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Characterization of changes in glia-related protein expression following a history of excessive cocaine taking.

A dissertation submitted in partial satisfaction of the

requirements of the degree Doctor of Philosophy in Psychology

by

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Abstract

Characterization of changes in glia-related protein expression following a history of excessive cocaine taking.

by

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The majority of addiction research examining the brain has centered on neuronal adaptations both resulting from cocaine use and contributing to the development of addiction. However, neurons constitute a small minority of the total cells within the central nervous system (CNS) and the role of glial cell types, which make up the vast majority of CNS cells, in cocaine addiction is largely unexplored. Imaging studies have revealed white matter deficits within the corpus callosum and post-mortem tissue analysis has revealed decreased oligodendrocyte-specific protein expression within the brains of chronic cocaine users. The extended access (6h/day) cocaine self-administration paradigm conducted in rodents is used to model the escalation of cocaine intake seen within human cocaine users and is critical for gaining insight to the molecular adaptations that result from excessive cocaine use. The series of experiments detailed in this dissertation utilize this model to address three specific aims: (1) characterize changes in glial-specific proteins and mRNA expression in cortical and limbic brain regions of rats with a history of extended access cocaine self-administration, (2) characterize the behavioral consequences of decreased myelin basic protein on a variety of prefrontal cortex-dependent tasks that are impaired following a history of cocaine use, and (3) to determine the functional significance of decreased myelin basic protein expression on behavioral measures in an animal model of cocaine addiction, including the acquisition and maintenance of intravenous cocaine, dose sensitivity, as well as the extinction and

reinstatement of drug-seeking. Taken together, the experiments detailed herein provide further support for an enduring effect of cocaine on the expression of glial-specific proteins within the forebrain and highlight a role for myelin basic protein within the ventromedial prefrontal cortex in regulating impulsive choice, but not other cocaine addiction- related behaviors.

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List of Abbreviations

5-HT	Serotonin
6-OHDA	6-hydroxydopamine
ACC	Anterior cingulate cortex
ACh	Acetylcholine
Agm	Agranular frontal cortex
AGS3	Activator of G protein signaling 3
AMPA	α-amino-3-dydroxy-5-methyl-isoxazol-4-propionic acid
ATP	Adenosine 5'-triphosphate
BA	Brodman's area
BDNF	Brain derived neurotrophic factor
BLA	Basolateral amygdala
BOLD	Blood-oxygen level dependent
CAL	Calnexin
Caspr	Contactin-associated protein, a member of the neurexin family
CC	Corpus callosum
CD11b	Cluster differentiation molecule 11B (aka Integrin alpha M)
cDNA	Complimentary DNA
CHOP	C/EBP Homologous protein (molecule in endoplasmic reticulum stess-mediated apoptosis).
CLN	Claudin-11 aka oligodendrocyte specific protein
CNP	2',3'-cyclic nucleotide 3' phosphodiesterase
CNS	Central nervous system
COC6h-3	Cocaine 6 hour self-administration 3 day withdrawal
COC6h-30	Cocaine 6 hour self-administration 30 day withdrawal
COC6h-cue-tested	Cocaine 6 hour self-administration cue-tested prior to sacrifice
COC6h-untested	Cocaine 6 hour self-administration untested prior to sacrifice
CP	Caudate-putamen
CPA	Conditioned place aversion
CPP	Conditioned place preference
CS	Conditioned stimulus
Cx	Connexin
DA	Dopamine
dB	Decibel
dlPFC	Dorsolateral prefrontal cortex
DNMS	Delayed non-matching to sample
dPFC	Dorsal prefrontal cortex
DTI	Diffusion tensor imaging
ER	Endoplasmic reticulum
Expt	Experiment
FA	Fractional anisotropy
FI	Fixed-interval
fMRI	Functional magnetic resonance imaging
FR	Fixed ratio
GABA	γ-Aminobutyric acid

GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GFAP	Glial fibrillary acidic protein
GLT-1	(aka) EAAT2 -Excitatory amino acid transporter 2
GS	Glutamine synthetase
GSH	Glutathione
GT	Gambling Task
HPA	Hypothalamic pituitary axis
HPC	Hippocampus
IL	Infralimbic
IL-2	Interleukin 2
IL-6	Interleukin 6
IL-β	Interleukin beta
IM	Intramuscular
inf	Infusion
IP	Intraperitoneal
IP3	Inositol-triphosphate
IV	Intravenous
LC	Locus coeruleus
LTD	Long term depression
MAG	Myelin associated glycoprotein
MBP	Myelin basic protein
MD	Mediodorsal
MDD	Major depressive disorder
MDmc	Magnocellular portion of MD of the thalamus
mGluR	Metabotropic glutamate receptor
mld	MBP mutant mice
MOBP	Myelin-oligodendrocyte basic protein
MOG	Myelin oligodendrocyte glycoprotein
mPFC	Medial prefrontal cortex
MRI	Magnetic resonance imaging
mRNA	Messenger RNA (spell out)
NAc	Nucleus accumbens
Na_v	Voltage gated sodium channel
NE	Norepinephrine
NF155	Neurofascin 155
NG2+	NG2 Chondroitin sulfate proteoglycan
NMDA	N-methyl-d-aspartate
NRT	No reverse transcriptase (negative control)
NTC	No template control (reaction mixture without cDNA)
OFC	Orbitofrontal cortex
OL	Oligodendrocyte
OMgp	Oligodendrocyte-myelin glycoprotein
PAG	Periaqueductal grey
PET	Positron emission tomography
PFC	Prefrontal cortex
PKC	Protein kinase C

PL	Prelimbic
PLP	Proteolipid protein
PND	Postnatal day
PPF	Methylxanthine propentofylline
qPCR	Quantitative polymerase chain reaction
qRT-PCR	Quantitative real time polymerase chain reaction
ROS	Reactive oxygen species
RT	Reverse transcriptase
SA	Self-administration
SAL1h-3	Saline 1 hour self-administration 3 day withdrawal
SAL1h-30	Saline 1 hour self-administration 30 day withdrawal
SAL6h-3	Saline 6 hour self-administration 3 day withdrawal
SAL6h-30	Saline 6 hour self-administration 30 day withdrawal
SAL1h-cue-tested	Saline 1 hour self-administration cue-tested prior to sacrifice
SAL1h-untested	Saline 1 hour self-administration untested prior to sacrifice
TEMPOL	4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl
TNF-α	Tumor necrosis factor alpha
TTX	Tetrodotoxin
UGT8	UDP glycolsyltransferase 8
vmPFC	Ventromedial prefrontal cortex
VP	Ventral pallidum
VS	Ventral striatum
VTA	Ventral tegmental area
WD	Withdrawal
zif268	Zinc finger protein 225; (aka) EGR-1, Early growth response protein 1

Chapter 1 General Introduction

"…the user is gripped by a visceral emotional state, experiences a highly focused incentive to act, and is remarkably unencumbered by the memory of the negative consequences of drug taking."
(Childress et al., 1999)

1.1 Human Cocaine Addiction

1.1.1 Prevalence of Cocaine Addiction

Cocaine addiction is a chronic disorder characterized by drug craving, compulsive drug-seeking and persistant drug-taking behavior despite serious negative consequences (Camí & Farré, 2003). In 2013, the National Survey on Drug Abuse and Health for 2010-2012, was published revealing 1.1 million Americans are either dependent upon or abuse cocaine and 16.5% of adults aged 26 and older have used cocaine in their lifetime (Substance Abuse and Mental Health Services Administration, 2013a). Of the 656,025 estimated emergency room visits for illicit drug use in 2011, over 40% were for cocaine-related adverse reactions including convulsions, hyperthermia, and sudden cardiac death (Dietrich, 2009; O'Leary & Hancox, 2010; Substance Abuse and Mental Health Services Administration, 2013b). Highlighting the importance of the substance use/abuse problem within the United States, of those 23.1 million Americans who needed treatment in 2012, only approximately 2.5 million (~1%) received treatment in a specialized facility (Substance Abuse and Mental Health Services Administration, 2013a). High rates of relapse, even after extended abstinence, and the lack of effective treatment for cocaine addiction remain significant obstacles to recovery. While much knowledge has been gained through human neuropsychological testing, imaging and animal models of addiction, there are currently no approved pharmacotherapies for cocaine addiction.

1.1.2 Cocaine Associated Cognitive Deficits in Humans

Higher organisms are driven by internal motivational states that are kept under control by the inhibitory mechanisms of the frontostriatal regions, which augment behavior to allow for the attainment of far-removed goals (Jentsch & Taylor, 1999; Miller, 2000). Dysfunction of this frontostriatal system is thought to reduce inhibition on underlying reward circuitry, allowing habitual or compulsive drug-seeking and taking to continue despite the increase in personal and social costs (Everitt & Robbins, 2005; Jentsch & Taylor, 1999). Chronic cocaine intake results in impaired prefrontal cortex (PFC) function, resulting in deficits in executive processes such as behavioral inhibition and/or impulsivity, working memory, and decision-making (Antoine Bechara & Damasio, 2002; Jentsch & Taylor, 1999). However, some of these deficits are apparent in individuals prior to beginning their drug intake and predict, to some extent, future substance abuse. Block, Erwin, & Ghoneim (2002) have shown that performance on the fourth grade Iowa Test of Basic Skills is poorer for individuals who later develop major substance abuse problems. Additionally, individuals who later develop stimulant use perform poorer than alcohol or polydrug users. When these same individuals were tested in early abstinence, stimulant users performed more poorly than controls and other substance users on tests of verbal and mathematical skills and abilities, abstraction ability, verbal memory and imagery, even after controlling for premorbid cognition, and these deficits failed to improve even after 3 months of abstinence.

Chronic cocaine users show behavioral inhibition deficits as measured by a go/no-go task, whereby they have a decreased probability of inhibiting a response on a stop-signal and a slower reaction time to the stop signal than controls, even when showing similar reaction times to go-signals and an equivalent number of choice errors (Fillmore & Rush, 2002). Additionally, current and abstinent cocaine users discount monetary rewards at a steeper rate

than control participants and alcohol abusers, suggesting a higher propensity to make impulsive choices of smaller immediate rewards while forgoing larger delayed rewards (Heil, Johnson, Higgins, & Bickel, 2006; Kirby & Petry, 2004).

Imaging studies utilizing fMRI and PET technologies corroborate executive functioning deficits with alterations in neural activity in cocaine users. Castelluccio, Meda, Muska, Stevens, & Pearlson (2014) used BOLD fMRI to reveal hyperactivity in Brodman's areas (BA) 25 (cingulate gyrus, pregenual) and 40 (lateral agranular/supramarginal gyri) in response to no-go signals on the go/no-go task in current cocaine users, compared to drug-free participants. Interestingly, hyperactivity negatively correlated with self-reported measures of impulsivity, indicating that cocaine users with higher behavioral impulsivity have lower neural activity associated with omitting a go response when a no-go signal is present. In another go/no-go task by Kaufman, Ross, Stein, & Garavan (2003) cocaine users made significantly more errors of omission and commission than healthy controls, and exhibited significantly less activation of the anterior cingulate cortex (ACC; BA 32) for successful and failed inhibitions for stop signals. However, they did not differentiate between left and right ACC activation, and previous authors have found discrepant activation based on laterality (Kaufman et al., 2003). The right ACC (BA 32) has also been shown to have increased activity, while the left ACC and lateral PFC have decreased activity in response to word-color conflicts in a modified Stroop task conducted in 23-day abstinent cocaine users compared to controls (Bolla et al., 2004). Moreover, decreased activity in the dorsal ACC for error signals in both male and female cocaine users predicted relapse (Luo et al., 2013). Although, these studies do not necessarily replicate each other, a consistent trend is altered function of the PFC of cocaine users and recovering addicts compared to healthy controls.

The neuropsychological tests of delayed non-matching to sample (DNMS) and various gambling tasks are sensitive to damage to the the dorsolateral and ventromedial PFC (dlPFC and vmPFC, respectively) (GT), respectively. These tests are not only useful in distinguishing the area of prefrontal damage, but also in detecting decreased function in relation to substance use. Bechara & Martin (2004) studied working memory and decision-making in individuals with substance dependence using a DNMS and GT. Compared to healthy controls, individuals abusing alcohol and stimulants (methamphetamine and/or cocaine) made a lower percentage of correct responses across all delay time points on the DNMS and chose more cards from the disadvantageous decks in the GT. Consistently, individuals abusing alcohol and stimulants perform more poorly on decision-making tasks (e.g. the GT) than controls, but not as poorly as individuals with lesions in the vmPFC, indicating a dysfunction of the vmPFC without complete loss of function (Bechara et al., 2001; Bechara & Damasio, 2002). Moreover, lesions of the dorsomedial PFC or dlPFC do not show this same deficit, revealing this process is dependent on a functional vmPFC (Rogers et al., 1999). One study did find similar impairments in decision-making for individuals with vm- or dl-frontal lobe damage on the Iowa Gambling task when the large reward cards were placed first, followed by large losses later in the deck (Fellows & Farah, 2005). When the cards were shuffled so that large losses were experienced before large wins, eliminating the need for reversal learning, only those patients with dm-frontal lobe damage continued to show impairments in decision-making, suggesting vm-frontal lobe patients may have a deficit in reversal learning and not overall problems with decision-making *per se*. Lastly, Ernst et al. (2002) has shown activation of the "anterior cingulate cortex" (BA 32 and

24) during the GT of normal healthy subjects, which is interesting given the vmPFC, and not more dorsomedial PFC, lesions lead to deficits in this task.

