ng/0.5 µl) or the full D₁ receptor agonist SKF81297 (10–1000 ng/0.5 µl) administered alone or co-administered with cAMP inhibitor Rpt-cAMP (doses of 100 or 500 ng/0.5 µl). At testing, rats were placed on a narrow, neutral gray zone that separated the test environments. Time spent in each environment was digitally recorded for 10 min. This procedure was repeated during the second CPP test day, with bilateral infusions of saline vehicle. A cue-induced CPP test was conducted on

the third testing day; 5 min before assessing place preference, rats received intra-PLC infusions of SKF81297 (100 ng/0.5 μ l) as well as i.p. injections of morphine (5 mg/kg). Times spent in each environment were scored separately for each animal over a 10 min test session. Experimental procedures for the morphine CPP experiments are schematically summarized in Fig. 4.1D.

4.2.4 Drug administration

The DA D₁ receptor agonist, SKF81297 (Tocris), DA D₁ receptor antagonist, SCH23390 (Tocris), DA D₂ receptor agonist, quinpirole (Sigma), morphine (morphine hydrochloride, MacFarlane-Smith), cyclic adenosine monophosphate (cAMP) inhibitor, Rp-cAMP (Biomol), were dissolved in physiological saline (pH adjusted to 7.4). For mPFC microinfusions, stainless steel guide cannulae (22 gauge) were implanted bilaterally into the PLC through a 28 gauge microinfusion injector (Plastics One). Bilateral microinfusions were performed over 1 min through a Hamilton syringe. Following this, injectors were left in place for an additional 1 min to ensure adequate diffusion from the tip.

4.2.5 Fear conditioning drug administration

Bilateral intra-PLC microinjections of either saline vehicle, the selective full D₁ receptor agonist, SKF81297 (Tocris; 10–100 ng/0.5 μ l), the D₂-like receptor agonist quinpirole (100–1000 ng/0.5 μ l), or the cAMP inhibitor, Rp-cAMP (Tocris; 100–500 ng/0.5 μ l) were microinfused immediately prior to the testing phase (expression challenge). We have previously reported that this *in vivo* dose range for SKF81297 is

behaviourally active in the olfactory fear conditioning paradigm (Chapter 2). While no previous studies have specifically examined the effects of intra-PLC quinpirole on the expression of fear memory or morphine CPP, this dose range for intra-cranial microinfusions of the D₂-like receptor agonist quinpirole, has been reported previously to produce behavioural effects in rats in terms of increased oral water consumption (Amato et al., 2012) and sustain intra-accumbens self-administration behaviours *in vivo*, in rats (Ikemoto et al., 1997).

4.2.6 Conditioned place preference drug administration

For experiments examining the effects of intra-mPFC D₁ receptor activation, D₂ receptor activation, or cAMP inhibition on morphine reward memory conditioning, we used a previously established conditioning dose of morphine (5.0 mg/kg, i.p.), which produces robust morphine CPP (Bishop et al., 2010, Sun et al., 2011 and Lintas et al., 2011). Intra-mPFC microinjection of either saline vehicle, or the highly selective full D₁ receptor agonist, SKF81297 (dose 100 ng/0.5 µl), D₂-like agonist quinpirole (dose 1000 ng/0.5 µl), or cAMP inhibitor Rp-cAMP (dose range 100–500 ng/0.5 µl) were given immediately prior to testing for morphine reward behaviour.

4.2.7 Histology

Following completion of the experiment rats were transcardially perfused, brains were removed and stored in formalin with 25% sucrose solution for at least 24 h. Brains were then sectioned into 40 µm coronal slices, mounted, and stained using cresyl violet to allow for histological analysis of the site of injection. The majority of

mPFC placements were localized within the boundaries of the prelimbic cortical area (PLC). Rats found to have injectors located outside the anatomical boundaries of the PLC were excluded from experimental analyses.

4.2.8 Data analysis

Data were analyzed with one or two-way ANOVA followed by Fisher's Least Significant Difference (LSD) tests or student's t-tests, where appropriate. Non-parametric data was analyzed with Friedman's ANOVA Tests and Wilcoxon Signed-Rank Tests where appropriate.

4.3 RESULTS

4.3.1 Histological analysis

Histological analysis revealed microinfusion injector cannulae placements to be bilaterally localized within the anatomical boundaries of the PLC division of the mPFC region, as determined by the Atlas of Paxinos and Watson (1996). In Fig. 4.1A, we present a microphotograph showing a typical, representative injector placement within the PLC. In Fig. 4.1B, we present a schematic illustration showing representative intra-PLC bilateral cannulae placements along the rostral–caudal axis. Rats found to have placements outside the anatomical boundaries of the PLC were removed from analysis.

4.3.2 Intra-PLC dopamine D₁ receptor activation blocks fear memory expression through a D₁ receptor-specific substrate

We have reported previously that DA D₁ receptor stimulation in the PLC dose-dependently blocks the acute expression of previously acquired fear memory over an order of magnitude dose range (10–100 ng/0.5 µl) with a lower dose (10 ng/0.5 µl) producing no block in fear memory expression vs. a higher dose (100 ng/0.5 µl) that completely blocks acute memory expression (Lauzon et al, 2009). In the present study, we selected our previously established behaviourally effective dose of intra-PLC SKF81297 (100 ng/0.5 µl) to confirm that intra-PLC D₁ receptor stimulation induces a block in the acute expression of an associative olfactory fear memory. In addition, we repeated the lower dose of SKF81297 (10 ng/0.5 µl) to confirm it was not sufficient to block acute expression of olfactory fear memory. Furthermore, to examine receptor specificity, in a separate experimental group, we co-administered the selective D₁ antagonist (SCH23390; 1000 ng/0.5 µl) with SKF81297 (100 ng/0.5 µl) immediately prior to fear conditioning memory expression testing. Analysis of conditioned freezing behaviour with two-way ANOVA revealed a significant interaction between group and treatment ($F_{(2,47)} = 6.53$; $p < 0.01$) on times spent freezing to CS+ vs. CS– cue presentations. *Post hoc* analysis revealed that rats in the saline ($n = 8$) control group and the low dose SKF81297 (10 ng/0.5 µl, $n = 8$) demonstrated significant fear conditioning at testing, with greater amounts of time spent freezing to CS+ relative to CS– presentations (p 's < 0.01).

In contrast, rats receiving intra-PLC SKF81297 (100 ng/0.5 µl) demonstrated no behavioural expression of fear memory, demonstrated by the absence of

associative freezing behaviour in response to CS+ vs. CS- presentations ($n = 8$; $p > 0.05$). For the group receiving co-administration of the D₁ antagonist SCH23390 with SKF81297 ($n = 8$), fear memory expression was restored, demonstrated by associative freezing to CS+ relative to CS- presentations ($p < 0.01$) (Fig. 4.2A). Non-parametric analysis (Friedman's ANOVA) of spontaneous exploratory behaviour during presentations of either CS+ or CS- revealed a significant effect of treatment ($\chi^2_{(7)} = 24.95$, $p < 0.01$). *Post hoc* analysis revealed that both vehicle control rats ($n = 8$) and rats receiving the lower dose of SKF81297 (10 ng/0.5 μ l, $n = 8$) demonstrated significant conditioned suppression of exploratory behaviour in response to CS+ presentations (p 's < 0.05). In contrast, *post hoc* analysis revealed a significant suppression of fear-related exploratory behaviour in the group receiving intra-PLC SKF81297 (100 ng/0.5 μ l) alone, demonstrated by the absence of conditioned suppression of exploratory behaviour in response to CS + presentations ($n = 8$; $p > 0.05$). However, in rats receiving co-administration of SCH23390 with SKF81297 ($n = 8$), conditioned suppression of exploratory behaviour was present following CS+ presentations ($p < 0.01$), demonstrating that the blockade of acute fear memory expression via SKF81297 was mediated through a D₁ receptor substrate (Fig. 4.2B).

4.3.3 Dopamine D₂ receptor activation in the PLC does not block the expression of olfactory fear memory

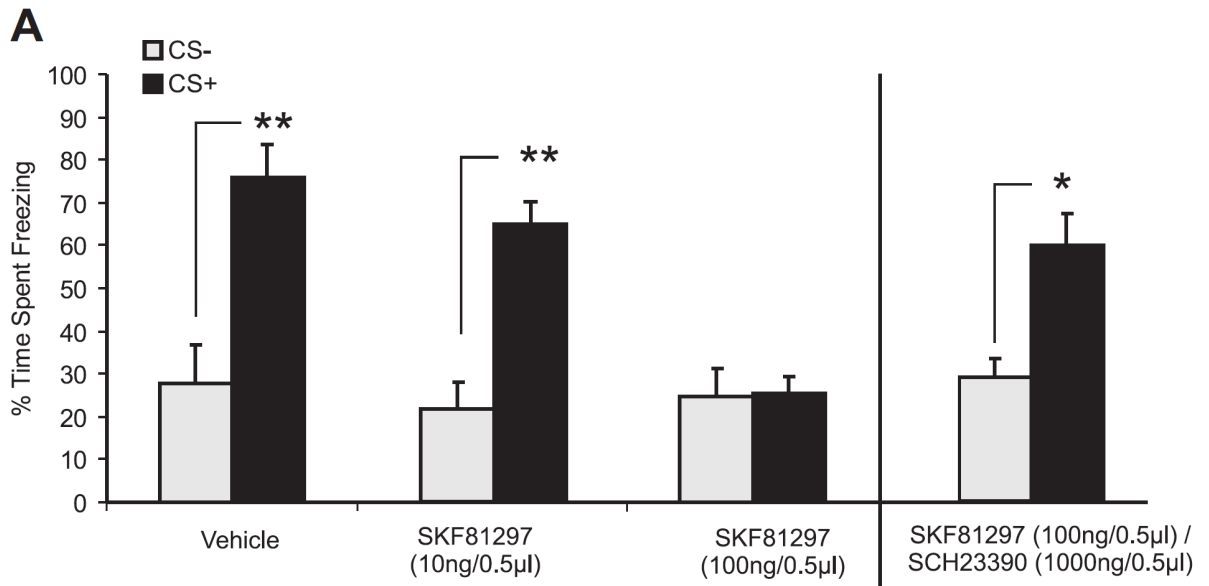
To further examine receptor specificity, we examined the effects of intra-PLC D₂ receptor activation on expression of fear memory, comparing the effects of intra-PLC microinfusions of either vehicle, SKF81297 (100 ng/0.5 μ l) alone, or the selective D₂

receptor agonist quinpirole (100–1000 ng/0.5 μ l) immediately prior to fear conditioning testing. Two-way ANOVA revealed a significant interaction between group and treatment ($F_{(2,45)} = 7.79$; $p < 0.01$) on times spent freezing to either CS+ or CS– cue presentations at testing. *Post hoc* analysis revealed a block in fear memory expression by SKF81297 (100 ng/0.5 μ l) alone, with no significant difference in times spent freezing to CS+ vs. CS– presentations ($n = 8$; $p > 0.05$). In contrast, *Post hoc* analysis revealed that vehicle control rats ($n = 8$), rats receiving intra-PLC quinpirole (100 ng/0.5 μ l; $n = 7$) and rats receiving quinpirole (1000 ng/0.5 μ l; $n = 7$) demonstrated significant fear conditioning at testing, with greater amounts of time spent freezing to CS+ vs. CS– presentations (p 's < 0.01 ; Fig. 4.3A). Non-parametric analysis (Friedman's ANOVA) of spontaneous exploratory behaviour during presentations of either CS+ or CS– revealed a significant effect of treatment ($\chi^2_{(7)} = 37.17$, $p < 0.001$). *Post hoc* analysis revealed a block of conditioned suppression of exploratory behaviour by SKF81297 (100 ng/0.5 μ l) demonstrated by no significant difference in exploratory behaviour in response CS+ vs. CS– presentations ($n = 8$; $p > 0.05$). In contrast, *Post hoc* analysis revealed that vehicle control rats ($n = 8$), rats receiving intra-PLC quinpirole (100 ng/ μ l; $n = 7$) and rats receiving quinpirole (1000 ng/ μ l; $n = 7$) demonstrated associative suppression of exploratory behaviour in response to CS+ vs. CS– presentations (p 's < 0.01 ; Fig. 4.3B). Thus, D₂ receptor activation in the PLC with quinpirole failed to attenuate fear memory expression over an order of magnitude dose range, including a dose 10-fold higher than the behaviourally effective dose of the D₁ agonist, SKF81297.