Consistent with the cognitive deficits seen in substance abusers, imaging studies reveal altered metabolic rates of brain areas controlling executive function and implicated in addiction processes in a variety of cued studies to elicit craving (Grant et al., 1996; Volkow et al., 1999). In drug-free states, glucose utilization measured in positron emission tomography (PET) studies reveal an increase in the orbitofrontal cortex (OFC; both whole OFC and medial component of the OFC) in a control condition where non-drug stimuli are presented to cocaine users compared to the activity of control participants, and under baseline scanning conditions (Grant et al., 1996; London, Ernst, & Grant, 2000; Stapleton et al., 1995). Additionally, in studies using drug-associated stimuli or acute administration of the stimulant methylphenidate, enhanced glucose utilization is seen in the OFC and is correlated with self-reported craving (Grant et al., 1996; Volkow & Fowler, 2000; Volkow et al., 1999; Wang et al., 1999). Acute administration of cocaine in cocaine-dependent individuals leads to enhanced blood-oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) signal activity in the lateral frontal cortices and medial frontal cortex (BA 32) (Breiter et al., 1997). The activity increases in the PFC were also correlated with subjective ratings of "rush".

Several regions of the human PFC have an integral role in cocaine addiction, however, inconsistent findings could partly result from differences in how the brain was "sectioned" to compare for differences in function. The following sections will discuss the correspondence between different PFC regions in the human and rodent brains, and highlight the importance of clear definitions and consistency of naming conventions when making

connections between animal models of addiction-related behaviors and relating those findings to the human condition. Regardless, given the overwhelming evidence from clinical studies implicating the importance of the PFC in executive function and addiction, and consistent alterations in activity following cocaine abuse, the focus of this dissertation will be on the role of the corresponding rat PFC in cocaine-taking, -seeking, and cognitive deficits resulting from cocaine-induced changes in physiology.

1.2 Ventromedial Prefrontal Cortex Circuitry

1.2.1 Defining the vmPFC by reciprocal connectivity with the mediodorsal nucleus of the thalamus

The vmPFC is defined by the reciprocal connections with the mediodorsal (MD) nucleus of the thalamus and contains the prelimbic (PL) and infralimbic (IL) subregions (Conde, Maire-lepoivre, Audinat, & Crepel, 1995; Hoover & Vertes, 2007; Rose & Woolsey, 1948; Takagishi & Chiba, 1991; Uylings & van Eden, 1990). A common feature of many neural networks is a topographical organization of inputs and outputs, as seen in sensory input from the retina through the thalamus and into the primary visual cortex (Bear, Connors, & Paradiso, 2007) Likewise, the MD thalamic projections to the PFC are organized in a lateral to medial projection onto the dorsal to ventral medial PFC, though there is overlap concerning the input to the medial agranular frontal cortex (AGm) (Hoover & Vertes, 2007; Uylings & van Eden, 1990). Retrograde tracing studies in rat using fluorogold show almost exclusive input of the ventromedial MD of the thalamus to the IL; central MD of the thalamus input to the PL, lateral MD of the thalamus input to the ACC; while the AGm receives input from the extent of the MD of the thalamus (Hoover & Vertes, 2007).

Similar findings have been observed using non-human primates where reciprocal connections are found with the medial magnocellular portion of the MD of the thalamus

(MDmc) and Brodman's areas 11-14 (OFC), area 25 (approximately the IL in rat), area 32 (approximately the PL in rat), and ventral portions of area 24 (approximately the ventral PL in rat) (Giguere & Goldman-Rakic, 1988; Uylings & van Eden, 1990; Wise, 2008). Interestingly, this shift of medial MD thalamic projections to ventral portions of the OFC in primates is also reflected in a shift in the medial PFC efferents to the ventral striatum, in comparison to the pattern seen in rodent connectivity (Wise, 2008).

The vmPFC of rats has reciprocal projections to the MD of the thalamus; anterograde tracers into the IL of the rat almost exclusively labeled the medial portion of the MD of the thalamus with a shift to the central division of this nucleus for injections encompassing the dorsal IL and ventral PL (Takagishi & Chiba, 1991). Likewise, a further shift in injection site dorsally (dorsal PL and ACC), reveals terminals in the central to lateral divisions of the MD of the thalamus (Uylings & van Eden, 1990). In both non-human primates and rodents, the PFC is reciprocally connected with many other thalamic nuclei, though to a lesser degree than the MD, including the anteromedial, central lateral, central medial, interanteromedial, paracentral, paratenial, paraventricular, nucleus reuniens, rhomboid, and ventromedial (Heidbreder & Groenewegen, 2003; Hoover & Vertes, 2007).

1.2.2 vmPFC Connectivity with Other Brain Reward Centers

The vmPFC is intimately connected with many brain areas known to be involved in various aspects of goal-directed and addiction related-behaviors, including the ventral striatum (VS), basolateral amygdala (BLA), ventral tegmental area (VTA), ventral pallidum (VP), and interconnections with prefrontal cortical areas (Kalivas & Volkow, 2005; Tzschentke, 2000). Cortico-cortical efferent projections of the IL innervate the medial orbital cortex, PL, IL, ventral agranular insular area, piriform, entorhinal, perirhinal, and to a

lesser extent, the ACC; the PL innervates the same targets, often in more dorsal aspects (Barbas & Pandya, 1989; Heidbreder & Groenewegen, 2003; Hoover & Vertes, 2007; Takagishi & Chiba, 1991). The vmPFC is reciprocally connected with the BLA, a region known to be involved in reinforcer valuation and the ability of conditioned stimuli to guide behavior (Hoover & Vertes, 2007; Jentsch & Taylor, 1999; Takagishi & Chiba, 1991). Additionally, the vmPFC has reciprocal connections with VTA dopamine (DA) neurons, a cell group that also sends projections to the nucleus accumbens (NAc) (Carr & Sesack, 2000). This mesoaccumbens DA projection from the VTA to the NAc is a key element in the generation of reward and goal-directed behavior (Tzschentke, 2000).

A distinct difference becomes apparent when examining the vmPFC efferents to the ventral striatum in the rat, compared to tracing studies of the Macaque monkey. Tracing studies in rat reveal a dorso-ventral gradient of outputs to the lateral to medial subregions of the NAc, respectively (Gorelova & Yang, 1997; Sesack, Deutch, Roth, & Bunney, 1989; Takagishi & Chiba, 1991). Rostral-caudal and dorsal portions of the PL send a majority of their projections to the core of the NAc, while ventral PL and IL send the majority of their efferents to the shell of the NAc. However, there is not a distinct division, and all regions send some efferents throughout the extent of the NAc. In line with this dorsal-ventral, lateral to medial trend, the IL also sends a dense projection to the medial olfactory tubercle, the most ventral region of the ventral striatum and also a region involved in reward (Ikemoto & Donahue, 2005; Sesack et al., 1989; Takagishi & Chiba, 1991). Anterograde tracer injections into various subregions of the orbital cortex only reveal sparse labeling in the NAc core (from the ventral orbital area) and the most lateral part of the NAc shell (from ventral lateral

and lateral orbital injections), while the densest projections are throughout the caudate-putamen (CP) (Schilman, Uylings, Galis-de Graaf, Joel, & Groenewegen, 2008).

In the non-human primate, the division of afferents to the ventral striatum occurs more ventrally and laterally in portions of the OFC. Retrograde tracer injections into the NAc shell reveal dense labeling in areas 32 and 25 (approximately the PL and IL in rat); into the NAc core labeling occurred in orbital areas 11-14 and areas 32 and 25 (Haber, Kunishio, & Mizobuchi, 1995). Similarly, Ferry, Ongür, An, & Price (2000) found areas 32 and 14r to project to the CP and NAc core, while area 25 was the only region to project to the NAc shell, along with similar projections to the CP and NAc core. These authors suggested a "medial" and "orbital" network of prefrontal-striatal projections, rather than a strict division based upon BAs; this is consistent with the medial MD thalamic projection fields in the mPFC expanding ventrally in the primate to encompass portions of the orbital cortex, and points to a similarity of connections between the rodent and primate mPFC.

1.2.3 Homology Considerations From Primate to Rodent PFC

Conclusions drawn based on research across species, without a consideration of homology of brain regions of interest, has the potential to lead to misconceptions about the function of those areas. Campbell & Hodos (1970) proposed a list of principles for inferring homology among brain regions across species, including afferent and efferent connections, topography, topology, cytoarchitectonic characteristics, electrophysiological properties, immunocytochemical properties, and embryologic development. While the primate and rodent fiber connections between the MD of the thalamus and the medial PFC are quite similar, differences do arise in the OFC of the primate and rat in regards to efferent projections to the NAc. Additionally, the cytoarchitectonic features of these two species

differ across medial and orbitofrontal areas. The rodent PFC only includes allocortex and agranular cortex, the later encompassing the PL and IL found on the medial surface (Wise, 2008). This is in contrast to the macaque and human PFC, which includes various types of granular cortex (Wise, 2008). Taking these architectonic features into consideration, IL, PL, agranular insular, agranular orbital, and ACC cortices in rats have presumed homologous regions in primates (Wise, 2008). The areas of the primate OFC (BA 14) that similarly projected to the NAc core, could have some functional similarities with the ventral orbital cortex in rat; an area with minor input to the NAc core. Other areas of the primate OFC, including 11-13, are defined as dysgranular cortex and most likely evolved later in primate evolution (Wise, 2008). Therefore, outside the presumed homologous areas discussed, conclusions drawn across species for other brain areas must be done with care.

Caution must also be taken when drawing inferences from human imaging data using generalized terminology for large areas of the brain. Examples of such inconsistent terminology include naming of parts of BA 10 as medial PFC, which only include granular tissue, compared to other references of mPFC that include ACC, PL and IL cortices (Hare, O'Doherty, Camerer, Schultz, & Rangel, 2008; Sanfey, 2007). Many imaging studies will refer to OFC activity without distinguishing medial from lateral activation, an important distinction considering the differences in connectivity with other limbic structures. Bechara, Damasio, Tranel, & Anderson (1998) use the "ventromedial prefrontal cortex" in reference to ventral and low mesial areas of the frontal lobe of humans with major input to the limbic system and those structures processing reward and punishment; areas including ventral BA 32 & 24 (ACC), BA 25 (IL), BA 11, 13 and 14 (medial OFC) (BA references, Wise, 2008). This definition coincides well with the tracing studies, as well as with areas that are

presumably homologous to rodents, and therefore accessible for study in this species. Taking into consideration the similarities between the human, non-human primate, and rodent vmPFC, and the human evidence of dysfunction within this brain area following prolonged cocaine use, the target of study for this dissertation is the vmPFC of the rodent as defined at the beginning of this section.

1.3 The vmPFC in Pre-clinical Studies of Reward

A variety of experimental methods and animal models have been employed to elucidate the functions of the PFC in reward and addiction, with the understanding that the human PFC has evolved to a greater extent than that of non-human primates and rodents. Three paradigms are commonly used to assess the importance of brain areas in reward and whether drugs or treatments have rewarding properties: self-stimulation, conditioned place-preference, and self-administration (Tzschentke, 2000) and the current status of our knowledge regarding the role for the PFC in reward processing are summarized below.

1.3.1 Self-stimulation studies of the PFC

The classic demonstration of Olds & Milner (1954) that rats will learn to self-stimulate discrete regions of the brain provided the basis for using this approach to delineate the neurocircuitry of reward behavior. Some of the first studies to reveal a role of the PFC in goal-directed or reward behavior came from self-stimulation studies conducted in rodents. Sites that sustain self-stimulation include the PFC (especially the PL-IL interface), MD of the thalamus, medial forebrain bundle-lateral hypothalamus, VTA, locus coeruleus (LC), and periaqueductal grey (PAG); all of which have connections with the PFC and most connections being reciprocal (Mora & Cobo, 1990; Mora & Ferrer, 1986; Vives, Morales, & Mora, 1986). Additionally, lesions to the VTA, MD of the thalamus, CP, and BLA all

decrease self-stimulation of the PFC in rats (Ferrer, Cobo, & Mora, 1987; Phillips & Fibiger, 1978; Vives et al., 1986), indicating an important role for these efferents in regulating reward-related processing within PFC.

The VTA is known to supply DA to both the PFC and other reward-related structures (e.g. NAc) and lesions within the VTA or depletion of DA terminals within the mPFC result in a significant decrease in self-stimulation behavior (Phillips & Fibiger, 1978; Vives et al., 1986). Self-stimulation of the medial PFC also enhances the activity of VTA DA neurons and causes an elevation of extracellular levels of DA in the NAc (You, Tzschentke, Brodin, & Wise, 1998). Dopamine release into the NAc from VTA afferents initially results from the experience with a rewarding drug, and subsequently transfers to cues that predict the delivery of reward (Jacobs, Smit, de Vries, & Schoffelmeer, 2003; Kalivas et al., 2005). Additionally, drugs that are thought to produce their rewarding effects through the mesolimbic DA system also enhance the rewarding effects of mPFC self-stimulation (Tzschentke, 2000). McGregor, Atrens, & Jackson (1992) found that a single systemic injection of cocaine enhanced mPFC self-stimulation rates under a FI-5 schedule of reinforcement by an average of 269%; enhanced responding continued for 48hrs after the single cocaine administration, and medial PFC self-stimulation was dependent on DA D1 receptors as shown by attenuated self-stimulation after the administration of a D1 receptor antagonist.

Rats withdrawn from extended non-contingent and contingent administration of cocaine show an increased resting membrane potential and lack of the characteristic bistable neuronal membrane property within the mPFC (Trantham, Szumlinski, McFarland, Kalivas, & Lavin, 2002). However, the PL neuronal response to intravenous (IV) cocaine self-administration is heterogeneous. PL neurons respond by excitatory or inhibitory anticipatory

responses to lever-presses for cocaine infusion, exhibit either excitation or inhibition following cocaine infusion, or exhibit no change in the firing rate related to anticipatory behavior or cocaine being on-board (Chang, Janak, & Woodward, 1998; Chang, Sawyer, Paris, & Kirillov, 1997). Thus, cocaine not only enhances self-stimulation in the mPFC, but over time, it alters the excitability of these neurons and affects the PL in a heterogeneous manner.

1.3.2 Conditioned Place Preference Studies of the PFC

The majority of studies examining mPFC and reward have used self-stimulation and self-administration methods, but a handful of conditioned place-preference (CPP) studies have supported the role of this region in reward. In the CPP paradigm, the motivational properties of a drug or some other reinforcing treatment are paired with a neutral set of environmental stimuli, while a control treatment is paired with a distinct set of neutral environmental stimuli (Tzschentke, 2007). Over the course of repeated pairings of reinforcing treatments with their respective environments, these environmental stimuli can acquire secondary motivational properties such that they can elicit approach or withdrawal when then animal is subsequently exposed to them in a "treatment-free" state (Tzschentke, 2007). An example of such conditioning is demonstrated by electrical stimulation of the PL in rats, which results in preference for the side of the testing chamber paired with the rewarding stimulation; this effect is dependent on DA as the drug haloperidol dose-dependently blocked preference for the stimulation-paired side (Duvauchelle & Ettenberg, 1991).

In regards to conditioning with cocaine, one must be cognizant of methodological differences as the dose administered, number of pairings, length of time for each pairing, the

time between administration of drug and placement into the conditioning chamber, and the time of day testing occurred, can result in either CPP or conditioned place-aversion (CPA) (Tzschentke, 2007). Brabant, Quertemont, & Tirelli (2005) administered either 4, 8, or 12 mg/kg intraperitoneal (IP) injections of cocaine to mice and immediately placed them in the conditioning chamber for 20 minutes with 1, 2, or 4 total pairings. While all doses elicited a CPP for the drug-paired side of the chamber, a minimum of 2 pairings was necessary to show a preference, with 4 pairings showing the strongest effect. However, a delay of 15, but not 5, minutes between a 0.75 mg/kg IV cocaine injection and placement in the conditioning chamber for a 5-minute session results in a significant CPA (Ettenberg, Raven, Danluck, & Necessary, 1999). Mice administered 10 mg/kg (IP) of cocaine and immediately placed in the conditioning chamber for 30 minutes showed a clear CPP when testing was conducted during the day, but the conditioned reward was significantly diminished when testing was conducted at night (Kurtuncu, Arslan, Akhisaroglu, Manev, & Uz, 2004).

Taking the above methodological differences into consideration in addition to differences in the size, location and technique used to lesion the mPFC, the majority of CPP studies have shown inconsistent results with respect to its role in regulating drug reward. Isaac, Nonneman, Neisewander, Landers, & Bardo (1989) lesioned large portions of the ACC and PL by aspirating tissue and found that this manipulation promoted the development of a CPA elicited by 6 pairings of 5 mg/kg subcutaneous cocaine with immediate placement into the apparatus for 20-minute sessions, while this conditioning procedure elicited a CPP in sham-lesioned controls. Thus, large lesions of the more dorsal aspects of the mPFC appear to promote the aversive properties of cocaine. In contrast, animals with lesions to the orbital or precentral frontal cortex exhibited neither CPP nor CPA in this study, indicating that these

regions are important for the manifestation of cocaine's rewarding properties. Other studies found more specific lesions of the PL, using quinolinic acid to damage cell bodies but spare fibers of passage, to attenuate cocaine CPP, without eliciting CPA. Specifically, three cocaine (15 mg/kg, IP) injections immediately followed by 40 minute conditioning sessions resulted in an attenuation of CPP for PL impaired animals while a significant cocaine-induced CPP was seen for sham-operated animals (Tzschentke & Schmidt, 1998).

Consistently, serotonin depletion of the mPFC, encompassing the PL subregion, prevents the acquisition of cocaine-induced CPP [10 mg/kg (IP) X 4, 30-min, pairings immediately upon injection; (Pum, Carey, Huston, & Müller, 2008). However, Zavala, Weber, Rice, Alleweireldt, & Neisewander, (2003) found that quinolinic acid lesions to the same PL region did not affect the acquisition of cocaine CPP (either one or three, 30 minute conditioning sessions following 15 mg/kg, IP cocaine injection), or the rate of extinction of the conditioned response, but did attenuate cocaine-primed reinstatement of CPP.

The cocaine-induced CPA seen in the Isaac et al. (1989) study may be due to the majority of the extraction being much more posterior than the subsequent inactivation studies and also completely eliminated the ACC. While Zavala et al. (2003) did not find an effect on PL inactivation on CPP acquisition, a number of procedural differences exist between this and other studies, including the use of tactile, visual and olfactory stimuli. Additionally, Zavala et al. (2003) conducted the conditioning session during the dark phase of the rat's circadian cycle, where the previous studies were conducted during the light phase of the cycle. As mentioned earlier, Kurtuncu et al. (2004) demonstrated that the time or phase of the light cycle affects the strength of cocaine-induced CPP and thus, timing of the conditioning may partially explain why Zavala and colleagues failed to find an inactivation effect on

acquisition. While methodological differences make defining a specific role of the mPFC in cocaine-conditioned reward difficult, there is some evidence to support a contribution of this region to this phenomenon.

1.3.3 Cocaine Self-Administration Studies of the PFC

1.3.3.1 Acquisition and Maintenance

The mPFC also supports various self-administration procedures, including the direct administration of drugs into this region and systemic administration of drugs. Rats will intracranially self-administer cocaine, phencyclidine, and some NMDA receptor antagonists into the mPFC (McBride, Murphy, & Ikemoto, 1999; Tzschentke, 2000). Goeders & Smith (1986) demonstrated that rats would self-administer cocaine intracranially into the PL subregion. This effect is DA-dependent as 6-OHDA lesions of the PL or co-infusion of the D2 receptor antagonist sulpiride attenuated intracranial cocaine self-administration. Moreover, rats will traverse a run-way to self-administer cocaine (12.5, 25, & 50 μg/0.5 μl per side) directly into the PL, a treatment that does not result in enhanced retreat behavior over time as seen for rats that are trained under run-way procedures for intravenous or intracerebroventricular injections of cocaine (Guzman, Moscarello, & Ettenberg, 2009).

In contrast to the equivocal results from cocaine CPP studies (see above), lesions of the PL with quinolinic acid or 6-OHDA result in enhanced acquisition and responding for IV cocaine (0.083 mg/inf or 0.25 mg/kg/inf), compared to sham-lesioned control rats (Schenk, Horger, Peltier, & Shelton, 1991; Weissenborn, Robbins, & Everitt, 1997). Moreover, 6-OHDA lesions induced after the establishment of stable cocaine self-administration also facilitated responding for low cocaine doses versus sham-lesioned controls (Schenk et al., 1991). Lesions of the mPFC (ACC, PL and IL) also produce elevated response rates for light

stimuli previously paired with cocaine under second-order schedules of reinforcement (Weissenborn et al., 1997). Additionally, Di Ciano, Benham-Hermetz, Fogg, & Osborne (2007) have assessed the role of discrete mPFC subregions on responding for drug-paired conditioned reinforcers, using reversible pharmacological inactivation approaches. Baclofen/muscimol microinjections into the PL attenuated lever-responding for the cocaine-conditioned light cue (in the absence of cocaine delivery) on the first day of acquisition, but failed to have a lasting effect as animals quickly learned the behavior in subsequent sessions. In contrast, inactivation of the IL on the first day of lever-press acquisition, again in the absence of cocaine delivery, resulted in slightly lower response levels than vehicle controls but these IL-inactivated animals failed to increase responding and acquire behavior directed towards the cue-paired lever upon subsequent training. Extinction/reinstatement procedures were conducted in which the conditioned cues were no longer presented to reduce responding on the CS-paired lever and then, were subsequently reinstated by CS presentation; animals with PL inactivation failed to reinstate lever-pressing behavior, suggesting the PL may have a role in the ability of cocaine-paired cues to invigorate goal-directed behavior (Di Ciano et al., 2007).

1.3.3.2 Extinction and Reinstatement

Several inactivation studies have implicated the PL and IL subregions in extinction and reinstatement of drug-seeking behavior (Gass & Chandler, 2013). In animals with cocaine self-administration experience, GABA agonists microinjected into the IL immediately following short extinction sessions (30 minutes each and a total of 5 sessions) impaired extinction learning, while infusions of an AMPA receptor potentiator (administered in the same manner as GABA agonists) enhanced extinction training (LaLumiere, Niehoff, &

Kalivas, 2010). Thus, the IL appears to bidirectionally regulate animal's ability to learn to suppress drug-seeking behavior in the absence of cocaine. Interestingly, when the PL or IL are inactivated by the microinfusion of baclofen/muscimol just prior to initiation of the first extinction session, inhibition of neither region impacts cocaine-seeking/extinction learning during a subsequent extinction session (Peters, LaLumiere, & Kalivas, 2008). Taken together, all of these data suggest that the PL and IL are not required for the ability to learn to suppress drug-seeking behavior *per se*, but are important for the consolidation of extinction memories that impact subsequent drug-seeking behavior.

Curiously, the results of the aforementioned studies contrast with effects of GABA agonist microinjection into the vmPFC observed in studies of drug-seeking following forced cocaine abstinence; microinjection just prior to the first extinction session conducted at 30 days of forced abstinence decreased the high rate of responding on the active lever seen in vehicle-injected animals (Koya et al., 2009). Moreover, GABA antagonists microinjected into the vmPFC just prior to the first extinction session conducted at day 1 of forced abstinence elevated drug-seeking, compared to vehicle injected controls (Koya et al., 2009). Several differences in experimental conditions may account for this discrepancy in findings between the Koya et al. (2009) study and those described above. These include: the length of self-administration (2-hr vs 6-hr), the location of agonist/antagonist administration (IL vs PL and IL, timing of microinjection (following extinction training vs prior to onset of training), number of extinction sessions (5 sessions with microinjections vs 1 session with microinjection), and the presence of forced abstinence prior to the onset of extinction training (Koya et al., 2009; LaLumiere, Smith, & Kalivas, 2012).

Examining extinction responding over one session may also account for the difference between these two studies as Peters, LaLumiere, and Kalivas (2008) failed to find a significant effect of GABA agonists in the vmPFC on drug-seeking during the first day of extinction training. Akin to the aforementioned negative findings for GABA agonists, mGluR5 antagonists microinjected into the IL subregion also do not affect extinction responding within the first training session, but animals failed to recall extinction learning from the previous day when tested subsequently (Fontanez-Nuin, Santini, Quirk, & Porter, 2011). Consistent with this, acute microinjections of mGluR1/5 antagonists into the vmPFC (IL-PL interface) after 3 days of forced abstinence from cocaine self-administration did not affect within-session drug-seeking behavior during a 30-min extinction session; however, akin to the results of Fontanez-Nuin et al. (2011), there was no recall of that extinction learning the next day nor was there recall of the extinction learning the next day when the mGluR1/5 antagonists were infused immediately after the 30-min extinction sessions (Ben-Shahar et al., 2013). These findings indicate that inhibiting the vmPFC at the level of the IL-PL interface does not prevent extinction learning but rather interferes with the consolidation of extinction memories. Conversely, acute stimulation of mGluR1/5 within the vmPFC after 30 days forced abstinence from cocaine self-administration also failed to influence drug-seeking behavior during a 30-min extinction session; however, this prior stimulation facilitated the development of extinction on a subsequent test, while that of vehicle-infused controls was normally impaired (Ben-Shahar et al., 2013). At least in the case of extinction, increased glutamate signaling and stimulation of mGluR1/5 receptors facilitates extinction learning when animals are tested in subsequent extinction sessions while decreased vmPFC activity either by increased GABA signaling or antagonizing mGluR1/5 receptors either prior

to or immediately following the first extinction session is sufficient to impair the manifestation of extinction learning the next day (Ben-Shahar et al., 2013; Fontanez-Nuin et al., 2011; Peters, LaLumiere, et al., 2008). Thus, the vmPFC appears to play a role in the consolidation of new contingencies between reward and behavior, and/or habitual responding – both of which can impact the ability of a cocaine-experienced individual to suppress drug craving/seeking behavior.

The recidivism rate for cocaine use is high, even after prolonged periods of abstinence and the extinction-reinstatement model has been developed to study this phenomenon in experimental animals (Knackstedt & Kalivas, 2009). In this model, animals are trained to self-administer a drug under operant procedures. The operant response is then diminished or extinguished by allowing the animal to perform the response in the absence of the drug (extinction training). The animal is subsequently presented with a stimulus predictive of drug delivery, a stressor (e.g., foot-shock) or an injection/infusion of the drug itself to instigate or "reinstate" the operant response (Knackstedt & Kalivas, 2009).