Figure 4.2 Behavioural effects of bilateral intra-PLC DA D₁ receptor activation on the expression of olfactory fear memory.

(A) Rats receiving either intra-mPFC saline or the low dose D₁ agonist (SKF81297; 10 ng/0.5 µl) displayed normal expression of fear memory, as shown by significantly higher freezing behaviour in response to CS+ presentations compared to CS-. In contrast, administration of a D₁ agonist (SKF81297; 100 ng/0.5 µl) immediately before testing blocked expression of previously acquired fear memories demonstrated by the absence of associative freezing behaviour during testing. Receptor specificity was demonstrated using bilateral intra-PLC administration of SKF81297 (100 ng/0.5 µl) co-administered with a D₁ receptor antagonist (SCH23390; 1000 ng/0.5 µl) which rescued normal expression of fear conditioning memory with significantly higher freezing behaviour in response to CS+ presentations compared to CS-. B, Similar effects were observed in conditioned suppression of exploratory behaviour scores following CS+ vs. CS- presentations, with intra-mPFC D₁ receptor activation blocking conditioned suppression of exploratory behaviour following pre-testing microinfusions of SKF81297 (100 ng/0.5 µl), relative to D₁ agonist low dose (10 ng/0.5 µl) and saline control groups. In addition, normal expression of exploratory behaviour was rescued by co-administering the D₁ receptor antagonist SCH23390 (1000 ng/0.5 µl) with the D₁ receptor agonist, SKF81297 (100 ng/0.5 µl) with significantly lower exploratory behaviour in response to the CS+ compared to the CS-, similar to the saline control group. * $p < 0.05$; ** $p < 0.01$ for this and all subsequent figures.

FREEZING BEHAVIOUR



EXPLORATORY BEHAVIOUR

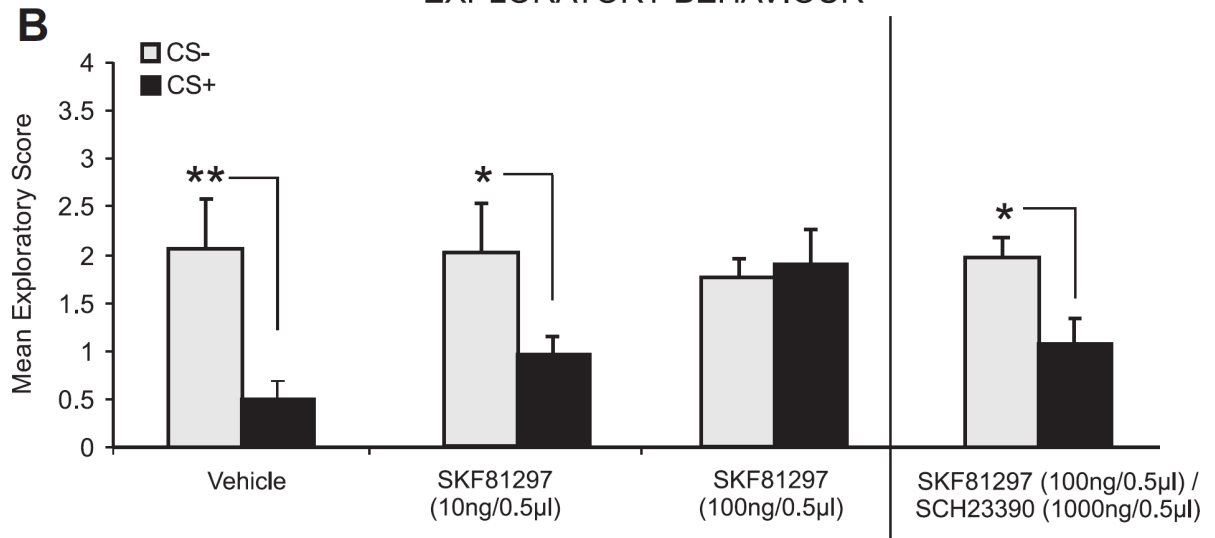
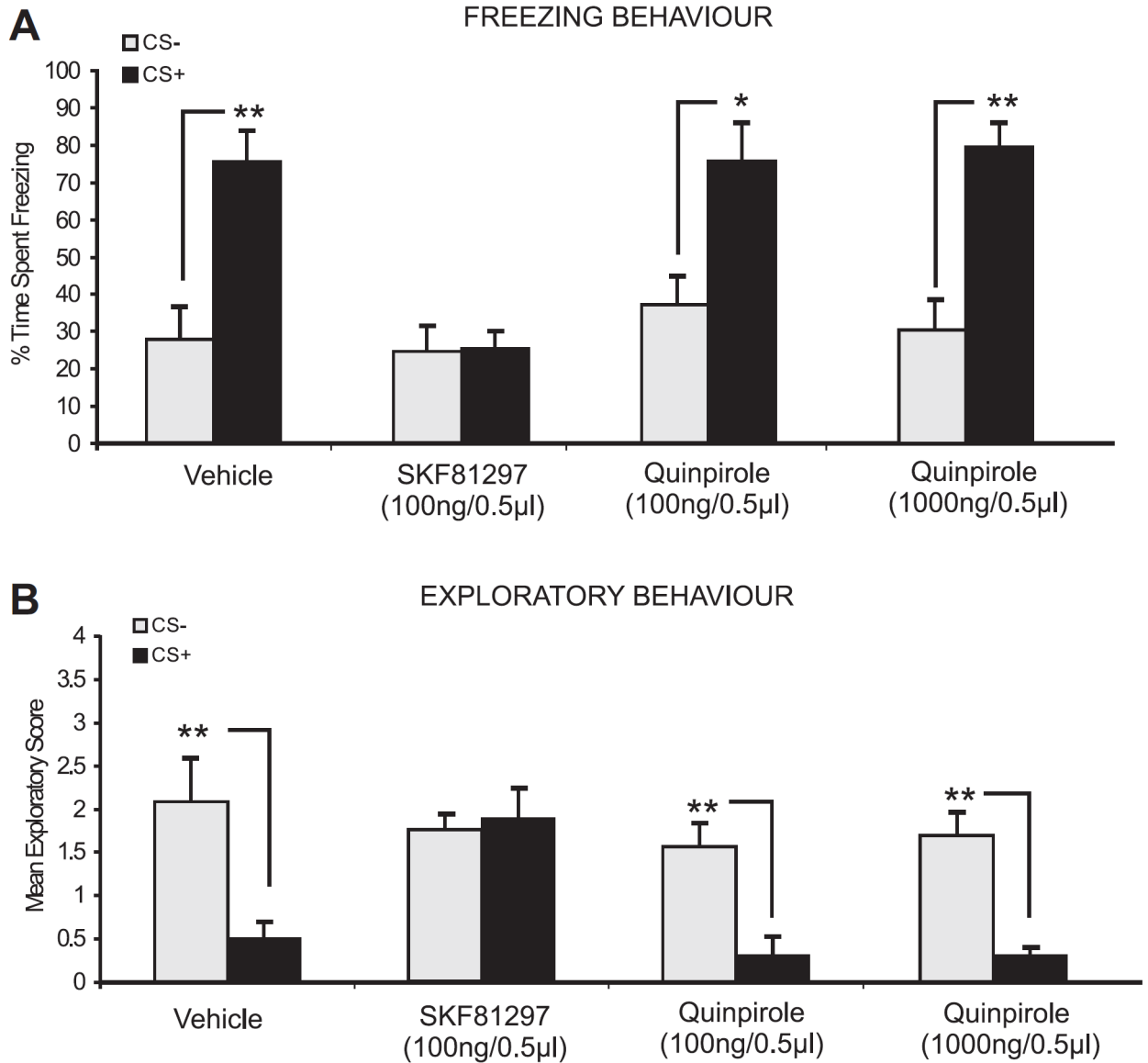


Figure 4.3 Behavioural effects of bilateral intra-PLC DA D₁ and D₂ receptor activation on expression of olfactory fear memory.

(A) Rats receiving intra-PLC saline displayed normal expression (expression) of salient fear conditioning when administered saline immediately before testing, as shown by a significantly higher freezing behaviour in response to CS+ presentations compared to CS-. In contrast, administration of a D₁ agonist (SKF81297; 100 ng/0.5 µl) immediately before testing, completely blocked the expression (expression) of previously acquired emotional learning with rats displaying no significant difference in freezing behaviour in response to CS+ relative to CS- at testing. In contrast, D₂ receptor activation (quinpirole 1000 ng/0.5 µl) had no effect on expression demonstrating normal expression of fear conditioning memory with significantly higher freezing behaviour in response to CS+ presentations compared to CS-. (B) Similar effects were observed in conditioned suppression of exploratory behaviour scores following CS+ vs. CS- presentations, with intra-PLC D₁ receptor activation blocking conditioned suppression of exploratory behaviour following pre-testing microinfusions of SKF81297 (100 ng/0.5 µl), relative to saline controls. In contrast, D₂ receptor activation (quinpirole 1000 ng/0.5 µl) had no effect on expression demonstrating normal expression of fear conditioning memory with significantly lower exploratory behaviour in response to CS+ presentations compared to CS-, similar to the saline control group.



4.3.4 Intra-PLC dopamine D₁ receptor-mediated block of fear memory expression is transient

Next, we examined D₁ receptor activation-mediated block of fear memory expression was transient in nature, or represented a permanent erasure of the original memory. In a separate experiment, we administered the previously established effective dose of intra-PLC SKF81297 (100 ng/0.5 µl) immediately prior to expression testing, followed by a second expression test 24 h later, using intra-PLC saline vehicle infusions immediately prior to the test. Two-way ANOVA revealed a significant interaction between group and treatment ($F_{(2,47)} = 3.59; p < 0.05$) on times spent freezing to either CS+ or CS- cue presentations during testing phases. *Post hoc* analysis revealed that vehicle control rats ($n = 8$) demonstrated robust fear memory expression at testing, with greater amounts of time spent freezing to CS+ relative to CS- presentations ($p < 0.01$). In contrast, fear memory expression was blocked in rats receiving intra-PLC SKF81297 (100 ng/0.5 µl) with no significant difference in time spent freezing to the CS+ relative to CS- presentations ($n = 8; p > 0.05$), during the first expression test (Fig. 4.4A). However, during the second expression test (intra-PLC vehicle), the same rats demonstrated robust expression of the memory, in the absence of intra-PLC D₁ receptor stimulation, showing significant associative freezing in response to CS+ presentations (Fig. 4.4A). Non-parametric analysis (Friedman's ANOVA) of spontaneous exploratory behaviour during CS+/CS- presentations (Fig. 4.4B) revealed a significant effect of treatment ($\chi^2_{(5)} = 13.43, p < 0.05$). *Post hoc* analysis revealed saline control rats' demonstrated significant associative suppression of exploratory behaviours in response to CS+ relative to CS-

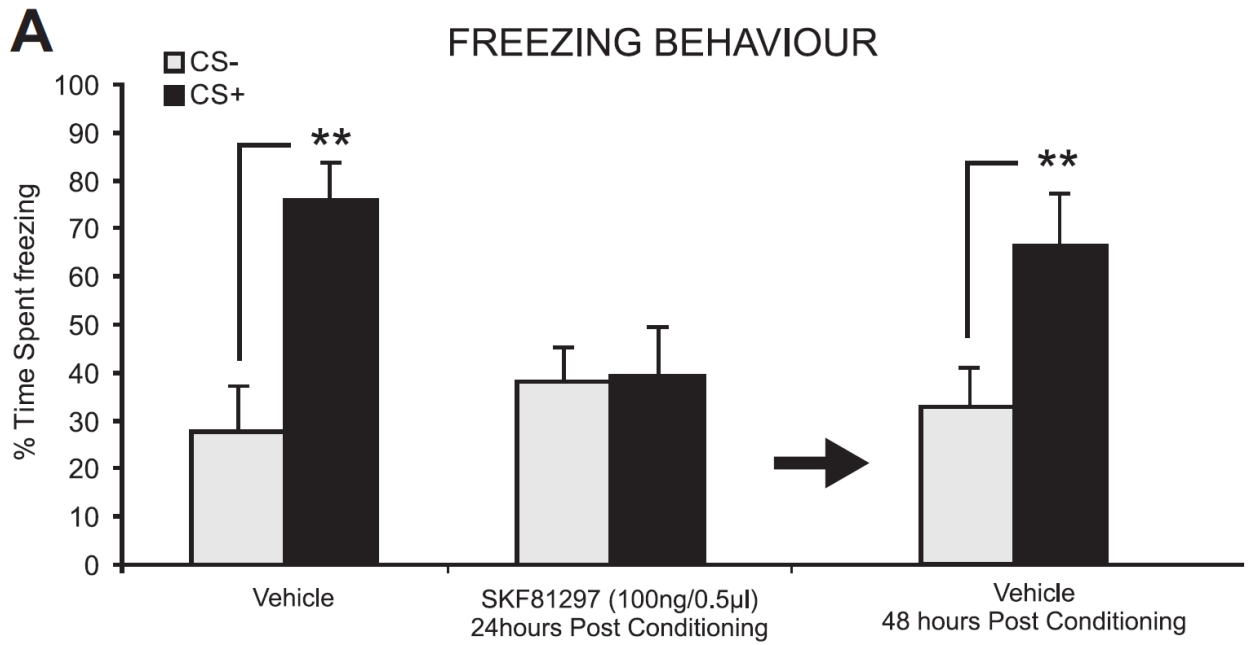
presentations ($p < 0.01$). In contrast, rats receiving intra-PLC SKF81297 demonstrated no associative suppression of exploratory behaviours ($n = 8$; $p > 0.05$). However, this same group demonstrated strong suppression of exploratory behaviour during the second expression test, in the absence of intra-PLC D₁ receptor activation ($p < 0.05$; Fig. 4.4B). These results demonstrate that intra-PLC D₁ receptor-mediated block of fear memory expression is transient in nature, and that the original memory is still intact and can be expressed in the absence of D₁ receptor stimulation.

4.3.5 D₁ receptor mediated block of fear memory expression is cAMP-dependent

Next, we examined the potential role of downstream cAMP signaling during D₁ receptor-mediated blockade of fear memory expression. Given that D₁ receptor activation strongly increases downstream cAMP signaling (Senogles et al., 1988), a separate experimental group received co-administration of the cAMP inhibitor, Rp-cAMP (100–500 ng/0.5 μ l), with the previously established effective dose of the D₁ agonist SKF81297 (100 ng/0.5 μ l) in order to examine if counteracting the potential effects of intra-PLC D₁ receptor activation on cAMP signaling may reverse the observed block of spontaneous fear memory expression. Intra-mPFC microinjections of either saline vehicle, SKF81297 (100 ng/0.5 μ l) alone, or SKF81297 (100 ng/0.5 μ l) co-administered with Rp-cAMP (100–500 ng/0.5 μ l) were given directly before expression testing.

Figure 4.4 Intra-PLC D₁ receptor activation transiently blocks expression of olfactory fear memory.

(A) Rats receiving intra-PLC vehicle displayed normal expression of fear memory, as shown by significantly higher freezing behaviour in response to CS+ presentations. As previously demonstrated, administration of a D₁ agonist (SKF81297; 100 ng/0.5 µl) immediately before testing (24 h post conditioning), completely blocked the expression of previously acquired fear memory with rats displaying no associative freezing behaviour in response to CS+ presentations. The same group was given a vehicle intra-PLC microinjection immediately before a second expression, 48 h post-conditioning. These animals demonstrate normal expression of fear conditioning memory with significantly higher freezing behaviour in response to CS+ presentations compared to CS-, illustrating that the D₁ mediated block of expression is transient in duration. (B) Similar effects were observed in exploratory behaviour scores. Vehicle control rats displayed normal expression of fear memory, shown by associative suppression of exploratory behaviour in response to CS+ presentations whereas intra-PLC D₁ receptor activation SKF81297 (100 ng/0.5 µl) blocked this effect. The same group tested 48 h post-conditioning displayed normal associative suppression of exploratory behaviour, similar to effects observed with freezing behaviour.



In addition, a separate control group received intra-PLC Rp-cAMP (500 ng/0.5 μ l) alone, to control for any potential non-specific effects of the cAMP inhibitor on memory expression ($n = 8$). Two-way ANOVA revealed a significant interaction between group and treatment ($F_{(4,79)} = 5.04$; $p < 0.01$) on times spent freezing to either CS+ or CS- cue presentations. *Post hoc* analysis demonstrated a block in associative fear conditioning expression by both SKF81297 100 ng/0.5 μ l alone ($n = 8$) and SKF81297 100 ng co-administered with Rp-cAMP 100 ng/0.5 μ l ($n = 8$) with no significant difference in time spent freezing to the CS+ relative to CS- presentations (p 's > 0.05). In contrast, *post hoc* analysis revealed that rats in the Saline ($n = 8$) control group, the group receiving SKF81297 100 ng/0.5 μ l co-administered with the high dose of Rp-cAMP 500 ng/0.5 μ l ($n = 8$), and the group receiving Rp-cAMP 500 ng/ μ l alone ($n = 8$) demonstrated significant fear conditioning at testing, with greater amounts of time spent freezing to CS+ relative to CS- presentations (p 's < 0.01 ; Fig. 4.5A). Non-parametric analysis (Friedman's ANOVA) of spontaneous exploratory behaviour during presentations of either CS+ or CS- revealed a significant interaction between group and treatment ($\chi^2_{(9)} = 37.80$, $p < 0.001$). *Post hoc* analysis illustrated a significant suppression of fear conditioning expression by both SKF81297 100 ng/0.5 μ l alone ($n = 8$) and SKF81297 100 ng co-administered with Rp-cAMP 100 ng/0.5 μ l ($n = 8$) demonstrated by no significant difference in exploratory behaviour in response CS+ relative to CS- presentations (p 's > 0.05). In contrast, *post hoc* analysis revealed that rats in the saline ($n = 8$) control group, the group receiving SKF81297 100 ng/0.5 μ l co-administered with the high dose of Rp-cAMP 500 ng/0.5 μ l ($n = 8$), and the group receiving Rp-cAMP 500 ng/ μ l alone ($n = 8$) demonstrated significant fear

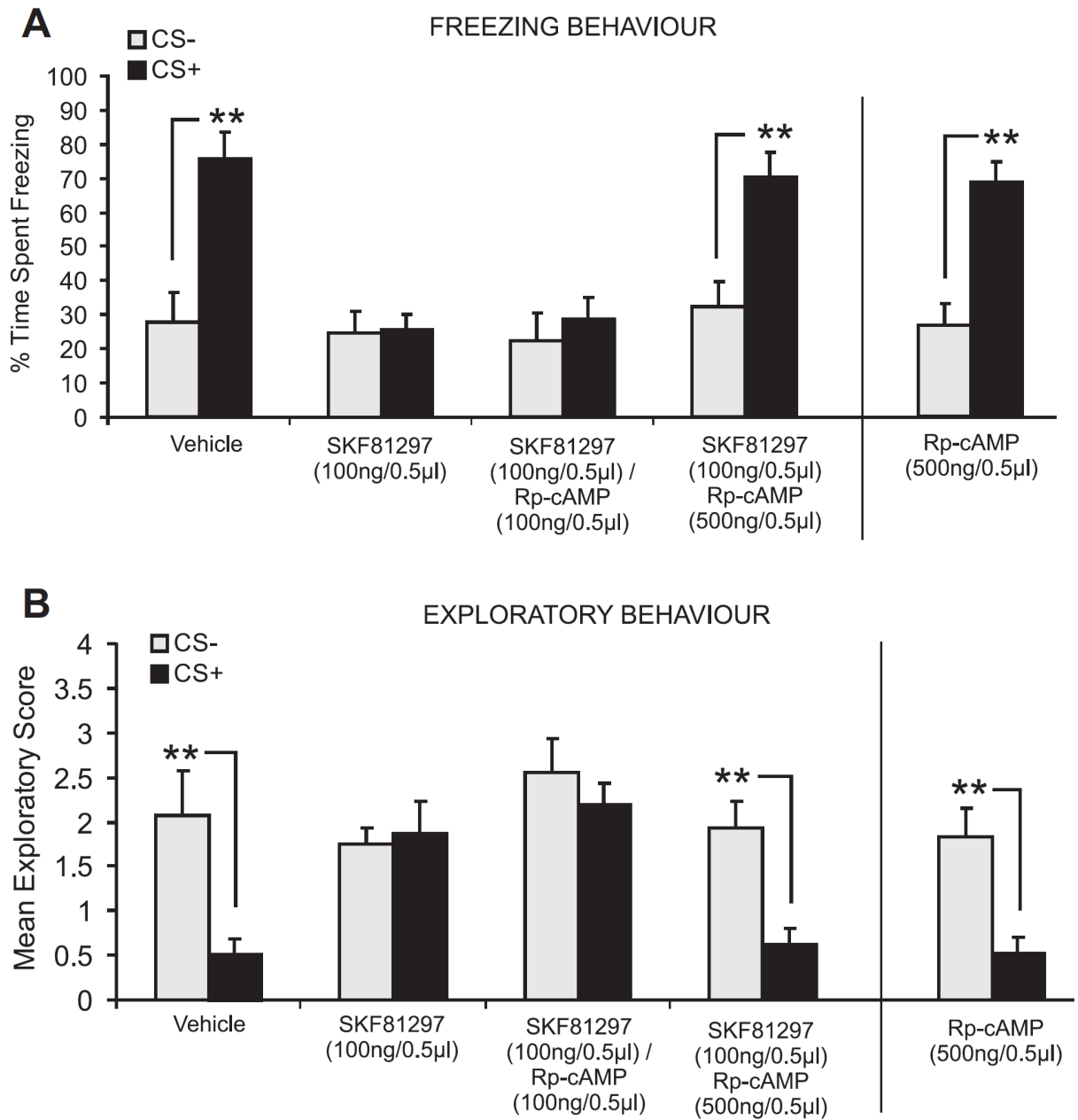
conditioning at testing, with significantly lower exploratory behaviour in response to CS+ relative to CS- presentations (p 's < 0.01; Fig. 4.5B). These results demonstrate that the D₁ receptor mediated block of expression during fear learning is dependent on downstream activation of cAMP with the mPFC, as inhibition of cAMP signaling dose-dependently rescued the effects of intra-PLC D₁ receptor-mediated block of fear memory expression, while having no effects in and of itself on acute fear memory expression.