The PL and IL have also been shown to be involved in various forms of reinstatement of drug-seeking following extinction of self-administration. For example, inactivation of the PL with tetrodotoxin (TTX) or lidocaine reduces context-, conditioned stimulus-, foot-shock, and cocaine-induced reinstatement of drug-seeking (Capriles, Rodaros, Sorge, & Stewart, 2003; Di Pietro, Black, & Kantak, 2006; Fuchs et al., 2005; Fuchs, Eaddy, Su, & Bell, 2007; McLaughlin & See, 2003). The Kalivas laboratory has consistently shown inhibition of the dmPFC with baclofen/muscimol microinjections or blockade of the intracellular effector, activator of G protein signaling 3 (AGS3), impedes reinstatement of drug-seeking following a cocaine priming injection (Bowers et al., 2004; McFarland & Kalivas, 2001; McFarland,

Lapish, & Kalivas, 2003; Peters, LaLumiere, et al., 2008; Peters, Vallone, Laurendi, & Kalivas, 2008; Shen, Gipson, Huits, & Kalivas, 2014). McFarland et al. (2001, 2003) trained rats to self-administer cocaine under short-access conditions (2 hr sessions), followed by extinction procedures. Then baclofen/muscimol was microinjected into the dmPFC prior to a 10 mg/kg IP cocaine priming injection to reinstate drug-seeking. These GABA agonists inhibited cocaine-primed reinstatement of active lever-pressing when localized to the ACC and dorsal PL, but not when the vmPFC (PL and IL interface) was targeted. Additionally, a more specific neurochemical basis for dPFC-mediated blockade of reinstatement was shown by D1/D2 receptor antagonism hindering cocaine-primed reinstatement and a direct injection of DA into this same region was sufficient to reinstate drug-seeking in the absence of cocaine (McFarland & Kalivas, 2001).

The dmPFC projects to the NA core via glutamatergic efferents and causes a rise in extracellular glutamate following cocaine-primed reinstatement procedures (McFarland et al., 2003). Baclofen/muscimol injections into the dPFC prevented this rise in extracellular glutamate following a 10 mg/kg IP cocaine-priming injection, providing further evidence for the role of the dPFC in the reinstatement of cocaine-seeking (McFarland et al., 2003). Consistent with this latter study, Shen et al. (2014), using similar cocaine self-administration, extinction and reinstatement procedures, found PL inactivation with baclofen/muscimol to block cocaine-primed reinstatement. Bowers et al. (2004) used a similar short-access (2 hr) cocaine self-administration-extinction-reinstatement model to show reduced AGS3 expression via administration of oligonucleotides against AGS3 within the ventral PL and dorsal IL to attenuate cocaine-primed (10 mg/kg IP) reinstatement, an effect that was reversed upon cessation of oligonucleotide administration, additional extinction training and

identical reinstatement procedures. Together, these data support a necessary role for the ACC, both dorsal and ventral PL and the dorsal IL in the reinstatement of cocaine-seeking, a conventional model of drug relapse.

However, other reports in the literature provide discrepant results regarding the direction of effects of mPFC inactivation upon measures of relapse. For example, when rats were trained to self-administer cocaine under short (2 hr) access self-administration procedures, followed by extinction training, and roughly 28 days of forced abstinence in the home cage, injections of baclofen/muscimol into the IL, but not PL, enhanced the spontaneous recovery of cocaine-seeking when animals were run through an additional extinction session (Peters, Vallone, et al., 2008). This is consistent with additional studies by Peters and colleagues using similar self-administration and extinction procedures, where IL inactivation induced cocaine-seeking while activation of this region inhibited cocaine-primed reinstatement (Peters, LaLumiere, et al., 2008; Peters, Vallone, et al., 2008). Moreover, IL inactivation-induced cocaine-seeking relies on an activated PL, as baclofen/muscimol administered into both regions prior to reinstatement inhibits drug-seeking behavior (Peters, LaLumiere, et al., 2008). These results are consistent with the findings of LaLumiere et al. (2010) discussed above for extinction, in that inactivation of the IL inhibited extinction learning, as seen by enhanced cocaine-seeking, and activation of the IL via AMPA administration facilitated extinction learning (decreased cocaine-seeking). Despite some inconsistencies in results, the neuropharmacological evidence described above implicates both the PL and IL regions of the rat in drug reward and goal-directed behavior. Thus, these structures are the focus of the following series of studies described within this dissertation.

1.4 The Limited Study of Glial Cells in the Etiology of Addiction

1.4.1 Astrocytes

The best characterized glial cell types that reside in the adult CNS tissue include astrocytes, microglia, and oligodendrocytes (OL) (Fields, 2008). Astrocytes are the most numerous cells in the human brain and each cell can support and modulate the function of up to an estimated 2 million synapses (Oberheim, Wang, Goldman, & Nedergaard, 2006;Verkhratsky, 2009). They have essential roles in the regulation of ion and water homeostasis, blood-brain barrier maintenance, regulation of blood flow, proliferation of stem cells, neurotransmission, neurotransmitter metabolism, supply of energy metabolites, and the development and formation of synaptic connections (Miguel-Hidalgo, 2009; Oberheim et al., 2006; Ullian, Sapperstein, Christopherson, & Barres, 2001). Astrocytes express receptors for most neurotransmitters and peptides, including but not limited to DA, glutamate, GABA, ATP, adenosine, glycine, acetylcholine, oxytocin and vasopressin, serotonin, and opioid making these cell types sensitive to neurochemical changes in the extracellular milieu (Verkhratsky, 2009; Volterra & Meldolesi, 2005).

While astrocytes are not electrically excitable, they respond in a receptor-dependent manner to neural activity by the propagation of calcium waves that can result in the release of glutamate, serine, and ATP capable of affecting neighboring neuronal and glial physiology (Oberheim et al., 2006). Astrocytic calcium signaling is mediated predominantly through metabotropic receptors on the cell surface whose activation initiates phospholipase C-mediated formation of inositol-triphosphate (IP3) triggering endoplasmic reticulum release of calcium stores (Deitmer, Singaravelu, & Lohr, 2009). Calcium waves can be initiated within microdomains surrounding synapses and propagate along the processes to other regions of the astrocyte, whereby both calcium and IP3 can permeate gap junctions connecting

neighboring astrocytes regenerating and furthering the signaling wave (Scemes & Spray, 2009; Deitmer, Singaravelu, & Lohr, 2009). Therefore, astrocytes are not only capable of sensing neuronal signaling via receptor-dependent mechanisms, but are also capable of signaling through calcium waves to neighboring cells and releasing transmitters that form a feedback loop on a wide network of connected neurons.

While limited, evidence of astrocyte dysfunction in neuropsychiatric disease is beginning to accumulate even if primarily correlative in nature. The first instance of a disease with astroglial origin is Alexander disease distinguished by a mutation in the glial fibrillary acidic protein (GFAP) gene, which encodes the intermediate filament found exclusively in astrocytes (Brenner, Goldman, Quinlan & Messing, 2009). The most common symptoms include seizures, megaencephaly, failure to meet physical and intellectual milestones, spasticity, poor coordination, paralysis, vomiting, difficulty swallowing, and this disease ultimately results in premature death (Brenner et al., 2009). A speculative role of astrocytes has also been proposed based on the finding of reactive gliosis in conjunction with neuronal loss within sclerotic hippocampi of deceased temporal lobe epilepsy patients (Binder & Steinhauser, 2009). Given the role of astrocytes in glutamate uptake from synapses, and their ability to directly excite neighboring neurons via calcium-dependent glutamate release, it is plausible an astrocytic abnormality could drive the excessive excitability underlying seizure disorders (Binder & Steinhauser, 2009).

Tissue collected from post-mortem patients with major depressive disorder (MDD) and examined via microarray and quantitative polymerase chain reaction (qPCR) technologies have revealed significant reductions in the astrocyte-specific transcripts GFAP, S100 calcium binding protein, gap junction protein α1 and β6, and aquaporin-4 in the LC,

but not in tissue collected from individuals diagnosed with bipolar disorder (Bernard et al., 2011). Another study of post-mortem dlPFC tissue of suicide-completers compared to control subjects matched for cause of death, found a decrease in the astrocyte-specific gap junction proteins connexin-30 and 43 (Cx30 and Cx43, respectively) mRNA transcripts in a microarray analysis of global gene transcription (Ernst et al., 2011). A causal role for Cx30 in normal brain function has been established in a gene knock-out mouse model where mice show decreased motor coordination on the Rotarod task, decreased number of rearings in the open field and object exploration tasks, and greater anxiety-like behavior as assessed by increased avoidance of the center and corner preference in the open field test (Dere et al., 2003).

In regards to cocaine addiction, cocaine administration and withdrawal results in increased expression of GFAP within the dentate gyrus, PFC, and NAc (Bowers & Kalivas, 2003; Fattore et al., 2002). Bowers and Kalivas (2003) and Fattore et al. (2002) reported significant modifications in astrocyte number, size and shape complexity following cocaine experience in animal models. Microdialysis experiments examining the effect of 3 weeks of withdrawal from experimenter administered cocaine on basal extracellular levels of glutamate within the NAc found a cocaine-induced decrease in levels dependent upon the decreased function of the astrocyte-specific cystine/glutamate exchanger, a protein known to modulate the extracellular levels of glutamate within this brain region (Baker, Shen, & Kalivas, 2002; Baker, Xi, Shen, Swanson, & Kalivas, 2002). Additionally, reinstatement of drug-seeking promoted by a cocaine-priming injection was diminished in animals with prior cocaine experience and withdrawal when an intracranial (NAc) perfusion of cystine or systemically administered N-acetylcystine was given to normalize glutamate levels prior to

reinstatement (Baker et al., 2003). Pretreatment of rats with systemic N-acetylcystine before daily long-access (6 hr/day) cocaine self-administration sessions or experimenter administered cocaine injections, followed by 2-3 weeks of withdrawal, prevented the development of escalated drug intake, behavioral sensitization, reduced cocaine-primed reinstatement, and prevented the decrease in cystine/glutamate exchanger activity, returning basal glutamate levels to normal (Kau et al., 2008; Madayag et al., 2007). In line with alterations in the cystine/glutamate exchanger following cocaine, cystine uptake into astrocytes is the rate-limiting step in the synthesis of glutathione (GSH), a molecule important in balancing cellular redox in response to oxidative stress and with a potential role in decreasing the oxidative stress induced by cocaine administration (Uys et al., 2011). Experimenter administered cocaine followed by three-weeks withdrawal resulted in a decrease in GSH-S-transferase pi (GSTpi; the primary catalyst for glutathionylating proteins which generate GSH), and mimicking this decrease with a knock-out mouse model or administering an agent to inhibit GSTpi, facilitated cocaine CPP and locomotor sensitization (Uys et al., 2011).

GLT1 (a.k.a. EAAT2) is a sodium-dependent transporter located exclusively on astrocytes within the CNS and is responsible for 90% of the removal of extracellular glutamate, it was therefore hypothesized to play a role in cocaine relapse as an increase in glutamate transmission facilitates this feature of addiction (Sari, Smith, Ali, & Rebec, 2009). To examine for an effect of GLT1 on relapse, Sari et al. (2009) trained rats to self-administer cocaine (2 hr/day, 10-14 days) followed by five days of extinction training with post-session injections of ceftriaxone, a β-lactam antibiotic thought to increase GLT1 expression. While cocaine experience itself did not increase the expression of GLT1 within the PFC or NAc,

administration of ceftriaxone attenuated cue-induced reinstatement of cocaine seeking and resulted in enhanced expression of GLT1 within the PFC and NAc. Cocaine self-administration (2 hr/day, 14 days) followed by three-weeks of extinction training, did however, decrease the expression of GLT1 within the NAc but not the PFC of rats (Knackstedt, Melendez, & Kalivas, 2010). Moreover, ceftriaxone treatment restored GLT1 levels, and prevented cue-induced and cocaine-induced reinstatement. Fischer-Smith, Houston, & Rebec (2012) extended findings of cocaine-induced changes in GLT1 expression when examining rats with long-access (6 hr/day; 11 days) cocaine self-administration followed by short (1 day) versus long (45 days) withdrawal within the NAc core and shell. They found both NAc regions to have decreased expression of GLT1, but that NAc core expression at the long withdrawal time point was significantly reduced to a greater extent than expression at either withdrawal time points for the NAc shell.

Another approach for examining the role of GLT1 was with the administration of the atypical methylxanthine propentofylline (PPF), which is an adenosine uptake and phosphodiesterase inhibitor and has been linked with increased expression of GLT-1 (Reissner et al., 2014). Systemic administration of PPF prior to a cocaine-induced reinstatement test inhibited cue- and cocaine-induced reinstatement (Reissner et al., 2014). This effect is potentially due to enhanced GLT-1 function buffering against cue-induced stimulated glutamate release in the NAc (Kalivas et al., 2005). Astrocytes are not the only glial cells in position to affect neurotransmission, as it is well established that microglial cells promote a healthy brain and OL membrane processes form the myelin sheaths that are required for much of mammalian neuronal communication.