4.3.6 Intra-PLC D₁ receptor activation blocks expression of spontaneous, but not morphine-primed opiate memory expression

We next examined the effects of intra-PLC D₁ receptor activation on reward memory expression using our previously established morphine CPP procedure (Laviolette and van der Kooy, 2003, Bishop et al., 2010 and Lintas et al., 2011). CPP conditioning was performed using a dose of systemic morphine (5 mg/kg, i.p.) which produces robust CPP (Bishop et al., 2010 and Lintas et al., 2011) and the effects of D₁ activation were tested by administering SKF81297 (10–100 ng/0.5 μ l) directly into the PLC immediately before CPP testing. Two-way ANOVA revealed a significant interaction between group and treatment ($F_{(2,43)} = 3.84$; $p < 0.05$) on times (s) spent in the morphine vs. saline-paired testing environments. *Post hoc* analysis revealed during CPP testing animals receiving intra-mPFC saline ($n = 6$) or the lower dose of D₁ agonist, SKF81297 (10 ng/0.5 μ l; $n = 8$) showed a significant preference for the morphine paired compartment (p 's < 0.01).

Figure 4.5 Inhibition of cAMP signaling rescues D₁-receptor mediated blockade of fear memory expression.

(A) Rats receiving intra-mPFC vehicle displayed normal expression of fear memory, demonstrating associative freezing behaviour in response to CS+ presentations. In contrast, intra-PLC administration of SKF81297 (100 ng/0.5 µl) alone or co-administered with a lower dose of the cAMP inhibitor (Rp-cAMP; 100 ng/0.5 µl) before testing blocked expression of fear memory with rats displaying no associative freezing behaviour in response to CS+ presentations. However, contrast, intra-PLC co-administration of a higher dose of Rp-cAMP (500 ng/0.5 µl) with the D₁ agonist (100 ng/0.5 µl) reversed D₁ mediated memory expression blockade, with rats showing normal associative fear-related freezing. In a separate control experiment, intra-PLC Rp-cAMP (500 ng/0.5 µl) alone did not influence the expression of fear memory, with significantly higher freezing behaviour in response to the CS+ presentations. (B) Similar effects were observed in spontaneous exploratory behaviour, as rats receiving intra-PLC vehicle showing significantly lower exploratory behaviour in response to CS+ presentations. Administration of SKF81297 (100 ng/0.5 µl) alone or co-administered with the lower dose of cAMP inhibitor (Rp-cAMP; 100 ng/0.5 µl) blocked conditioned suppression of exploratory behaviour in response to CS+ presentations. In contrast, intra-mPFC co-administration of the higher dose of Rp-cAMP (500 ng/0.5 µl) with the D₁ agonist (100 ng/0.5 µl) reversed D₁ mediated suppression of expression, shown by significantly lower CS+-related conditioned suppression of exploratory behaviour. Intra-PLC Rp-cAMP (500 ng/0.5 µl) alone does not influence conditioned suppression of exploratory behaviour during expression testing.



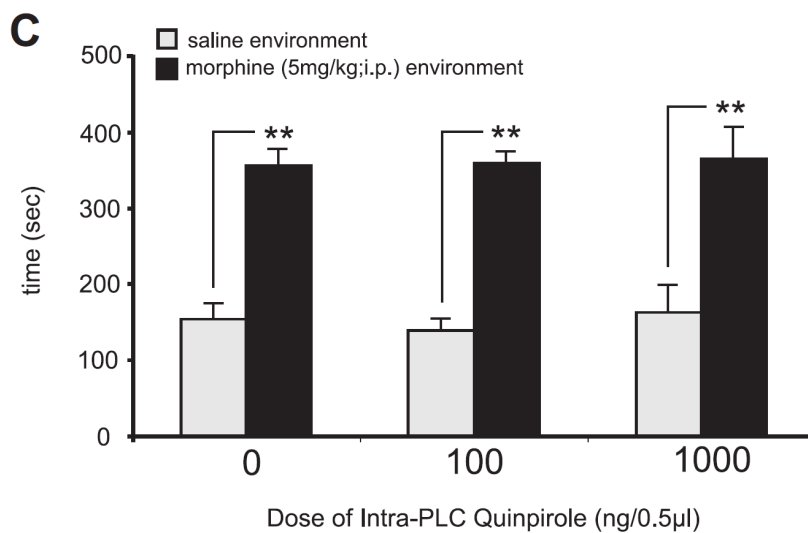
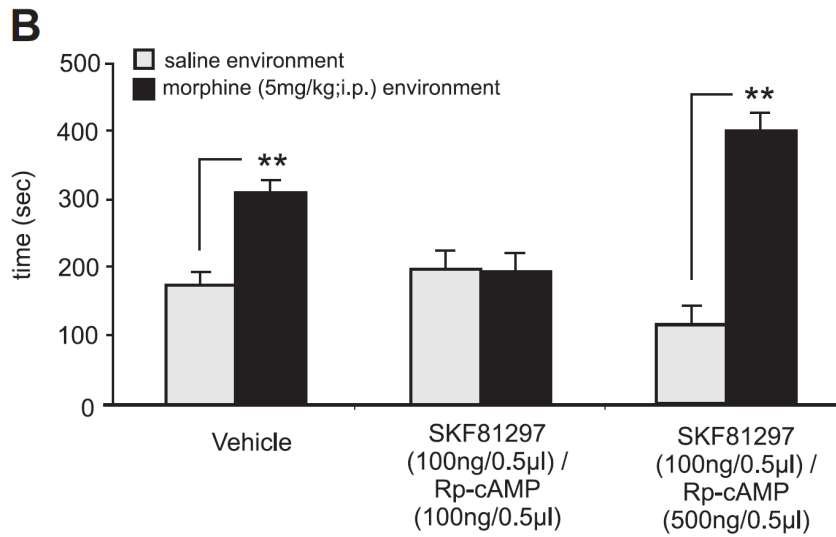
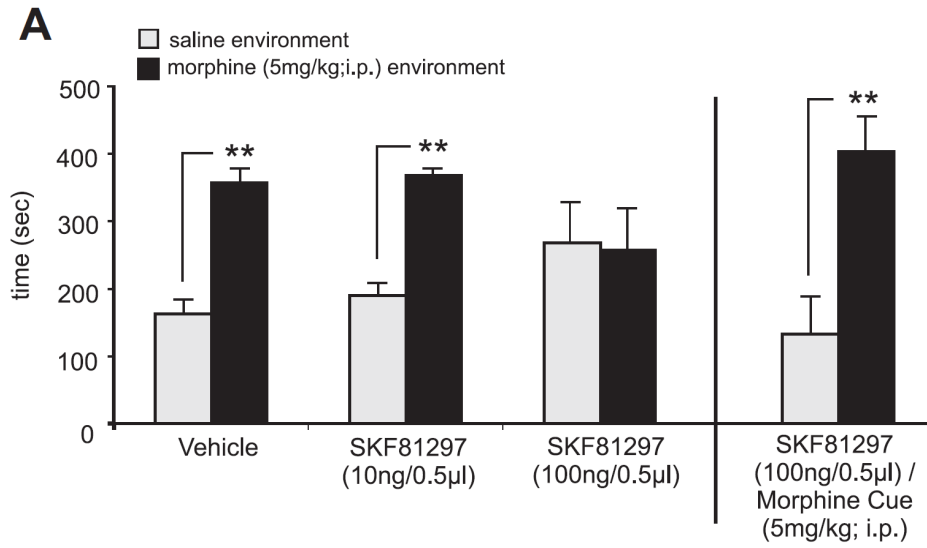
In contrast, animals receiving the higher dose of SKF81297 (100 ng/0.5 μ l; $n = 8$) demonstrated no morphine CPP ($p > 0.05$). In a subsequent test, we tested the same group for CPP expression following a priming injection of morphine (5 mg/kg; i.p.) following intra-PLC administration of the previously determined effective dose of SKF81297 (100 ng/0.5 μ l; $n = 8$). Following morphine priming, we found that intra-PLC D₁ receptor activation failed to block the expression of morphine CPP ($t_{(14)} = 5.13$; $p < 0.01$), with the same rats demonstrating a significant preference for the morphine-paired environment (Fig. 4.6A). These results demonstrate that intra-PLC DA D₁ receptor hyperstimulation transiently inhibits the spontaneous expression of morphine CPP, but does not block morphine-primed memory expression.

4.3.7 D₁ receptor mediated block of associative opiate reward memory is dependent on cAMP activity

We next examined the possible role of downstream cAMP modulation as a potential mediator of intra-PLC D₁ receptor-mediated block of spontaneous opiate associative reward memory expression. Accordingly, we co-administered a cAMP inhibitor, Rp-cAMP, with the highest effective dose of SKF81297 (100 ng/0.5 μ l) immediately prior to CPP expression testing. Two-way ANOVA revealed a significant interaction between group and treatment ($F_{(2,47)} = 17.83$; $p < 0.001$) on times (s) spent in the morphine-paired or saline-paired side of the testing apparatus. Post hoc analysis revealed that groups receiving intra-PLC saline ($n = 8$) or SKF81297 (100 ng/0.5 μ l) co-administered with the higher dose of Rp-cAMP (500 ng/0.5 μ l) showed a significant preference for morphine-paired environments (p 's < 0.01).

Figure 4.6 Effects of intra-mPFC D₁ receptor activation, cAMP inhibition, and D₂ receptor activation on morphine reward CPP expression.

(A) Intra-PLC vehicle controls and rats receiving the lower dose of the D₁ agonist SKF81297 (10 ng/0.5 μ l) displayed significant morphine CPP. In contrast, morphine CPP expression was blocked in rats receiving a higher dose of intra-PLC SKF81297 (100 ng/0.5 μ l). However, rats receiving intra-PLC SKF81297 (100 ng/0.5 μ l) following a morphine priming injection (5 mg/kg; i.p.) displayed a significant morphine CPP. (B) Vehicle controls and rats receiving a lower dose of the cAMP inhibitor Rp-cAMP (100 ng/0.5 μ l) co-administered with the effective dose of SKF81297 (100 ng/0.5 μ l) displayed significant morphine CPP. In contrast, morphine CPP expression was blocked in animals receiving a higher dose of intra-PLC Rp-cAMP (500 ng/0.5 μ l) co-administered with SKF81297 (100 ng/0.5 μ l). (C) In contrast, vehicle controls and or receiving intra-PLC microinfusions of the D₂ receptor agonist quinpirole (100–1000 ng/0.5 μ l) displayed significant morphine CPP.



In contrast, animals receiving the D₁ agonist, SKF81297 (100 ng/0.5 µl) co-administered with a lower dose of Rp-cAMP inhibitor (100 ng/0.5 µl) did not show morphine CPP, with no significant difference in times spent in morphine vs. saline-paired environments ($p > 0.05$) (Fig. 4.6B). Thus, similar to effects observed during olfactory fear memory expression, inhibition of cAMP signaling dose-dependently rescued the memory expression blocking effects of intra-PLC D₁ receptor activation during opiate reward memory expression testing.

4.3.8 Opiate reward memory expression is not dependent upon dopamine D₂ receptor activation

In a separate experiment, we next examined the potential role of intra-PLC DA D₂ transmission during the expression of associative opiate reward memory, by performing bilateral intra-PLC micro-infusions of the D₂ selective receptor agonist, quinpirole (100–1000 ng/0.5 µl) prior to morphine CPP testing, as previously described. Statistical analysis revealed a significant effect of treatment ($F_{(1,43)} = 81.6308$; $p < 0.0001$) on times (s) spent in morphine vs. saline-paired environments during CPP testing. *Post hoc* analysis revealed that DA D₂ receptor activation had no effect on behavioural expression of morphine CPP in neither saline controls ($n = 8$), nor in groups receiving intra-PLC quinpirole over an order of magnitude dose range (100–1000 ng/0.5 µl; n 's = 8), with all groups demonstrating significant CPP for morphine-paired environments (p 's < 0.05 ; Fig. 4.6C). Thus, similar to effects observed during olfactory fear memory expression, block of spontaneous opiate

reward memory expression appears to be independent of D2 receptor signaling in the PLC.

4.4 DISCUSSION

The activation of neuronal networks within the mPFC is associated with the expression of various forms of memory. For example, neuronal populations in the PLC division of the mPFC encode and express associative information related to opiate reward-related memories (Sun et al., 2011 and Sun and Laviolette, 2012). Furthermore, neurons within the mPFC can encode and express associative learning related to aversive stimulus conditioning, such as olfactory fear-related memories (Laviolette et al., 2005 and Laviolette and Grace, 2006a). These results demonstrate that the acquisition and expression of emotionally salient associative memories engages mPFC neuronal networks across rewarding and aversive emotional valences. Furthermore, considerable evidence suggests that these processes are dependent upon specific DA receptor-subtype transmission (Laviolette et al., 2005, Lauzon et al., 2009 and Pezze et al., 2003). However, less is understood regarding how DAergic transmission within the mPFC can control the spontaneous expression of previously acquired emotionally salient memories, particularly across memory expression across rewarding vs. aversive conditioning events.

DAergic modulation of mPFC neuronal networks is known to control the encoding of working memory and the temporal sequencing of behavioural output (Goldman-Rakic, 1995, Goldman-Rakic et al., 1989 and Seamans et al., 1998).

Previous investigations have demonstrated that intra-mPFC activation of D₁ receptors impairs spatial working memory performance (Zahrt et al., 1997) and delayed response performance (Arnsten et al., 1994 and Cai and Arnsten, 1997). Current theories of mPFC DA function have suggested that D₁ receptor transmission may be critical for the preservation and expression of memories over temporal delay periods (Durstewitz and Seamans, 2008) although few studies have examined directly the role of mPFC D₁ receptor transmission during the expression of emotionally salient associative memories. Seamans et al. (1998) reported that blockade of mPFC D₁ receptors blocked the ability of rodents to use previously acquired spatial memory during a delayed performance radial arm task, while having no effect on non-delayed memory expression. These studies suggest that executive memory functions within mPFC neuronal networks may require an optimal level of DA D₁ signaling, specifically during periods of memory expression, rather than during associative memory encoding. While no previous studies have reported that intra-PLC stimulation of D₁ receptor signaling may block spontaneous expression of either aversive or rewarding associative memories, the present results are consistent with the concept that an optimal level of D₁ receptor signaling is required for spontaneous expression of previously acquired associative learning. Previous research has implicated the importance of optimal D₁ receptor signaling by demonstrating an inverted 'U' shaped function for the effects of D₁ receptor stimulation on PFC neuronal activity and correlated cognitive performance in primates (Vijayraghavan et al., 2007). Thus, while low level D₁ receptor stimulation tended to enhance neuronal spatial tuning during a spatial working memory task, high stimulation levels impaired

performance both at the neuronal and behavioural levels. We have previously reported no effect of intra-mPFC D₁ receptor stimulation on the acquisition of fear-related memory, over an order of magnitude dose range in rats (Lauzon et al., 2009). In addition, in the present study, lower doses of intra-PLC D₁ agonists had no apparent memory enhancing effects in terms of behavioural performance. However, both fear conditioning and CPP represent relatively simple, Pavlovian conditioning procedures, whereas previously cited studies used more complex cognitive tasks in both primate and rodent behavioural assays. While future studies are required to examine these differences, one possibility is that the cognitive enhancing effects of low level D₁ receptor stimulation may be observed more specifically in tasks requiring greater cognitive load, as opposed to more basic associative memory procedures.

4.4.1 D₁ receptor activation modulates memory expression through a cAMP-dependent substrate

Functionally, DA transmission within the mPFC influences neuronal network activity in a biphasic manner, with D₂-like receptor activation decreasing the activity of GABAergic interneurons, and D₁ receptor activation increasing the intrinsic excitability of these inhibitory interneurons (Seamans et al., 2001). Specifically, activation of D₁ receptors increases the activity of adenylate cyclase, in turn increasing intracellular levels of cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA; Kebabian and Calne, 1979). Within mPFC neuronal circuits, these events are believed to lead to increased activity of GABAergic interneurons and

feedforward inhibition of mPFC pyramidal output neurons (Starr, 1987, Kebabian and Calne, 1979, Laviolette and Grace, 2006b, Lauzon et al., 2009 and Seamans et al., 2001). Thus, intra-mPFC D₁ receptor activation may ultimately trigger a downstream cascade, increasing feedforward inhibition of mPFC neuronal networks required for the acute expression of associative memories. Anatomically, the rodent mPFC is highly heterogeneous, comprising various subpopulations of interneuronal and pyramidal output neuron populations. In terms of DA transmission, D₁ and D₂ receptors are found ubiquitously throughout these neuronal populations. However, some studies have suggested a preferential localization of the D₁ receptor on non-pyramidal, presumptive interneuron mPFC populations (Vincent et al., 1993 and Sesack and Bunney, 1989). Given that neuronal populations within the mPFC can store associative memories linked to both aversive and rewarding events, increasing cAMP/PKA signaling within the mPFC via D₁ receptor activation may lead to increased feedforward inhibition on mPFC pyramidal neurons, thereby impairing the expression of olfactory fear associations or opiate reward associations (Starr, 1987, Seamans et al., 2001, Laviolette and Grace, 2006b and Lauzon et al., 2009). Furthermore, given the critical involvement of the BLA in the processing of both fear and reward-related associative memories (Lintas et al., 2011 and Tan et al., 2011), D₁ receptor activation within the PLC may modulate memory recall via descending influences on BLA neuronal populations.

In addition to the traditional D₁-AC-cAMP pathway, it is important to note that D₁ receptor transmission can produce bidirectional modulation of neuronal activity

via different intracellular cascades. DA input provides a state dependent dynamic regulation of intracellular cascades allowing for different mechanisms of intracellular Ca^{2+} potentials by acting through PKA or protein kinases C (PKC) pathways. It has been demonstrated within pyramidal neurons. In terms of the present findings, simultaneous inhibition of cAMP rescued DA D_1 -receptor mediated block of both aversive and rewarding associative memories, suggesting a common molecular pathway within the PLC involving D_1 /cAMP signaling. Our findings are consistent with a previous report demonstrating that co-administration of a cAMP activator can similarly rescue the cognitive impairment induced by intra-mPFC D_1 receptor overstimulation in primates performing a delayed spatial memory task, further demonstrating the important functional link between intra-cortical D_1 receptor transmission and cognitive processing (Vijayraghavan et al., 2007). Interestingly, in the context of reward-related learning and memory, previous evidence has demonstrated that cAMP/PKA stimulation or inhibition directly within the nucleus accumbens, can disrupt the acquisition of reward-related memory, specifically, the acquisition of amphetamine-conditioned CPP (Beninger et al., 2003). Thus, cAMP/PKA signaling may be critical not only for the expression of drug reward-related memories, but may be necessary for the acquisition of these memories as well.

Although the spontaneous expression of opiate reward memory CPP was dose-dependently blocked by acute intra-PLC D_1 receptor activation, when rats were given a morphine priming injection immediately prior to expression testing, concomitant with intra-PLC D_1 receptor activation, we observed normal expression of the CPP

behaviour. This demonstrates that the ability of D₁ receptor activation to disrupt morphine reward memory expression can be prevented by the presence of the morphine conditioning prime. Importantly, this result also demonstrates that D₁ receptor activation was not preventing behavioural expression of the morphine CPP via performance disruption, since the same dose of SKF81297 failed to block morphine CPP expression in the presence of the morphine priming dose. Previous research has suggested that the retrieval of morphine-related associative memories is more likely to occur in the presence of the morphine conditioning cue during expression testing (Izquierdo and Dias, 1983 and Darbandi et al., 2008). In addition, morphine state-dependent memory retrieval can be mediated by neurotransmitter systems other than DA, including muscarinic acetylcholine receptors within the amygdala and ventral tegmental area (Darbandi et al., 2008 and Rezayof et al., 2009). Thus, the present results may suggest that the administration of a morphine priming injection may counteract the effects of intra-PLC D₁ receptor-mediated memory expression suppression. Alternatively, exposure to the morphine conditioning prime during CPP testing may activate a state-dependent memory pathway that triggers behavioural expression of the original memory, bypassing D₁ receptor-dependent expression mechanisms within the PLC.

4.4.2 Potential implications for memory-related neuropsychiatric disorders

The obtrusive and spontaneous expression of associative memories linked to either traumatic or highly rewarding experiences may be a critical factor underlying the

pathological features of disorders such as PTSD or addiction. PTSD patients may associate a wide variety of sensory stimuli with intense fear (Rothbaum and Davis, 2003). While the specific role of D₁ signaling in the context of PTSD has not been extensively examined in clinical populations, abnormally low levels of cAMP signaling have been found to be a persistent correlate of PTSD in clinical populations (Kolb, 1989) leading to the suggestion that lowered cAMP signal transduction may serve as a clinical biomarker for PTSD (Lerer et al., 1987). DA D₁ receptor stimulation is critically linked to downstream regulation of cAMP (Senogles et al., 1988) and in the present study, the effects of intra-PLC D₁ receptor stimulation on blockade of spontaneous fear memory expression were reversed by concomitant suppression of cAMP activity. Together with the well-established profound decreases in cAMP levels observed in PTSD clinical populations, the present results may have implications for understanding how DA D₁-mediated inhibition of cAMP levels may modulate spontaneous expression of traumatic associative memories through PFC-dependent memory circuits. Indeed, considerable evidence points to neuropathological abnormalities within frontal cortical regions of PTSD patients as potential clinical correlates of PTSD-related memory disturbances and lack of control over traumatic memory expression (Tavanti et al., 2012 and Carrion et al., 2009).

In the context of addiction-related memories, neurons within the mPFC both encode and express associative memories linked to the rewarding properties of opiates (Sun et al., 2011 and Sun and Laviolette, 2012). Furthermore, activation of mPFC neuronal circuits is linked to various drug-seeking behaviours (Daglish et al.,

2001, Luo et al., 2004, Koya et al., 2006 and Langleben et al., 2009). The formation and persistence of these drug-related associative memories can trigger drug-seeking behaviours or relapse in response to drug-associated stimuli (de Wit and Stewart, 1981 and Ehrman et al., 1992). The present findings demonstrate that transmission through a D₁ receptor-specific substrate within the PLC is involved critically in the spontaneous expression of opiate-related reward memories. Indeed, considerable evidence has linked D₁ receptor transmission to psychostimulant drug-related memory expression. For example, D₁ receptor activation has been shown to decrease cocaine-seeking behaviour in rats, whereas D₂ receptor activation triggers cocaine-seeking behaviour and relapse (Self et al., 1996). In addition, D₁ receptor activation has been reported to induce extinction like behaviours in rats that have previously acquired cocaine-related self-administration behaviours, suggesting that stimulation of D₁ transmission may degrade previously acquired drug-related associative memories (Self et al., 2000). While the current experiments examined only the acute expression of a previously acquired associative opiate reward memory, an interesting possibility is that intra-PLC D₁ receptor stimulation may trigger a memory extinction-like process for opiate-related associative memory over longer term administration. In the present study, D₁ receptor stimulation directly within the PLC blocked the spontaneous expression of a morphine-related associative reward memory.