1.4.2 Microglia

This review of microglial cells will be brief, as my examination of this cell type in the following series of studies is minor. Microglial cells are of mesodermal origin and retain many of the features of macrophages, including the initiation of inflammatory responses and innate immune signaling (Miguel-Hidalgo, 2009; Morris, Clark, Zinn, & Vissel, 2013). Microglial morphology exists two distinct forms depending on activation state; in the "resting" state cells are highly ramified with long processes and small cell bodies compared to the "activated" state where they appear as globular and "amoeboid" in shape (Morris et al., 2013). In response to injury or pathogenic events, microglial cells change their morphology, migrate to the site of injury and perform a variety of functions including neuroprotection, support neurogenesis, direct the invading vasculature, remove apoptotic cells, influence synaptogenesis, and mediate developmental apoptosis (Morris et al., 2013). While previously thought of as dormant in their resting state, this "resting" state is a misnomer as microglia are dynamic surveyors of their environment, expanding and retracting their thin processes to contact neighboring neurons, astrocytes, blood vessels, sometimes engulfing tissue, and increasing their activity in response to neural activity (Morris et al., 2013). Microglial cells also express most of the receptors to neuropeptides, neurotransmitters, and astrocytic transmitters (Miguel-Hidalgo, 2009).

The vast majority of research on microglia and addiction has centered on alcohol and methamphetamine administration and reveals these drugs result in microglial activation and enhance innate immune gene induction (i.e. IL-1β, IL-6, TNF-α). However, only a handful of studies have examined the effect of cocaine on the CNS microglial response (*see reviews* Clark, Wiley, & Bradberry, 2013; Crews, Zou, & Qin, 2011). In post-mortem tissue from

human chronic cocaine users, elevated levels of activated macrophages and microglial cells in the anterior midbrain were detected via immunohistochemistry (Little et al., 2009).

Animal models using both experimenter-administered cocaine and self-administration procedures have revealed an increase in several inflammation related transcripts throughout the PFC, NAc, and striatum (Ahmed et al., 2005; Clark, Wiley, & Bradberry, 2013b; Piechota et al., 2010). For example, some of the findings of Ahmed et al. (2005), included increased expression of IL-6, IL-4, TNF within the PFC, a variety of microglial transcript increases in the VTA, septum, amygdala, lateral hypothalamus, and NAc using oligonucleotide arrays and qRT-PCR in rats with either 18 days of short access (1 hr/day) or extended-access (6 hr/day) cocaine self-administration (0.25 mg/0.1 ml infusion, IV), compared to animals undergoing the same experimental procedures excluding cocaine experience (further data analysis conducted by Clark et al. (2013) for microglial-related genes). Additional analyses of gene array data collected by Piechota et al. (2010) and analyzed by Clark et al. (2013), revealed a single injection of 25 mg/kg cocaine (IP) into mice sacrificed 1 hr post-injection to enhance the expression of several cytokine receptors (e.g., IL-6r and TNFα), oxidative stress molecules (e.g. nitric oxide synthase), and growth factors whose expression further increased with increased delay between injection and sacrifice time points (1, 2, 4 or 8 hr post-injection) within the rostral portion of the CP and NAc.

Activated microglial cells release reactive oxygen species (ROS), including superoxide and nitric oxide, which have a role in facilitating the apoptotic process (Clark et al., 2013; Costa, Yao, Yang, & Buch, 2013). In a microglial cell culture assay, cocaine dose dependently decreased cell viability via pro-apoptotic pathways, an effect mediated by ER

stress-related proteins (Costa et al., 2013). Specifically, CHOP, a protein that signals the pro-apoptotic pathway, had increased expression levels following cocaine and inhibiting this increase, by siRNA mediated knockdown, increased cell viability in the presence of cocaine. Additionally, rats treated with IP injections of cocaine for 14 days (20 mg/kg) were shown to have enhanced CHOP protein levels in brain, validating the enhancement seen in cell culture. Consistently, cocaine self-administering rats were shown to dose-dependently decrease active lever responding when a ROS scavenger or TEMPOL (to reduce oxidative stress) was systemically administered or TEMPOL was microinjected into the NAc (Jang et al., 2014). While this last study only revealed oxidative stress within neurons, and not astrocytes, OLs or microglial cells, it is tempting to speculate that treatment with either of these drugs could potentially reduce the ER stress in microglial cells which may impact the course of cocaine addiction.

1.4.3 Oligodendrocytes

The vertebrate nervous system requires myelination for efficient and reliable action potential propagation that mediates communication within and between brain regions (Wake, Lee, & Fields, 2011). Myelination provides increased membrane resistance and lowers capacitance, thereby increasing nerve impulse velocity (Bhat et al., 2001; Kidd & Trapp, 2010; Min et al., 2009; Murray & Steck, 1984). A single OL can wrap up to 60 internodal segments, contacting many different neurons simultaneously (Mathey, Arthur, & Armati, 2010). The relationship between OLs and neurons is mutual with cell-specific changes impacting the structure and function of the other connected cell. Friede (1972) demonstrated that the rate of axonal growth determined the rate of myelin formation, one mechanism being glutamate and ATP released by nerve conduction and sensed via OL NMDA, AMPA, and

mGluR 1 & 5 receptors (Fields, 2008; Kelland & Toms, 2001; Luyt, Varadi, & Molnar, 2003; Wake et al., 2011). Changes in OL-specific protein expression can also affect axonal diameter, as seen in myelin oligodendrocyte basic protein (MOBP)-deficient mice with increased axonal diameters compared to wild type controls (Sadahiro et al., 2000). These mice showed no gross behavioral abnormalities, though only motor coordination was tested on Rotarod and forced bar-crossing tests (Yamamoto et al., 1999).

Additionally, changes in OL specific proteins (e.g., oligodendrocyte-myelin glycoprotein (OMgp), proteolipid protein (PLP), & myelin basic protein (MBP)), can result in abnormal or absent nodes of Ranvier and decreased nerve conduction velocity (Boiko et al., 2001; Nie et al., 2006; Tanaka et al., 2009). Boiko et al. (2001) used immunohistochemistry to label retinal ganglion cells for sodium channels ($Na_v1.2$ and $Na_v1.6$) of normal Sprague Dawley rats, *Shiverer* mice and wild-type mice. *Shiverer* mice have a mutation in the MBP gene resulting in a severe deficiency in the formation of compact myelin (Chernoff, 1981). Along with hypomyelination, *Shiverer* mice have few or absent nodes of Ranvier distinguished by the clustering of $Na_v1.6$ sodium channels in the nodal region and restriction of the $Na_v1.2$ form to the intermodal, myelinated regions (Boiko et al., 2001). Additionally, the $Na_v1.6$ isoform was rarely detected and then only in clusters adjacent to Caspr-labeling (an indicator of nodes of Ranvier).

OMgp is detected in clusters at the nodes of Ranvier in wild-type mice by immunohistochemical techniques, but is distributed diffusely along myelinated axons of *Shiverer* and PLP-null mice (Nie et al., 2006). Nie et al. (2006) also used electron microscopy to demonstrate abnormal node-paranode junctions in OMgp mutant mice, immunoblotting to show reduced Na channel α subunit expression, and compound action

potentials recorded from the spinal cord revealed significantly decreased conduction velocity of OMgp-transgenic compared to wild-type mice. Moreover, PLP-null transgenic mice were shown to have reduced conduction velocities within various tracts of the spinal cord estimated from regression analyses of latencies recorded at three or more segments and the distances between recording sites compared to wild-type controls (Tanaka, Ikenaka, & Isa, 2006). In the same experiments, PLP-null transgenic mice were also found to have reduced sodium and potassium channel clustering around paired clusters of Caspr labeling (to identify nodes of Ranvier) revealed by immunohistochemistry compared to their wild-type counterparts, similar to what has been found with *Shiverer* mice and OMgp-transgenic mice discussed above (Tanaka et al., 2006). These findings would suggest that the differential distribution of sodium channel isoforms is not mediated intrinsically by neurons alone, but requires the formation of proper axoglial junctions. In addition to decreased nerve conduction velocity resulting from OL protein changes, activation of OLs via glutamate induces depolarization, resulting in increased conduction velocity in the correspondingly wrapped axonal process, as assessed with electrophysiology (Yamazaki et al., 2007). This effect may be mediated by OL process swelling, which increases the conduction velocity by increasing membrane resistance (Morán & Mateu, 1983).

Only a few laboratories have conducted behavioral testing with myelin-deficient mice. *Shiverer* mice with MBP gene mutations show severe motor deficits that become exacerbated with age, including a generalized tremor, paralysis of the hindquarter, progression to frequent seizures and death at around 50 days (Chernoff, 1981). Interestingly, these animals have been shown to have a normal ability to learn the location of a food reward in a T-maze; however, when the location of the reward is reversed to the opposite arm, they

show a deficit in reversal learning and make a greater number of errors by returning to the location of the original food-paired arm (Inagawa, Watanabe, & Tsukada, 1988). Heterozygous MBP-mutant mice (mld) showed a similar deficit in reversal learning compared to wild-type mice, but to a lesser degree than *Shiverer*, indicating a partial recovery when at least half the expression of MBP was returned to normal and myelination was less severely impacted (Inagawa et al., 1988).

PLP-null mice have been examined for a variety of behavioral deficits including Rotarod, wire hanging and grip strength tests, Open field, Light/dark transition, hot plate, social interaction test in a novel environment, startle response/prepulse inhibition, porsolt swim test, elevated plus-maze, the Barnes spatial navigation task, and T-maze forced alternation tasks (Tanaka et al., 2009, 2006). PLP-mice exhibit an age-related decline in latency to fall in the Rotarod motor test that is correlated with the progressive demyelination observed in these animals (Tanaka et al., 2006). While there were no significant differences observed in the wire hanging, grip, open-field or pain nociception tests, PLP-null mice exhibited abnormal anxiety-like behavior (Tanaka et al., 2009). Tanaka et al. (2009) showed PLP-null mice to make significantly less transitions between the light and dark boxes, had a greater number of entries and time spent in the open arms of the elevated plus-maze, and a tendency towards a decreased total number of contacts in the social interaction test compared to wild-type controls. Moreover, PLP-null mice exhibit a mildly impaired prepulse inhibition when a 78 dB prepulse sound precedes both a 110 and 120 dB startle pulse. In the spatial navigation test, PLP-null mice show less exploration around the target hole (hole containing food reward on the acquisition trial), a greater number of errors to reach the target hole, spent a lesser percentage of time near the target, and also tended to travel greater distances to reach

the target than wild-type controls. PLP-null mice exhibited a robust deficit in working memory revealed in significantly greater errors made during training sessions of the forced alternation T-maze, and when a 30 sec or 60 sec delay was added between the forced and choice trials (requiring animals to remember the forced arm and alternate their entry to the rewarded arm). These studies provide evidence for links between myelin-specific protein expression, the structural integrity of myelin and axons, neuronal signaling, and behavioral consequences of altering OL specific genes.

Direct manipulation of OL-specific protein expression on cocaine taking in animal models has yet to be examined, however, several labs have examined the effect of cocaine in various animal models and methods of administration on the expression of OL -density, -specific proteins, and -mRNA transcripts. White matter from the anterior (precommisural) striatal level of rhesus monkeys with prolonged self-administration (30 injections of 0.3 mg/kg each injection, for 300 sessions (days)) of cocaine showed a reduction in both PLP and MBP mRNA and protein expression levels (Smith, Beveridge, Nader, & Porrino, 2014).

Within the dmPFC and OFC of rats with extended-access to cocaine self-administration (0.5 mg/kg per 100 ml cocaine, 6-20 sessions of 1 hr/session followed by a minimum of 85 sessions of 6 hr/day) was a decrease in the density of OL cells, which correlated with working memory impairments in the delayed non-matching to sample T-maze (George, Mandyam, Wee, & Koob, 2008). However, one caveat to this study is the use of the protein NG2+ as the marker for OL cells in their immunohistochemistry, as NG2+ positive cells have also been found to differentiate into gray matter astrocytes, neurons, or remain as NG2+ cells that are distinct from OLs (Mathey, Aurthur, & Armati, 2010). Cocaine self-administration (14 consecutive days, 3 hr/day, 0.75mg/kg/0.1ml, IV) has also

resulted in the hypomethylation of the *Sox10* gene within the corpus callosum at 30 days of forced abstinence compared to drug-free, sham surgery controls, a gene which encodes a transcription factor essential in regulating OL differentiation (Nielsen et al., 2012).

Experimenter-administered cocaine (continuous infusion of intradermal cocaine, ~40 mg/kg/day for 28 days without withdrawal) resulted in decreased volume and expression of MBP in the splenium of the corpus callosum (CC) as measured by differences in fractional anisotropy (FA) seen on diffusion tensor imaging (DTI) and post-mortem histological analysis of CC size and immunohistochemistry for MBP (Narayana et al., 2009). Consistently, 14 days of 15 mg/kg (IP/day) cocaine followed by 30 days of abstinence in rats, also decreased MBP, PLP, myelin oligodendrocyte glycoprotein (MOG), and myelin associated glycoprotein (MAG) in the NAc (Kovalevich, Corley, Yen, Rawls, & Langford, 2012). Interestingly, mice given daily injections of ceftriaxone (GLT-1 activator) with cocaine injections or only during the withdrawal period, saw no significant loss of myelin proteins at 30 days withdrawal compared to saline control animals, and ceftriaxone given alone had no effect on myelin protein expression (Kovalevich et al., 2012). Due to the limited understanding of the molecular changes that result from extended-access (6 hr/day) cocaine self-administration, the focus of this dissertation is on cocaine induced protein changes within the vmPFC of the rat and the cognitive consequences of altered glial physiology.