Interestingly however, D₁ activation did not block morphine reward memory expression in the presence of a morphine prime, suggesting that, similar to effects observed with fear conditioned memory, supra-normal stimulation of D₁ receptor

transmission within the PLC transiently prevents the expression of opiate-related reward memory, while leaving the original associative memory intact. Alternatively, the re-activation of the morphine associative memory in the presence of the morphine conditioning cue may be sufficient to overcome the effects of intra-PLC D₁ receptor activation, or activate the memory through a DA-independent memory mechanism. Similar to the effects observed with D₁-receptor mediated modulation of associative fear memory, the transient blockade of drug-related reward memory was dependent upon downstream cAMP signaling, as inhibition of cAMP rescued D₁-receptor activation mediated memory expression blockade. Alternatively, while we observed a block in the behavioural expression of previously acquired associative memories, considering that it is possible to dissociate memory recall from behavioural expression, it is important to consider the possibility that these conditioned memories may have been recalled, but were masked due to an inability to behaviorally express them during testing.

4.5 SUMMARY

We report that specific activation of D₁ receptor transmission within the PLC can transiently block the spontaneous expression of associative memories linked to either emotionally aversive or rewarding conditioning events. In both cases, D₁ receptor activation appeared to leave the original memory trace intact, as animals were able to express the original memory in the absence of D₁ receptor activation, or in the case of associative opiate reward memories, in the presence of the original drug cue. These effects were dependent upon downstream cAMP stimulation as simultaneous

inhibition of cAMP reversed the effects of D_1 activation on the blockade of memory expression. These findings demonstrate that supra-normal stimulation of D_1 receptors within the PLC can generally disrupt spontaneous associative memory expression, regardless of emotional valence. Together with previously discussed evidence, our results further implicate the critical role of intra-cortical D_1 receptor signaling during the expression of previously acquired learning and memory tasks, across a wide range of cognitive processes.

4.6 REFERENCES

- Arnsten AFT, Cai JX, Murphy BL, Goldman-Rakis PS (1994) Dopamine D₁ receptor mechanisms in the cognitive performance of young adult and aged monkeys. *Psychopharmacology* 116:143-151.
- Bishop SF, Lauzon NM, Bechard M, Gholizadeh S, Laviolette SR (2011) NMDA receptor hypofunction in the prelimbic cortex increases sensitivity to the rewarding properties of opiates via dopaminergic and amygdalar substrates. *Cereb Cortex*: 21(1):68-80.
- Blum S, Runyan JD, Dash PK (2006). Inhibition of prefrontal protein synthesis following recall does not disrupt memory for trace fear conditioning. *BMC Neurosci.* 7:67.
- Cai JX, Arnsten AF (1997) Dose-dependent effects of the dopamine D₁ receptor agonists A77636 or SKF81297 on spatial working memory in aged monkeys. *J Pharmacol Exp Ther*: 283(1):183-9.
- Carrion VG, Weems CF, Watson C, Eliez S, Menon V, Reiss AL. (2009) Converging evidence for abnormalities of the prefrontal cortex and evaluation of midsagittal structures in pediatric posttraumatic stress disorder: an MRI study. *Psychiatry Res.* 172:226-234.
- Daglish MRC, Weinstein A, Malizia AL, Wilson S, Melichar JK, Britten S, Brewere C, Lingford-Hughes A, Myles JS, Grasby P, et al. (2001) Changes in regional cerebral blood flow elicited by craving memories in abstinent opiate-dependent subjects. *Am J Psychol* 158:1680-1686
- de Wit H, Stewart J. (1981) Reinstatement of cocaine-reinforced responding in the rat. *Psychopharmacology* 75:134-43.
- Durstewitz D, Seamans JK (2008) The dual-state theory of prefrontal cortex dopamine function with relevance to catechol-O-methyltransferase genotypes and schizophrenia. *Biol Psychiatry*: 64(9):739-49.
- Darbandi N, Rezayof A, Zarrindast MR (2008) Modulation of morphine state-dependent learning by muscarinic cholinergic receptors of the ventral tegmental area. *Physiol Behav* 94: 604-610.
- Edwards S, Whisler KN, Fuller DC, Orsulak PJ, Self DW. (2007) Addiction-related alterations in D₁ and D₂ dopamine receptor behavioural responses following chronic cocaine selfadministration. *Neuropsychopharmacology.* 32:354-66.
- Ehrman RN, Robbins SJ, Childress AR, O'Brien CP. (1992) Conditioned responses to cocaine related stimuli in cocaine abuse patients. *Psychopharmacology*107:523-9.
- Goldman-Rakic PS., Leranthe, C., Williams S.M., Mons, N., Geffard M. (1989) Dopamine synaptic complex with pyramidal neurons in primate cerebral cortex. *PNAS* 86:9015-9019.
- Goldman-Rakic PS (1995) Cellular basis of working memory. *Neuron* 14:477-485.
- Izquierdo I, Dias RD (1983) Memory as a state dependent phenomenon: role of ACTH and epinephrine. *Behav Neural Biol* 38: 144-149.

- Kebabian JW, Calne DB (1979) Multiple receptors for dopamine. *Nature* 277: 93-96.
- Kolb LC (1989) Chronic post-traumatic stress disorder: implications of recent epidemiological and neuropsychological studies. *Psychological Med* 19:821-824.
- Koya E, Spijker S, Voorn P, Binnekade R, Schmidt ED, Schoffelmeer ANM, De Vries TJ, Smit AB (2006) Enhanced cortical and accumbal molecular reactivity associated with conditioned heroin, but not sucrose seeking behaviour. *J Neurochem*; 98: 905-915.
- Langleben DD, Ruparel K, Elman I, Busch-Winokur S, Pratiwadi R, Loughhead J, O'brien CP, Childress AR. Acute effect of methadone maintenance dose on brain fMRI response to heroinrelated cues. *Am J Psychol* 2008;165:390-394.
- Lauzon NM, Bishop SF, Laviolette SR (2009) Dopamine D₁ versus D₄ receptors differentially modulate the encoding of salient versus nonsalient emotional information in the medial prefrontal cortex. *J Neurosci* 29: 4836-4845.
- Lauzon NM, Ahmad T, Laviolette SR. (2011) Dopamine D₄ Receptor Transmission in the Prefrontal Cortex Controls the Saliency of Emotional Memory via Modulation of Calcium Calmodulin-Dependent Kinase II. *Cereb Cortex*. E-pub ahead of print, Nov 24, 2011.
- Lauzon NM, Laviolette SR. (2010) Dopamine D₄-receptor modulation of cortical neuronal network activity and emotional processing: Implications for neuropsychiatric disorders. *Behav Brain Res*.17:12-22.
- Laviolette SR, van der Kooy D (2003) Blockade of mesolimbic dopamine transmission dramatically increases sensitivity to the rewarding effects of nicotine in the ventral tegmental area. *Mol Psychiatry*: 8(1):50-9.
- Laviolette SR, Lipski WJ, Grace AA (2005) A subpopulation of neurons in the medial prefrontal cortex encodes emotional learning with burst and frequency codes through a dopamine D₄ receptor-dependent basolateral amygdala input. *J Neurosci* 25(26): 6066-6075.
- Laviolette SR, Grace AA (2006a) Cannabinoids potentiate emotional learning plasticity in neurons of the medial prefrontal cortex through basolateral amygdala inputs. *J Neurosci* 26:6458-6468.
- Laviolette SR, Grace AA (2006b) The roles of cannabinoid and dopamine receptor systems in neural emotional learning circuits: implications for schizophrenia and addiction. *Cell Mol Life Sci*. 63:1597-613.
- Lerer B, Ebstein RP, Shestatsky M, Shemesh Z, Greenberg D. (1987) Cyclic AMP signal transduction in posttraumatic stress disorder. *Am J Psych* 144:1324-1327.
- Lintas A, Chi N, Lauzon NM, Bishop SF, Gholizadeh S, Sun N, Tan H, Laviolette SR. (2011) Identification of a dopamine receptor-mediated opiate reward memory switch in the basolateral amygdala-nucleus accumbens circuit. *J Neurosci*. 31:11172-83.

- Luo F, Xi ZX, Wu G, Liu C, Gardner EL, Li SJ. (2004) Attenuation of brain response to heroin correlates with the reinstatement of heroin-seeking in rats by fMRI. *Neuroimage*; 22: 1328-1335
- Nader K, Schafe GE, Le Doux JE. (2000) Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature*. 406:722-6.
- Paxinos G, Watson C (1996) *The rat brain in stereotaxic coordinates*, Ed 2. San Diego: Academic Press.
- Pezze MA, Bast T, Feldon J (2003) Significance of dopamine transmission in the rat medial prefrontal cortex for conditioned fear. *Cereb Cortex* 13: 371-380.
- Rezayof A, Khajepour L, Zarrindast MR (2009) The amygdala modulates morphine-induced state-dependent memory retrieval via muscarinic acetylcholine receptors. *Neuroscience* 160(2): 255-263.
- Rosenkranz JA and Grace AA (2003) Affective conditioning in the basolateral amygdala of anesthetized rats is modulated by dopamine and prefrontal cortical inputs. *Ann N Y Acad Sci*: 985:488-91.
- Rothbaum BO, Davis M (2003) Applying learning principles to the treatment of post-trauma reactions. *Ann N Y Acad Sci*: 1008:112-21.
- Santini E, Ge H, Ren K, Peña de Ortiz S, Quirk GJ. (2004) Consolidation of fear extinction requires protein synthesis in the medial prefrontal cortex. *J Neurosci*. 24:5704-10.
- Seamans JK, Floresco SB, Phillips AG (1998) D₁ receptor modulation of hippocampal-prefrontal cortical circuits integrating spatial memory with executive functions in the rat. *J Neurosci*. 18:1613-1621.
- Seamans JK, Gorelova N, Durstewitz D, Yang CR (2001). Bidirectional dopamine modulation of GABAergic inhibition in prefrontal cortical pyramidal neurons. *J Neurosci* 21: 3628-3638.
- Seamans JK, Yang CR (2004) The principle features and mechanisms of dopamine modulation in the prefrontal cortex. *Prog Neurobiol* 74: 1-57.
- Self DW, Barnhart WJ, Lehman DA, Nestler EJ. (1996) Opposite modulation of cocaine-seeking behaviour by D₁- and D₂-like dopamine receptor agonists. *Science*. 271:1586-9.
- Self DW, Karanian DA, Spencer JJ. (2000) Effects of the novel D₁ dopamine receptor agonist ABT-431 on cocaine self-administration and reinstatement. *Ann N Y Acad Sci*. 909:133-44.
- Senogles SE, Amlaiky N, Berger JG, Caron MG (1988) Biochemical properties of D₁ and D₂ dopamine receptors. *Adv. Exp. Med. Biol.*, 235, 33-41.
- Sesack, S.R. & Pickel, V.M. (1990) In the rat medial nucleus accumbens,
- Starr M (1987) Opposing roles of dopamine D₁ and D₂ receptors in nigral gamma-[³H]aminobutyric acid release? *J Neurochem* 49: 1042-1049.

- Sun N, Laviolette S (2012) Inactivation of the basolateral amygdala during opiate reward learning disinhibits prelimbic cortical neurons and modulates associative memory extinction. *Psychopharmacology*. E-pub ahead of print Mar 21, 2012.
- Sun N, Chi N, Lauzon N, Bishop S, Tan H, Laviolette SR. (2011) Acquisition, extinction, and recall of opiate reward memory are signaled by dynamic neuronal activity patterns in the prefrontal cortex. *Cereb Cortex*. 21:2665-80.
- Tavanti M, Battaglini M, Borgogni F, Bossini L, Calossi S, Marino D, Vatti G, Pieraccini F, Federico A, Castrogiovanni P, De Stefano N. (2012) Evidence of diffuse damage in frontal and occipital cortex in the brain of patients with post-traumatic stress disorder. *Neurol Sci*. 33:59-68.
- Vijayraghavan S, Wang M, Birnbaum SG, Williams GV, Arnsten AF. Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. *Nat Neurosci*. 2007 Mar;10(3):376-84.
- Vincent S.L., Khan Y., Benes F.M. (1993) Cellular distribution of dopamine D1 and D2 receptors in rat medial prefrontal cortex. *J. Neurosci.*; 13: 2551–2564
- Zahrt J, Taylor JR, Mathew RG, Arnsten AF (1997) Supranormal stimulation of D₁ dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance. *J Neurosci* 17(21): 8528-8535.

Chapter 5:

5 General Discussion

5.1 SUMMARY OF RESULTS AND CONCLUSIONS

5.1.1 DA D₄ versus D₁ subtype receptor transmission within the mPFC plays distinct functional roles in emotional learning and memory

The findings in Chapter 2 begin to explain the complex role for DA transmission within the mPFC during the processing of emotional and cognitive information. Specifically, the existence of an optimal level of DA transmission in the mPFC function and that overstimulation of the DA D₁ or D₄ receptor subtypes in the mPFC can disrupt adaptive associative learning and memory. Given the ability of D₄ receptor activation to amplify DA-mediated neuronal activity within the mPFC (Ceci et al., 1999), we hypothesized that activation of mPFC D₄ receptors may potentiate the emotional salience of normally subthreshold sensory stimuli, leading to heightened emotional memory encoding. We found that specific activation of DA D₄ receptors within the mPFC strongly potentiates the salience of normally nonsalient emotional associative fear memories and blocks the encoding of suprathreshold conditioned fear associations. However, D₄ receptor activation has no effect on the recall of previously learned emotionally salient conditioned memories. In contrast, intra-mPFC D₁ receptor activation failed to increase the emotional salience of subthreshold fear stimuli but completely blocked the expression of previously learned emotionally relevant information, demonstrating that DA D₄ versus D₁ subtype receptor transmission within the mPFC plays distinct functional roles in the processing of emotionally salient versus nonsalient associative information and

differentially modulates the encoding versus recall phases of emotional memory within the mPFC (Lauzon et al., 2009).

5.1.2 CaMKII bidirectionally regulates fear memory salience via DA D₄ transmission in the mPFC

The intriguing and dynamic relationship of DA D₄ receptor activation within the prelimbic (PLC) division of the mPFC leads to a bidirectional modulation of emotional memory by strongly potentiating the salience of normally non-salient emotional memories, but blocking the acquisition of suprathreshold emotionally salient fear memories. These behavioural effects add to previous *in vitro* studies demonstrating that activation of cortical DA D₄ receptors can bidirectionally modulate levels of CaMKII, a molecule essential for learning and memory (Gu and Yan 2004; Gu et al. 2006). Taken together, we began our exploration into regulation of intracellular CaMKII levels as a possible mechanism underlying the functional, bidirectional effects of DA D₄ receptor activation. We report that CaMKII blockade prevents the ability of intra-mPFC DA D₄ receptor activation to potentiate the salience of subthreshold fear memory. In contrast, blockade of either cAMP/PKA or PP1 signaling pathways rescued the blockade of suprathreshold fear memory via intra-mPFC D₄ receptor activation. Our results demonstrate that modulation of emotional memory salience via intra-mPFC DA D₄ receptor transmission depends upon downstream signaling via CaMKII, cAMP/PKA, and PP1 substrates. These findings add to a growing body of evidence linking CaMKII signaling to the aberrant emotional regulation present in schizophrenia-related psychoses, and provide a

potential DA receptor-specific substrate through which CaMKII signaling may control emotional salience processing and memory formation (Malenka and Nicoll 1999; Frankland et al. 2001). Furthermore, the present results identify several downstream molecular targets of intra-PLC D₄ receptor substrates, such as CaMKII, which may serve as potential pharmacotherapeutic targets in the context of aberrant D₄ receptor signaling linked to dysregulated emotional processing and memory encoding in psychiatric illnesses such as schizophrenia, ADHD, and addiction (Lauzon et al., 2012).

5.1.3 D₁ receptor activation modulates associative memory recall through a cAMP-dependent substrate

In our final investigations of this thesis, we demonstrate that specific activation of DA D₁ receptors in the prelimbic (PLC) division of the mPFC causes a transient block in the recall of both aversive and rewarding associative memories. We report that intra-PLC microinfusion of a selective D₁ receptor agonist blocks the spontaneous recall of an associative olfactory fear memory without altering the stability of the original memory trace. Furthermore, using an unbiased place conditioning procedure (CPP), intra-PLC D₁ receptor activation blocks the spontaneous recall of an associative morphine (5 mg/kg; i.p.) reward memory, while leaving cue-induced morphine reward memories intact. Interestingly, both intra-PLC D₁-receptor mediated blockade of either fear-related or reward-related associative memories were dependent upon downstream cyclic-AMP (cAMP) signaling as both effects were rescued by co-administration of a cAMP signaling inhibitor. The blockade of both rewarding and

aversive associative memories are mediated through a D₁-specific signaling pathway, as neither forms of spontaneous memory recall were blocked by intra-PLC microinfusions of a D₂-like receptor agonist. Our results demonstrate that the spontaneous recall of either rewarding or aversive emotionally-salient memories share a common, D₁-receptor mediated substrate within the mPFC. Our findings have important implications for understanding disorders such as post-traumatic stress disorder and addiction, both of which are characterized by the obtrusive recall of memories linked to either aversive experiences, or drug-related reinforcement memories (Lauzon et al., 2013).

5.1.4 Limitations

Currently, neuroscientists are working to characterize the complex functions of monoamine neurotransmitter systems in various neural pathways. In most current investigations, this involves investigating the effect of a given monoamine in isolation. Although this approach has led to a wealth of knowledge, it is important to bear in mind that a given monoamine is seldom released in isolation. Moreover, DA, cannabinoid, and serotonin receptors coexist in the mPFC (Santana et al., 2004; Santana et al., 2009; Amargós-Bosch et al., 2004), and many of their intracellular signaling pathways overlap (Di Pietro & Seamans JK, 2007). At the molecular level, cannabinoid, serotonin, and DA receptors may synergistically interact with one another in the mPFC, potentiating each other's downstream effects through cyclic AMP and other signaling cascades, suggesting that these neurotransmitter systems may modulate emotional processing mechanisms through these functional interactions

(Iyer and Bradberry, 1996; Andersson et al., 2005; Kearn et al., 2005). Increasing evidence suggests that it is not only the case that multiple monoamines might be released simultaneously, but once released they interact in a complex functionally significant manner. Therefore, instead of thinking of one specific neurotransmitter and its related intracellular pathway as ‘the key’ modulator of emotional learning in the mPFC, it is more realistic to envision that multiple neurotransmitter systems and downstream molecular pathways together play supporting roles within the complex neural circuitry underlying normal cognitive functioning. The ultimate goal of my thesis is to contribute to a better understanding of differential DA receptor function within the mPFC, with the hope that this knowledge builds a more cohesive ‘picture’ of the neurobiology underlying the emotional associative learning process.

5.2 FUTURE DIRECTIONS

Many critical questions remain regarding the functional interrelationships within the mPFC, and specifically how DA receptor transmission modulates neuronal network dynamics within the mPFC. Future investigations will aim to further elucidate our findings that activation of mPFC D₄ receptors bi-directionally modulates the emotional salience of fear-conditioning stimuli during acquisition but not expression (recall) phases of associative learning. In addition, future investigations will explore D₄ receptor-mediated modulation of CaMKII, cAMP/PKA, and PP1 directly within the mPFC as potential underlying molecular mechanisms controlling the salience of emotional memory processing and acquisition. For example, *In vivo* electrophysiological evidence demonstrates that D₄ receptor agonists can decrease

feedforward inhibition on mPFC neurons receiving input from the BLA (Floresco and Tse, 2007), suggesting the possibility that our observed potentiation of emotional associative memory may be attributable to a BLA-dependent amplification of associative information input from the BLA to the mPFC, in effect “priming” neurons in the mPFC to receive emotionally salient associative information from the BLA.

To further elucidate the synaptic mechanisms underlying D_4 mediated modulation of emotional salience, we propose future investigations utilizing *in vivo* electrophysiological techniques. Alterations in synaptic connectivity between functionally connected brain regions are well established correlates of associative neuronal learning and memory processes. These synaptic changes can be measured in a variety of ways, but most typically in terms of long-term-potentiation (LTP) and or/ long-term-depression (LTD) of post-synaptic responsiveness to afferent stimulation. Recent *in vitro* work by Yuen and Yan (2011) has demonstrated that D_4 receptor-mediated modulation of CaMKII activity is tightly linked to AMPA receptor synaptic activity within the mPFC. Thus, during low mPFC neuronal activity levels, D_4 -mediated activation of CaMKII results in a potentiation in AMPA receptor synaptic efficacy, whereas during high neuronal activity levels, D_4 receptor activity decreases CaMKII levels concomitant with decreased AMPA receptor signaling. Given the essential role of AMPA-mediated glutamatergic signaling in learning and memory-related synaptic plasticity, this may provide another potential mechanism by which D_4 signaling within the PLC may control emotional memory formation and processing via CaMKII modulation. *In vivo*, extracellular, or intracellular neuronal recording

studies may allow us to more precisely determine emotional associative memory encoding and expression at the level of the single neuron (Laviolette et al., 2005; Laviolette & Grace, 2006; Rosenkranz & Grace, 2002). Furthermore, such studies will allow for a finer analysis of the functional relationships between different neuronal populations within the mPFC, such as inhibitory interneuron vs. pyramidal output neuron dynamics during specific DA receptor activation or blockade.

Previous results have demonstrated an interesting LTP effect following *in vivo* emotional associative learning in single neurons of the mPFC (Laviolette et al., 2005). These effects are not observed in neurons that do not show functional inputs from the BLA. This suggests that a specific functional circuit from the BLA to the mPFC may be potentiated as a direct consequence of associative learning *in vivo*. While our preliminary experiments have demonstrated this basic phenomenon up to 60 minutes post-conditioning, we further propose a series of pharmacological experiments to determine the possible role of DA D₁ and D₄ receptor subtype transmission in the modulation of this BLA↔mPFC learning induced plasticity. Thus, if our observed LTP effect along the BLA↔mPFC pathway is functionally related to this emotional associative learning process, we would expect a similar blockade of this LTP using DA D₄ receptor agonists at doses that prevent acquisition of associative neuronal encoding (Laviolette et al., 2005). Similar experiments could be conducted to analyze the potential involvement of CaMKII downstream pathways, including AMPA-mediated transmission in the mPFC. Indeed, current unpublished observations in our laboratory have demonstrated that D₄ receptor-mediated potentiation of sub-threshold

fear memories is dependent upon AMPA receptor transmission, while blockade of suprathreshold fear memory acquisition can be rescued by positive allosteric modulation of AMPA receptor transmission within the mPFC.