1.5 White matter in humans

1.5.1 White matter maturation and vulnerability to substance use

Magnetic resonance imaging (MRI) studies have revealed distinct white and gray matter volume changes across human development. A longitudinal study from preadolescence through post-adolescence (4-22 years) utilized MRI to measure cortical gray

and white matter volume within the frontal, temporal, parietal and occipital lobes of male and female children (Giedd et al., 1999). The net increase in white matter across this age range was 12.4%, increasing linearly with age. Gray matter, however, showed a region specific increase during preadolescence including maximum size in the frontal lobe at an average of 11-12 years, 10-11 years for the parietal lobe, 16-17 years for the temporal lobe, and linear increases in the occipital lobe without significant decline or leveling within the age-range examined. Beginning in adolescence, however, gray matter declines linearly with age while white matter continues to expand through the fourth to fifth decades of life, at least in males as females were not examined (Bartzokis et al., 2001). Bartzokis et al. (2001) used a cross-sectional study to examine males aged 19-76 years via volumetric MRI and confirmed an age-related linear loss in gray matter volume within the frontal and temporal lobes, while white matter continued to expand till age 44 for the frontal and 47 for the temporal lobes. The regional difference in maturation of white matter between the frontal and temporal lobes in the Bartzokis et al. (2001) study closely mirrors the age difference in gray matter increase within the same regions of the childhood study discussed above (roughly 3-4 years, with the frontal cortex reaching a maximum volume before temporal lobes).

Adolescents have been reported to exhibit deficient executive cognitive functioning and behavioral self-control in experiments examining planning, attention, foresight, abstract reasoning, judgment, and self-monitoring (Bernheim, Halfon, & Boutrel, 2013; Luna, Padmanabhan, & O'Hearn, 2010). The Dual Systems Model has been proposed to explain adolescent behavior guided predominantly by motivational systems which develop at an earlier stage than the slower maturing cognitive control (PFC) mechanisms, and suggest these differential maturation processes underlie the increased risky decision making and

reward seeking (Bernheim et al., 2013; Willoughby, Good, Adachi, Hamza, & Tavernier, 2013). Consistently, the majority of drug users are adolescents and young adults or adults whose use began as adolescents, and adolescent onset of drug use leads to a more rapid progression of abuse to dependency (Bernheim et al., 2013; Perry, Anderson, Nelson, & Carroll, 2007). Moreover, immature frontostriatal development in adolescence is associated with greater risk-taking, greater cocaine intake in adolescence lasting into adulthood, increased behavioral sensitization, and a decrease in the experience of the negative effects of drugs of abuse (Crews & Boettiger, 2009; Perry et al., 2007). Given the importance of the adolescence time point in normal brain maturation, it is important to sample this period for interactions between cocaine and development that may exacerbate cocaine use, cocaine-related cognitive and CNS deficits, and the vulnerability to develop addiction.

1.5.2 Imaging studies of chronic cocaine addicts

A frequently used method for *in vivo* imaging of white matter microstructural changes following cocaine use is DTI MRI, compared to more macrostructural changes measured more broadly as changes in gross volume. DTI measures the directionality of motion of water molecules and is based on the principle that water will move in all possible directions unless barriers exist in which it will preferentially travel along said barrier (e.g., axons or bundles of myelinated fiber tracts) (Moeller et al., 2005). This preferential movement along one axis is called anisotropic diffusion; organized structures like white matter fiber tracts exhibit a high degree of this form of water movement (Moeller et al., 2005). A reduction in anisotropy, or fractional anisotropy as measured in DTI, suggests a decrease in the organization of a particular structure and has been suggested to reflect subtle white matter pathology and/or a loss of integrity in fiber tracts (Moeller et al., 2005). An alternative explanation is an increase

in the number of fibers that cross in varying directions from the normal direction of the fiber tract, thereby resulting in a decrease in the preferential movement of water.

Investigators using DTI and volumetric MRI have revealed significantly lower volumes of white and gray matter, and abnormalities in white matter microstructure in chronic cocaine users compared to healthy age-matched controls; Table 1 lists descriptions of the subjects, their cocaine histories, and white matter changes (Bartzokis et al., 2002; Lim, Choi, Pomara, Wolkin, & Rotrosen, 2002; Moeller et al., 2007; Moeller et al., 2005). Specifically, Lim et al. (2008, 2002) found compromised white matter microstructure, as measured by a reduction in the fractional anisotropy, and a trend towards decreased gray and white matter volume (via volumetric MRI) in the inferior frontal brain regions of chronic cocaine addicts; an effect associated with the duration of cocaine use (Lim et al., 2008, 2002). Moreover, chronic cocaine addicts were found to have reduced white matter integrity of the corpus callosum, as measured by fractional anisotropy values from DTI, and a concomitant increase in behavioral measures of impulsive-behavior, both of which are reliable predictors of relapse (Moeller, et al., 2007; Moeller et al., 2005).

Study	Imaging method	Brain regions showing change relative to control	Subject Information				
			Group	Mean Age + SD	N / Sex	Mean years / range years cocaine use	Other substance use
Bartzokis et al., 2002	Volumetric MRI	*Correlation between age and WM increase, seen in control, but not cocaine-dependent individuals.*	Cocaine	37.5 + 6.1	37 / M	8.8 / 1-30	3 alcohol dependent (13 past dependence); 5 marijuana dependent (7 past dependence)
		Total WM ↑ Frontal WM ↑ Temporal WM ↑	Control	30.6 + 6.8	55 / M	0 / 0	Yes, no dependence
Lim et al., 2002	DTI fMRI	*Fractional anisotropy* Frontal white matter ↓ 0 mm above AC/PC ↓ 5 mm below AC/PC ↓ trend	Cocaine	44.17 + 5.09	12 / M	17.33 / 9-30	Yes, no dependence
			Control	40.35 + 6.80	10 / M; 3 / F	0 / 0	
Lim et al., 2008	DTI fMRI	*Fractional anisotropy* Inferior frontal WM ↓ Right ↓ Left ↓ Internal Capsule WM Right ↓ Left ↓ Splenium CC ↓ Body CC ↓	Cocaine	42.5 + 6.1	11 / M, 10 / F	18.9 / 3 - 38	6.9 + 6.7 days alcohol use per prior month
	Volumetric MRI	Inferior frontal GM ↓ Inferior frontal WM ↓ trend	Control	40.9 + 7.4	11 / M, 10 / F	0 / 0	3.90 + 7.2 days alcohol use per prior month
Moeller et al., 2005	DTI fMRI	*Fractional anisotropy* Genu ↓ Rostral Body ↓	Cocaine	33.1 + 8.7	14 / M, 4 / F	9.7 / 2-28	Yes, no dependence
			Control	30.3 + 7.7	14 / M, 4 / F	0 / 0	
Moeller et al., 2007	DTI fMRI	*Fractional anisotropy* Genu ↓	Cocaine	32.6 + 8.4	10 / M, 3 / F	10 / 2-28	Yes, no dependence
			Control	30.3 + 7.7	14 / M, 4 / F	0 / 0	

Table 1. Summary of Imaging Data from Chronic Cocaine Addicts. ↑ indicates increase in volume, ↓ indicates decrease in volume; AC, anterior commissure; CC, corpus callosum; COC, cocaine; CON, control; DTI, diffusion tensor imaging; F, female; GM, gray matter; M, male; MRI, magnetic resonance imaging; PC, posterior commissure; SD, standard deviation; WM, white matter.

An alternative approach to directly comparing volumetric differences in white matter following cocaine abuse is to compare the age-related increase in white matter volume normally seen in humans up to the fourth to fifth decades of life (Bartzokis et al., 2001). Bartzokis et al. (2002) used volumetric MRI to measure the frontal and temporal regions and conducted correlational analyses to evaluate the relationship between age and brain volumes while controlling for brain size variations due to overall body size, education, and race. By comparing the white matter growth slopes (e.g., the absolute rates of change per year in brain volumes) from cocaine dependent and control subjects, chronic cocaine users did not show the age-related increase in white matter within the frontal and temporal lobes as non-drug users and suggested cocaine may arrest normal white matter maturation. Studies of PFC damage in humans and animals consistently report that such damage leads to behavior dominated by impulses motivated by drive states, conditioned associations or habitual responses, therefore abnormal gray and white matter structure could also be implicated in these features that are involved in cocaine addiction (Damasio, 1996; Jentsch & Taylor, 1999).

Since chronic cocaine use has been consistently found to reduce white matter volume within humans measured with *in vivo* MRI techniques (Bartzokis et al., 2002; Lim et al., 2002; Liu, Matochik, Ph, Cadet, & London, 1998; Ma et al., 2009; Moeller et al., 2007; Moeller et al., 2005; Volkow, Valentine, & Kulkarni, 1988), this could also reflect a disruption in the connectivity and efficiency of signaling between the PFC and underlying reward circuitry; a disruption potentially underlying the cognitive and behavioral control deficits, as well as the abnormal drive for cocaine seen with cocaine dependence (Fields, 2008; Lim et al., 2002; Ma et al., 2009; Moeller et al., 2005). Post-mortem tissue analysis via

microarray technology of individuals with chronic cocaine use reveals significant mRNA reductions in the major myelin constituent, PLP, throughout the CP, internal capsule, NAc, and dlPFC (BA 46) (Albertson et al., 2004; Kristiansen, Bannon, & Meador-Woodruff, 2009; Lehrmann et al., 2003). Additionally, other myelin-specific mRNA for MBP, claudin-11 (CLN), MOBP, and transferrin are reduced following cocaine abuse (Albertson et al., 2004; Kristiansen et al., 2009), Table 2. While the area of study of white matter changes in the brain of cocaine addicts is limited and correlational in nature, it raises many important questions: do these differences precede drug exposure and predispose individuals to develop addiction, do they result from chronic drug use, are the non-neuronal components of white matter affected by drug use, and do these non-neuronal components have an affect on brain function? To address these questions, animal models must be utilized to examine for cause-effect relationships.

Study	Method	Brain Region / Changes compared to control	Subject Information					
			Group	Mean Age + SD	N / Sex	Mean years / range years cocaine use	Other substance use	Cause of death
Albertson et al., 2004	Microarray; verified by qRT-PCR	**NAc** MBP ↓ MOBP ↓ PLP ↓	COC*	25-50 range	8 / M; 2 / F	Tested *positive* at autopsy for cocaine and metabolites	Alcohol, not dependent	Matched between subjects; gun shot wounds, hypertension, or cardiovascular disease
			CON		8 / M; 2 / F	Tested *negative* for cocaine and metabolites		
Kristiansen et al., 2009	In situ hybridization	**Caudate** PLP ↓ (dorsal and ventral) CLN ↓ (dorsal only) **Putamen** PLP ↓ (dorsal and ventral) **IC** (dorsal and ventral) PLP ↓ (dorsal and ventral) **NAc** no changes	COC*	22-52 range	8 / M; 2 / F	Tested *positive* at autopsy for cocaine and metabolites	Alcohol, not dependent	5 cocaine-related deaths within COC group; others matched for gun shot wounds or cardiovascular disease
			CON		8 / M; 2 / F	Tested *negative* for cocaine and metabolites		
Lehrmann et al., 2003	Microarray; verified by qRT-PCR	**dlPFC (BA 46)** PLP ↓/↑ dependent upon whether cocaine only or cocaine and crack was used by COC subjects, respectively	COC	37.0 + 10.1	7 / M	Tested *positive* at autopsy for cocaine and metabolites	2 alcohol dependent	1 cocaine toxicity, 1 mixed drug intoxication; homocide
			CON	38.9 + 9.8	7 / M	Tested *negative* for cocaine and metabolites	No dependence	Natural or homocide

Table 2. Summary of myelin-specific RNA changes in post-mortem tissue analysis of chronic cocaine users. *= Cocaine subjects matched pairwise for age, gender, & brain pH; COC, cocaine; CON, control; dlPFC, dorsolateral PFC; F, female; IC, internal capsule; M, Male; NAc, nucleus accumbens

1.6 Animal Models of Cocaine Addiction

1.6.1 Active versus passive cocaine administration

Several methodologies exist for studying the effects of cocaine on the CNS, common forms of administration being either passive, experimenter administered cocaine, or active self-administration of drug. However, a wealth of data suggests that active self-administration of cocaine induces different neuroplasticity including changes in neurotransmitter release and turnover, protein expression, and gene expression (for review see Jacobs et al., 2003). For example, passively receiving yoked cocaine infusions led to a higher lethality rate than self-administration of the same amount of cocaine when rats had access to either 6-hr of 0.33 mg/infusion of cocaine or a maximum of 80 infusions over one session (Dworkin, Mirkis, & Smith, 1995). Additionally, Hemby, Co, Koves, Smith, & Dworkin (1997) utilized a yoked cocaine procedure where one rat was trained to self-administer IV cocaine (0.33 mg/infusion) over a 6-hr session (maximum of 60 daily infusions) till stable baseline responding (25 days of SA, run 7 days a week) was achieved and another rat received yoked infusions paired to one self-administering animal, and conducted microdialysis for DA concentration within the NAc. Both yoked and self-administering rats showed increased DA levels in the two-hr microdialysis session in response to cocaine delivery, but self-administering animals exhibited significantly greater concentrations than their yoked littermates. Moreover, on the following day both groups of animals experienced yoked cocaine infusions in the same pattern as the previous day, and while DA levels rose above baseline, the previously self-administering animals did not show the same increase above their yoked littermate when they were not actively administering.