Together, our behavioural investigations presented in this dissertation and proposed future *in vivo* electrophysiological studies are and will be critical to further elucidate the role of DAergic dysregulation in the pathophysiology of cognitive dysfunction in psychiatric disorders such as schizophrenia, ADHD, PTSD, and addiction, as well as novel therapeutics for cognitive impairments observed in them. Furthermore, it is hoped that these lines of research will ultimately lead to the identification of novel pharmacotherapeutics for the debilitating emotional and cognitive impairments in these disorders.

5.3 REFERENCES

- Amargós-Bosch M, Bortolozzi A, Puig MV, Serrats J, Adell A, Celada P. Co-expression and *in vivo* interaction of serotonin1A and serotonin2A receptors in pyramidal neurons of prefrontal cortex. *Cereb Cortex*, 2004; 14; 281–299
- Ceci A, Brambilla A, Duranti P, Grauert M, Grippa N, Borsini F. Effect of antipsychotic drugs and selective dopaminergic antagonists on dopamine-induced facilitatory activity in prefrontal cortical pyramidal neurons: an *in vitro* study. *Neuroscience* 1999; 93:107-115.
- Di Pietro NC, Seamans JK. Dopamine and serotonin interactions in the prefrontal cortex: Insights on antipsychotic drugs and their mechanism of action. *Pharmacopsychiatry*, 2007; 40; S27–S33
- Frankland PW, O’Brien C, Ohno M, Kirkwood A, Silva AJ. 2001. Alpha-CAMKII-dependent plasticity in the cortex is required for permanent memory. *Nature*. 411:309–313.
- Iyer RN, Bradberry CW. Serotonin-mediated increase in prefrontal cortex dopamine release: Pharmacological characterization. *J Pharmacol Exp Ther*, 1996; 277; 40–47.
- Lauzon NM, Bechara M, Ahmad T, Laviolette SR. (2013) Supra-normal stimulation of dopamine D1 receptors in the prefrontal cortex blocks spontaneous recall of both aversive and rewarding associative memories through a cyclic-AMP dependent signaling pathway. *Neuropharmacology*; 67:104-114
- Lauzon NM, Ahmad T, Laviolette SR. (2012) Dopamine D4 Receptor Transmission in the Prefrontal Cortex Controls the Salience of Emotional Memory via Modulation of Calcium Calmodulin-Dependent Kinase II. *Cerebral Cortex*; 22(11):2486-94
- Lauzon NM, Bishop SF, Laviolette SR. (2009) Dopamine D1 versus D4 receptors differentially modulate the encoding of salient versus nonsalient emotional information in the medial prefrontal cortex. *Journal of Neuroscience*; 29(15):4836-45
- Lauzon NM, Laviolette SR. (2010) Dopamine D4-receptor modulation of cortical neuronal network activity and emotional processing: Implications for neuropsychiatric disorders. *Behavioural Brain Research*; 208(1):12-22
- Laviolette SR, Lipski WJ, Grace AA. A subpopulation of neurons in the medial prefrontal cortex encodes emotional learning through burst and frequency codes through a dopamine D₄ receptor-dependent basolateral amygdala input. *J. Neurosci.* 2005; 25:6066-6075
- Laviolette SR, Grace AA. The roles of cannabinoid and dopamine receptor systems in neural emotional learning circuits: implications for schizophrenia and addiction. *Cell. Mol. Life Sci.* 2006; 63:1597-613.
- Malenka RC, Nicoll RA. 1999. Long-term potentiation—a decade of progress? *Science*. 285:325–334.

- Rosenkranz JA, Grace AA. The prefrontal cortex regulates lateral amygdala neuronal plasticity and responses to previously conditioned stimuli. *J Neurosci* 2002; 23:11054–11064.
- Santana N, Bortolozzi A, Serrats J, Mengod G, Artigas F. Expression of serotonin1A and serotonin2A receptors in pyramidal and GABAergic neurons of the rat prefrontal cortex. *Cereb Cortex*, 2004; 14; 1100–1109.
- Santana N, Mengod G, Artigas F. Quantitative analysis of the expression of dopamine D1 and D2 receptors in pyramidal and GABAergic neurons of the rat prefrontal cortex. *Cereb Cortex*, 2009; 19; 849–860.
- Yuen EY, Yan Z. 2011. Cellular mechanisms for dopamine D4 receptor-induced homeostatic regulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. *J Biol Chem*. 286:24957–24965.

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Submitted by: Kinchlea, Will D
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Lyons D, De Jaeger X, Ahmad T, **Lauzon NM**, Zunder J, Coolen LM, Rushlow W, and Laviolette SR (2013) Opiate Exposure and Withdrawal Induces a Molecular Memory Switch in the Basolateral Amygdala Between ERK1/2 and CaMKII α -Dependent Signaling Substrates [IN PRESS #JN-RM-1226-13]

Lauzon NM, Bechard M, Ahmad T, Laviolette SR. (2013) Supra-normal stimulation of dopamine D1 receptors in the prelimbic cortex blocks spontaneous recall of both aversive and rewarding associative memories through a cyclic-AMP dependent signaling pathway. *Neuropharmacology*; 67:104-14

Lauzon NM, Ahmad T, Laviolette SR. (2012) Dopamine D4 Receptor Transmission in the Prefrontal Cortex Controls the Salience of Emotional Memory via Modulation of Calcium Calmodulin-Dependent Kinase II. *Cerebral Cortex*; 22(11):2486-94

Lintas A, Chi N, **Lauzon NM**, Bishop SF, Sun N, Tan H, Laviolette SR (2012) Inputs from the basolateral amygdala to the nucleus accumbens shell control opiate reward magnitude via differential dopamine D1 or D2 receptor transmission. *European Journal of Neuroscience*; 35(2):279-90

Lintas A, Chi N, **Lauzon NM**, Bishop SF, Gholizadeh S, Sun N, Tan H, Laviolette SR. (2011) Identification of a dopamine receptor-mediated opiate reward memory switch in

the basolateral amygdala-nucleus accumbens circuit. *Journal of Neuroscience*; 31(31): 11172-83

Tan H, **Lauzon NM**, Bishop SF, Chi N, Bechard M, Laviolette SR. (2011) Cannabinoid transmission in the basolateral amygdala modulates fear memory formation via functional inputs to the prelimbic cortex. *Journal of Neuroscience*; 31(14):5300-12

Bishop SF, **Lauzon NM**, Bechard M, Gholizadeh S, Laviolette SR. (2011) NMDA receptor hypofunction in the prelimbic cortex increases sensitivity to the rewarding properties of opiates via dopaminergic and amygdalar substrates. *Cerebral Cortex*; 21(1):68-80

Lauzon NM, Laviolette SR. (2010) Dopamine D4-receptor modulation of cortical neuronal network activity and emotional processing: Implications for neuropsychiatric disorders. *Behavioural Brain Research*; 208(1):12-22 – [Invited review article]

Tan H, **Lauzon NM**, Bishop SF, Bechard MA, Laviolette SR. (2010) Integrated cannabinoid CB1 receptor transmission within the amygdala-prefrontal cortical pathway modulates neuronal plasticity and emotional memory encoding. *Cerebral Cortex*; 20(6):1486-96

Lauzon NM, Bishop SF, Laviolette SR. (2009) Dopamine D1 versus D4 receptors differentially modulate the encoding of salient versus nonsalient emotional information in the medial prefrontal cortex. *Journal of Neuroscience*; 29(15):4836-45

Tan H, Bishop SF, **Lauzon NM**, Sun N, Laviolette SR. (2009) Chronic nicotine exposure switches the functional role of mesolimbic dopamine transmission in the processing of nicotine's rewarding and aversive effects. *Neuropharmacology*; 56(4):741-51

Laviolette SR, **Lauzon NM**, Bishop SF, Sun N, Tan H. (2008) Dopamine signaling through D1-like versus D2-like receptors in the nucleus accumbens core versus shell differentially modulates nicotine reward sensitivity. *Journal of Neuroscience*; 28(32):8025-33

Abstracts:

Lauzon NM and Laviolette SR (2011) Dopamine transmission in the prefrontal cortex controls the salience of emotional experience by modulating CaMKII and cyclic-AMP activity. Society for Neuroscience (SfN). Washington D.C.; November 2011

Lauzon NM and Laviolette SR. (2011) Dopamine D4 transmission in the prefrontal cortex controls the salience of emotional experience by modulating CaMKII activity. International Brain Research Organization (IBRO) World Congress. Florence, Italy; July 2011

Lauzon NM Laviolette SR (2010) Dopamine D1 Receptors modulate expression of aversive and rewarding memories in the prefrontal cortex. Murray Barr - Anatomy & Cell Biology Research Day. October 2010

Lauzon NM and Laviolette SR (2010) Dopamine D1 Receptors modulate expression of aversive and rewarding memories in the prefrontal cortex. Society for Neuroscience (SfN). San Diego, CA; November 2010

Lauzon NM and Laviolette SR (2009) Dopamine D4 transmission in the prefrontal cortex controls the Salience of Emotional Experience by Modulating CaMKII Activity. Murray Barr - Anatomy & Cell Biology Research Day. London, ON; October 2009

Lauzon NM and Laviolette SR (2009) Dopamine D4 transmission in the prefrontal cortex controls the Salience of Emotional Experience by Modulating CaMKII Activity. Society for Neuroscience (SfN). Chicago IL.; October 2009

Lauzon NM and Laviolette SR (2008) Dopamine D1 versus D4 receptors differentially modulate processing of salient and non-salient emotional information in the pre-limbic cortex. Society for Neuroscience (SfN). Washington D.C; November 2008

Lauzon NM and Laviolette SR (2008) Dopamine D1 versus D4 receptors differentially modulate processing of salient and non-salient emotional information in the pre-limbic cortex. Southwestern Ontario Neuroscience Association (SONA). London, ON; May 2008

Lauzon NM and Laviolette SR (2008) Dopamine D1 versus D4 receptors differentially modulate processing of salient and non-salient emotional information in the pre-limbic cortex. Margaret Moffatt Graduate Research Day, London, ON; March 2008

Lauzon NM and Laviolette SR (2008) Dopamine D1 versus D4 receptors differentially modulate processing of salient and non-salient emotional information in the pre-limbic cortex Murray Barr - Anatomy & Cell Biology Research Day. London, ON; October 2008

Presentations:

Lauzon NM (2009) Controlling Emotional Memories: The Role of the Dopamine in the PFC. Murray Barr - Anatomy & Cell Biology Research Day. London, ON; 2009

Lauzon NM (2011) Dopamine Modulation of Emotional Learning in the Prefrontal Cortex. Anatomy & Cell Biology Departmental Lecture. London, ON; June 2011

Academic Service:

Vice-Chair, Anatomy & Cell Biology Student Council, London, ON; 2010, 2011, 2012

Reviewer, UWO Presidents Entrance Scholarships, London, ON; 2012

Recruiter, Schulich School of Medicine and Dentistry Graduate Studies Western University; London, ON; 2011

Consultant, UWO Graduate Scholarship Resource Library, London, ON; 2011

Recruiter, Schulich School of Medicine and Dentistry Graduate Studies, Guelph University; Guelph, ON; 2011

Recruiter, Schulich School of Medicine and Dentistry Graduate Studies, York University; Toronto, ON; 2011

Advisor, 'Consult the Experts' Scholarship Counseling, London, ON; 2010, 2011, 2012

Recruiter, Schulich School of Medicine and Dentistry Graduate Studies, McGill University; Montreal, QC; 2010

Student Host, Anatomy & Cell Biology Recruitment Weekend; 2009, 2010 & 2011

Recruiter, Department of Anatomy & Cell Biology; London, ON 2009, 2010

Departmental Representative, Schulich Graduate Open House, London, ON; 2008, 2009