A similar effect has been seen in mice with either yoked or active-self-administration of cocaine (5 days, 90 min session, 0.5 mg/kg/infusion), in that a passive IP injections of cocaine (5.0 mg/kg) following self-administration sessions failed to raise self-administering mice NAc dopamine levels above yoked controls assessed via *in vivo* microdialysis, however this group did not measure DA levels during a self-administration session (Zapata, Chefer, Ator, Shippenberg, & Rocha, 2003). Furthermore, rats trained to self-administer cocaine (0.25 mg/kg) or rats yoked to self-administering animals for 2-hr sessions till stable responding was achieved, then put through extinction procedures, and reinstated with 10 mg/kg IP cocaine injection prior to microdialysis procedures to assess glutamate and DA levels within the NAc, revealed both groups experienced elevated levels of DA in response to cocaine, but only animals with self-administration experience showed an increase in glutamate (McFarland et al., 2003). Therefore, while an elevation in DA increases in response to cocaine in general (either self-administered or non-contingently administered), a further increase requires cocaine to be actively administered, and glutamate increases only occur in self-administering animals, at least during reinstatement procedures.

Consistently, acetylcholine (ACh) levels within the NAc were found increase to a greater extent and remain higher during and after a 3 hr cocaine exposure session in animals allowed to self-administer cocaine compared to yoked-cocaine experienced and saline control rats; cocaine self-administering animals were allowed to administer cocaine (0.42 mg/kg/infusion) for three hour sessions over 13 consecutive days and yoked controls were matched for non-contingent cocaine delivery based on self-administering animals (Mark, Hajnal, Kinney, & Keys, 1999).

As stated previously, the method of cocaine administration also has an effect on neurotransmitter turnover. Dworkin, Co, & Smith (1995) examined neurotransmitter turnover rate in cocaine self-administering rats, yoked controls (0.33 mg/infusion; average 30 days drug administration; 6 hr sessions; 7 days a week), or saline controls by administering labeled [^{14}C]glucose, [^{3}H]tyrosine and [^{3}H]tryptophan through their jugular catheters 24 hours following the last self-administration session and 60-90 minutes prior to sacrifice. Using high-pressure liquid chromatography, they found decreases in DA, serotonin (5-HT), norepinephrine (NE), and glutamate turnover within the frontal cortex, NAc, motor-somatosensory cortex, dentate gyrus, and globus pallidus; increases in DA, 5-HT, NE, GABA, and aspartate acid turnover within the piriform cortex, temporal-auditory cortex, NAc, CP, pre-optic area- diagonal band, motor-somatosensory cortex, and cerebellum (not all neurotransmitters changed in all regions); and reversals depending on the contingency of cocaine administration (e.g., DA within the substantia nigra was decreased in the yoked-cocaine animals compared to yoked-vehicle, but cocaine self-administering animals had equivalent turnover to yoked-vehicle animals). This same group utilized the same self-administration and yoking procedures to access neurotransmitter turnover by pulse labeling for neurotransmitters via infusion of [^{14}C]glucose, [^{3}H]tyrosine and [^{3}H]tryptophan through the animals' catheters one hour into the 31st stable self-administration session, followed by sacrifice 60 to 90 minutes later (Smith, Koves, & Co, 2003). Self-administering compared to yoked-cocaine animals were found to have an increase in turnover of DA within the NAc, VP, septum, lateral hypothalamus and a decrease in the brain stem; a decrease in 5-HT within the medial hypothalamus and substantia nigra; a decrease in somatosensory cortex and lateral thalamus NE; a decrease in NAc, somatosensory cortex and ACC GABA; a decrease in

glutamate within NAc, VP, posterior cingulate cortex, entorhinal subicular cortex, brain stem, raphe, and visual cortex; and a decrease in entorhinal subicular cortex and brain stem aspartate turnover.

Protein expression within many brain regions is also differentially affected by the contingency of cocaine administration. Yoked-cocaine rats sacrificed 18 hr after the 30th yoked-cocaine session (0.33 mg/infusion; 6 hr/day, 7 days/week) exhibited decreased D1 receptor binding of [^{3}H] SCH23390 (indicating decreased receptor levels) within the NAc compared to self-administering and yoked-vehicle animals (Montis, Co, Dworkin, & Smith, 1998). Consistently, D2 receptor expression within the CP and NAc were reduced within yoked-cocaine animals compared to cocaine self-administering and yoked-saline rats as measured by *in vitro* quantitative autoradiography following 5 weeks of cocaine experience (0.3 mg/kg; 2 hr/day sessions; 5 sessions/week) (Stefański et al., 2007). Interestingly, when D2 receptor mRNA was assessed in the VTA by in situ hybridization, only rats actively self-administering cocaine showed increased expression. Fumagalli et al. (2013) examined for differences in BDNF protein expression within the NAc of rats either self-administering cocaine (0.25 mg/infusion; 2 hr sessions/day, 7 days/week; 14 total days), yoked-cocaine or yoked-saline immediately following, 24 hrs or 7 days after the last drug administration session. They found BDNF levels to be significantly elevated in the NAc of both yoked-cocaine and self-administering animals, with the yoked-cocaine animals expressing significantly greater levels than self-administering animals when levels were examined immediately following the last drug session but not at 24 hrs withdrawal.

Gene expression within hippocampus (HPC), NAc, CP, and BLA are also affected by active cocaine administration differently than passive drug experience (Mutschler, Miczek, &

Hammer, 2000). Specifically, rats trained to administer 0.5 mg/infusion of cocaine over 8 days, followed by a single 16 hr binge session and sacrificed either the day of the binge session (0-withdrawal), 24 hr or 14 days following the binge session showed decreased expression of zif268 mRNA within the HPC and VTA at the 0-withdrawal time point, and decreased in zif268 mRNA expression within the NAc, CP, HPC and BLA at the 14 day withdrawal time point compared to yoked-cocaine and saline controls. Zif268 mRNA expression was also found to be differentially expressed within the ACC, lateral and basal amygdala, NAc, and VTA dependent upon the contingency of cocaine infusions and whether discrete cues were paired with contingent cocaine infusions (Thomas, Arroyo, & Everitt, 2003). Specifically, rats were divided into four groups: **paired** (light paired with active lever depression) and **unpaired** (intermittent tone played with active lever depression) cocaine self-administration (0.25 mg/infusion; 2 hr/session; avg 10 consecutive sessions to stable responding), and **yoked-cocaine** and **yoked-saline** groups (received same light/cocaine pairings as the **paired** group). Following stable self-administration, rats were kept in their home cages for 3 days without drug, followed by a short (5 x 1 sec light presentations) exposure to light cues in the operant chambers without lever access and sacrificed one hour later. The **yoked-cocaine** group exhibited enhanced zif268 mRNA within the ACC, ventral orbital cortex, lateral orbital cortex, lateral and basal amygdala, NAc, and VTA compared to the **unpaired** cocaine self-administration group and **yoked-saline** groups. Additionally, increased expression was seen in the **yoked-cocaine** group compared to the **paired** cocaine self-administration group for the ACC, amygdala, NAc and VTA regions.

Clearly, the method of cocaine administration has an effect on various aspects of neuroplasticity and the conclusions drawn regarding the effects of cocaine or potential route

causes of drug addiction would be drastically different based on these experimental procedures. Therefore, in the following series of studies, cocaine self-administration is utilized to better model human self-administration and hopefully the underlying changes that result from contingent drug self-administration.

1.6.2 Length of self-administration session

The length of the self-administration session has been implicated in the progression from drug use to addiction, with longer sessions argued to better model this feature of the human condition (Ahmed & Koob, 1998; George et al., 2008). Specifically, rats given 6-hr versus 1-hr access to cocaine self-administration show an escalation of intake over time and only a short-lived return to baseline administration levels following abstinence before a reinstated escalation of intake (Ahmed & Koob, 1998). Direct comparisons of these two-session lengths reveals distinct neuroplasticity within the mPFC (Ben-Shahar et al., 2007, 2009). Rats were allowed to self-administer either cocaine (0.25 mg/infusion) or saline over 7 days of training (1-hr sessions), after which the cocaine self-administering group was split into 6-hr cocaine access and 1-hr cocaine access groups and all three groups were trained for an additional 10 days followed by 1, 14 or 60 days of withdrawal in their home-cages prior to sacrifice and immunoblotting procedures (Ben-Shahar et al., 2009). Within the mPFC at 1 day of withdrawal, select decreases in mGluR1 and mGluR5 protein expression were exhibited in the 1-hr access group compared to saline and 6-hr access groups, and homer 1b/c was increased in the 6-hr access group compared to the other two groups. At 14 days of withdrawal, only the 1-hr access group exhibited a decrease in homer 1b/c expression and NR2b was increased in the 6-hr group alone; at the 60-day withdrawal time point the only change was an elevation in NR2a within 6-hr access group.

In a similar cocaine self-administration and withdrawal experimental design, Ben-Shahar et al. (2007) demonstrated via receptor-autoradiography decreased NMDA, and increased D2 receptor densities within the mPFC of 1-hr access animals at a 20-minute withdrawal time point compared to saline controls, and increased expression of D1 receptors within the NAc shell of 6-hr access animals. Moreover, at 14 days withdrawal, there was a decrease in D2 receptors within the NAc shell of 1-hr animals compared to saline controls, an effect persisting into 60 days of withdrawal.

Given the data presented revealing distinct neuroplastic changes within the mPFC of rats undergoing 6-hr access conditions and the impact of self-administration procedures over experimenter administered cocaine, the current series of experiments utilizes an extended access (6-hr) cocaine self-administration model to gain insight to the molecular consequences of excessive cocaine intake of relevance to the neurobiology of cocaine addiction. Additionally, if cocaine is altering non-neuronal cells, we believe we would be more likely to detect such changes in a model of robust-drug taking than shorter-access conditions.

1.7 Summary

In summary, the data to date has suggested an integral role of the PFC underlying the reinforcing effects of cocaine and cocaine-induced behavioral and cognitive deficits. The similar afferent and efferent connections of the vmPFC in human and rodent with other brain regions implicated in addiction, cytoarchitectonic features, and related behaviors, make this region ideal for comparing the human condition with an animal model that can be more easily and reliably manipulated. The human literature is fraught with confounding subject factors, such as age of first drug use, duration of use, concurrent use of additional addictive substances, other environmental insults and/or physical and/or mental abuse, poverty,

coexisting mental disorders, etc, making it impossible to derive cause-effect relations between cocaine-taking and abnormal non-neuronal physiology. Given the limited evidence for a role of glial cells in addiction, and that experimental methods examining specifically for a glial role have demonstrated their influence on various aspects of cocaine-taking and seeking, it is imperative to further define the effects of cocaine on each of these cell types. Moreover, because these cell types express receptors able to sense the environment and have fundamental roles in maintaining the extracellular milieu, health of the brain, signaling efficiency, and so on, ignoring their role precludes any advancement in our neurobiological understanding of the etiology of cocaine addiction and related disorders, and impedes therapeutic progress for disease management.

1.8 Specific Aims

My dissertation seeks to address the following specific aims:

(1) *Characterize glial cell changes in protein and related-mRNA expression in rats with extended access to cocaine self-administration*. Based on previous clinical and preclinical findings of reduced OL myelin integrity, altered astrocytic glutamate transporters, and activated immune responses, I hypothesize that extended access to cocaine self-administration will decrease OL-specific proteins and related-mRNA expression levels, alter levels of astrocyte-specific proteins, and increase the levels of microglial markers within the vmPFC, relative to cocaine-naïve animals.

(2) *Characterize the behavioral consequences of decreased myelin basic protein (MBP) expression on a variety of PFC-dependent tasks, without cocaine treatment*. Based on the previously discussed importance in myelin integrity and

normal expression of specific myelin proteins on normal OL and neuronal function, I hypothesize that reducing MBP expression within the vmPFC will disrupt normal vmPFC processing resulting in decreased working memory function and enhanced impulsive choice, relative to animals without experimental manipulation of MBP within the vmPFC.

(3) *Determine the functional significance of decreased myelin basic protein on IV cocaine acquisition, maintenance, dose, extinction, and reinstatement*. Based on the role of the vmPFC on acquisition of cocaine self-administration, extinction and reinstatement tests, I hypothesize that reducing MBP expression within the vmPFC will result in enhanced acquisition, delayed attainment of extinction criterion and augmented cocaine-seeking under various reinstatement procedures compared to animals to animals without experimental manipulation of MBP within the vmPFC.

Chapter 2 Extended access to cocaine results in decreased oligodendrocyte-specific protein expression within the vmPFC

2.1 Introduction

As discussed in the General Introductiongeneral introduction, cocaine addiction is a chronic disorder characterized by impulsivity, compulsive drug-seeking and -taking, and serious negative consequences (Camí & Farré, 2003; Jentsch & Taylor, 1999). High rates of relapse, even after an extended period of abstinence, and the lack of effective treatment options impede lasting recovery. Persistent cocaine-induced neuroadaptations that increase the probability of relapse have been identified in animal models of addiction. However, only a small literature exists on the role of glial cell types in the etiology of this disease.

Several methodologies exist for studying the effects of cocaine on the CNS, common forms of administration being either passive, experimenter-administered cocaine, or active self-administration of drug. However, a wealth of data suggests that active self-administration of cocaine induces different neuroplasticity than either passive or experimenter-administered drug including: changes in neurotransmitter release and turnover, protein expression, and gene expression (see the General Introductiongeneral introduction for specific examples; Jacobs et al., 2003). Additionally, the length of the self-administration session has been implicated in the progression from drug use to addiction, with longer sessions argued to better model the escalation of drug intake in humans (Ahmed & Koob, 1998; George et al., 2008). Specifically, rats given 6-hr versus 1-hr access to cocaine self-administration show an escalation of intake overtime and only a short-lived return to baseline administration levels following abstinence before a reinstated escalation of intake (Ahmed & Koob, 1998).

A current trend in the pre-clinical study of addiction, particularly that conducted in rodents, is to incorporate a period of forced withdrawal to model periods of abstinence that occur frequently in addiction, either due to incarceration or during self-imposed cessation of

drug-taking (Fuchs, Branham, & See, 2006). Rodent studies have shown that prolonged periods of forced abstinence result in augmented cocaine-seeking behavior following re-exposure to drug-associated environments and cues. This phenomenon is termed, "incubation of craving" (Grimm, Hope, Wise, & Shaham, 2001; Tran-Nguyen et al., 1998). While we do not know if this phenomenon occurs in human cocaine addicts, an incubation of craving has been demonstrated for nicotine-, methamphetamine-, and alcohol-dependent humans (Bedi et al., 2011; Li et al., 2014; Wang et al., 2013). Given the data presented, the current study utilizes an extended access (6-hr) cocaine self-administration model to gain insight to the short- and long-term molecular consequences of excessive cocaine intake of relevance to the neurobiology of cocaine craving and relapse.

As discussed in the General Introductiongeneral introduction, the PFC is involved in regulating the motivational salience of stimuli and the intensity of behavioral responding (Jentsch & Taylor, 1999; Kalivas et al., 2005). Chronic cocaine intake results in aberrant activity of the frontal cortex, leading to impaired executive processes such as attention, impulsivity, problem-solving and decision making – cardinal features of addiction (Jentsch & Taylor, 1999; Kasperski et al., 2011; Lim et al., 2002; Moeller et al., 2005). A neural projection particularly important for drug craving and seeking includes efferent fibers from the PFC to the NAc (Kalivas et al., 2005). The PFC sends glutamatergic efferents to the NAc shell and coreA neural projection particularly important for drug craving and seeking includes glutamatergic efferent fibers from the PFC to the NAc core and shell (Kalivas et al., 2005). Cocaine experience results in amplified PFC glutamate signaling in the NAc because of reduced basal glutamatergic tone, resulting in less autoinhibition on PFC efferents and increased stimulated glutamate release (Baker et al., 2003; Kalivas et al., 2005). These

signals, combined with dopamine signaling from the VTA, lead to a reinstatement of cocaine-seeking when drug-related cues or drug-priming injections are administered in cocaine-withdrawn animals (Kalivas, Volkow, & Seamans, 2005; Kalivas et al., 2005; LaLumiere et al., 2012). Manipulations that reduce the activity of distinct PFC and NAc subregions have been shown to attenuate cocaine seeking in cocaine-withdrawn and/or behaviorally extinguished animals (Koya et al., 2009; LaLumiere et al., 2012; Ma et al., 2014). Specifically, microinjections of baclofen/muscimol to induce inactivation of the vmPFC of rats decreased responding during an extinction test conducted at 30 days of forced abstinence from long-access (6-hr/day; 10 days) cocaine self-administration, demonstrating a neutralizing effect to the incubation of craving phenomemon normally seen at this time point of forced-abstinence (Koya et al., 2009). The incubation of craving phenomenon has also been linked to distinct connections between subregions of the PFC and NAc, and the maturation of silent-synapses within the NAc over a period of protracted withdrawal, whereby the formation of new synapses are composed mainly of NMDA receptors and their maturation includes the recruitment of calcium-permeable AMPA receptors (Ma et al., 2014). Within the NAc shell, reversal of silent-synapse maturation by optogenetic induced LTD of IL efferents, results in enhanced cue-induced cocaine seeking at a 45 day withdrawal time point when rats were previously trained to self-administer cocaine (0.75 mg/mg; 40 infusions during a single overnight session followed by 5 days of 2-hr/day access). Interestingly, when the NAc core silent-synapse maturation was reversed by the same optogenetic induced LTD of PL afferents, cue-induced cocaine craving at 45 days withdrawal was decreased to an even lower level than seen on day 1 of withdrawal. Therefore, the projections from the PFC to NAc subregions provide a neural circuit where malfunction in glial cell types could have an

impact on both intra- and intercellular communication resulting in excessive drug taking and seeking behavior.

As reviewed in the general introduction, glial cells have been shown to have essential roles in the regulation of neurotransmission, conduction of nerve impulses, neurotransmitter metabolism (including release), supply of energy metabolites, development and formation of synaptic connections, and healing damage (Crews et al., 2011; Miguel-hidalgo, 2009; Ullian et al., 2001). Astrocytes, OLs, and microglia also express receptors for many neurotransmitters and peptides (e.g., DA, glutamate, & GABA), making these cell types sensitive to neurochemical changes resulting from the administration of cocaine (Volterra & Meldolesi, 2005). Indeed, cocaine administration and withdrawal from cocaine both have been shown to increase the expression of GFAP, along with significant modifications in astrocyte number, size and shape complexity, within the dentate gyrus, PFC and NAc (Bowers & Kalivas, 2003; Fattore et al., 2002). Consistently, alterations in the cystine/glutamate exchanger, which permits astrocytic control over basal extracellular glutamate levels, alters glutamate signaling within the NAc and mediates reinstatement of drug seeking (Baker et al., 2003).

While astrocytes do not show classical electrical signaling characteristics of neuronal activation (i.e. action potentials), they have a form of calcium signaling initiated both spontaneously and through receptors on the cell surface (Araque, 2008). Calcium waves can be generated through stimulation of ionotropic receptors, allowing direct entry of calcium from the extracellular space, or through stimulation of metabotropic receptors, which activate IP3-dependent pathways (Káradóttir & Attwell, 2007). These calcium waves can propagate through the exchange of IP3 via gap junctions composed of connexin 30 and 43 proteins

(Cx30 and Cx43, respectively); hemichannels composed of these proteins are known to release ATP into the extracellular space leading to further calcium wave spread among neighboring astrocytes (Perea & Araque, 2002; Stout, Costantin, Naus, & Charles, 2002). Based on the existing literature of cocaine-mediated astrocyte alterations in cystine/glutamate exchange and astrocyte calcium signaling, we examined for changes in astrocyte specific proteins. Glutamine synthetase (GS), Cx30 and Cx43 expression levels were measured to determine whether glutamate recycling and intracellular communication were perturbed following long access to cocaine self-administration in short-term withdrawal and during long-term withdrawal during which animals exhibit incubated cocaine-seeking behavior.

As previously mentioned, chronic cocaine use in humans has been consistently correlated with reduced white matter volume (Bartzokis et al., 2002; Lim et al., 2002; Moeller et al., 2007; Moeller et al., 2005). This could reflect a disruption in the connectivity and efficiency of signaling within and between the PFC and underlying reward circuitry, and possibly account for the cognitive and behavioral control deficits leading to the abnormal drive for cocaine. Microarray analyses of post-mortem tissue from human cocaine addicts revealed reductions in the expression of myelin-related genes including myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte basic protein (MOBP), and myelin and leukocyte T-cell differentiation protein 2 within the NAc (Albertson et al., 2004). The abundance of MBP transcripts alone was sufficient to classify subjects as cocaine abusers with 80% accuracy (Albertson et al., 2004). Therefore, OL-specific proteins relating to myelin integrity were also examined for cocaine-induced decreases in expression during withdrawal from long access cocaine self-administration.

Microglial cell activation has been linked to depression, a common co-morbid affective disorder with addiction (Crews et al., 2011). Alcohol, methamphetamine, depression and stress all have been shown to enhance innate immune gene induction within microglia (Crews et al., 2011). Stress has also been widely used as a mechanism to initiate relapse to drug seeking within cocaine experienced animals, and both stress and drugs of abuse lead to microglial activation that promotes microglial proliferation (Bossert, Marchant, Calu, & Shaham, 2013; Graeber, 2010). There is evidence that repeated alcohol use leads to cycles of immune gene activation which correlates with a decrease in positive affect and an increase in anxiety-like behaviors in rodents; common features seen in animals following a period of withdrawal from abused substances (Crews et al., 2011). Additionally, cells exposed to cocaine in culture and rats self-administering cocaine result in enhanced inflammation related-transcripts and markers of oxidative stress (Ahmed et al., 2005; Clark et al., 2013; Costa et al., 2013; Jang et al., 2014; Piechota et al., 2010). Microglia and macrophages express CD11b, an integrin important in migration, adhesion, and phagocytosis and a marker often used for identifying these cells types (Davis & Carson, 2013). To examine possible changes in microglial cell numbers following cocaine self-administration, the levels of microglial specific protein, CD11b, were measured.

It was hypothesized that long-access to cocaine self-administration would lead to changes in glial protein expression within the NAc and PFC. Additionally, it is hypothesized that varying withdrawal periods would produce differential effects on these protein changes, which might relate to the incubation of craving phenomenon.

2.2 Methods

2.2.1 Animals

Adult (275-325 g) male Sprague-Dawley rats (Charles River Laboratories, Hollister, CA) were housed in a colony room controlled for temperature (25 °C) and humidity (71%), under a 12-hr reverse cycle room (lights on at 20:00 hrs). Animals were given *ad libitum* access to food and water, except during lever response training for food reinforcement. Animals were allowed to acclimate to the colony room for three days following arrival then were handled daily till self-administration procedures were complete. All animal procedures were in accordance with the guidelines of the National Institutes of Health *Guide for Care and Use of Laboratory Animals* (Publication 80-23, revised 1996) and were reviewed and approved by the University of California Santa Barbara, Institutional Animal Care and Use Committee.

2.2.2 Lever Response Training

To encourage lever-pressing behavior for saline and cocaine, animals were first trained to lever press for food reinforcement using identical methods to those described by our group (Ben-Shahar et al., 2013; 45 mg pellets; Noyes, Lancaster, NH) on a FR1 schedule in sound-attenuating operate conditioning chambers (30 x 20 x 24 cm high; Med Associates Inc., St. Albans, VT) during either an overnight training session or during daily 1-hr training sessions (depending on the availability of operant chambers). Animals were food deprived 24-hr prior to the initiation of training and maintained on a restricted diet for the duration of food training. The operant chambers contained two retractable levers with a stimulus light located above each lever, a food pellet dispenser located outside the operant chamber, a food trough between the levers, a house light on the opposite wall to the levers, a speaker

connected to a tone generator (ANL-926, Med Associates), and a fan to provide ventilation and mask extraneous noise. During the session, pressing the active lever resulted in the delivery of a food pellet and pressing the inactive lever had no programmed consequence. Animals that failed to reach the criterion of a minimum of 200 responses on the active lever during the overnight session, or a minimum of 100 responses on the active lever during two consecutive 1-hr sessions, received additional food training sessions until criterion was reached. Following successful acquisition of lever-pressing behavior (1 to 2 overnight sessions or 5-7 days of 1-hr daily sessions), food was freely available in the animal's homecage for the remainder of the study.

2.2.3 Surgery

Following lever response training, animals underwent surgical implantation of chronic IV catheters as previously described by our group (e.g., Ben-Shahar et al., 2013). Under ketamine/xylazine anesthesia (56.25 and 7.5 mg/kg, respectively, administered intramuscularly (IM); Abbott Laboratories, North Chicago, IL), animals were implanted with a chronic silastic catheter (13 cm long; 0.3 mm inner diameter, 0.64 mm diameter; Dow Coming Corporation, Midland, MI) into the right jugular vein. Each catheter ran subcutaneously around the shoulder to back where it was secured to a threaded 22-gauge metal guide cannula (Plastics One, Roanoke, VA), which surfaced from the midline of the animal's back between the shoulder blades. A plastic plug covered the open end of the cannula to protect from contamination, and a smooth metal nut was secured around the threading of the cannula to prevent animals from chewing the catheter port. The cannula was held in place with a small square of Bard Mesh (C. R. Bard Inc., Cranston, RI) to which it was cemented, and the mesh was laid flat subcutaneously on the animal's back. Banamine (2

mg/kg, subcutaneous, Butler Schein Animal Health, Dublin, OH) was administered to treat post-surgical pain. All animals were allowed a minimum of 5 days for recovery and IV catheter patency was maintained by flushing daily with 0.1 ml of sterile heparin (60 IU/ml; Sagent Pharmaceuticals, Schaumburg, IL) and timentin/saline (100 mg/ml; GlaxoSmithKline, Research Triangle Park, NC) or 0.1 Gentimicin/Cefazolin (1 mg/ml, Butler Schein Animal Health, and 5 mg/ml, Westward Pharmaceuticals, Eatontown, NJ, respectively).

2.2.4 Self-Administration Training

Following surgical recovery, animals (n=10-15 per group at the start of each experiment) were trained to self-administer IV cocaine (0.25 mg/0.1 ml/infusion; National Institute on Drug Abuse, Bethesda, MD) during daily 6-hr sessions, or saline (0.1 ml/infusion) during 1-hr or 6-hr sessions, on a FR1 schedule of reinforcement. At the beginning of each session, the animal's catheter was connected to a motorized pump (located outside of the sound attenuated chamber) via a liquid swivel as previously described (e.g., Ben-Shahar et al., 2013). Active lever responses elicited a 5-sec activation of the infusion pump and a 20-sec presentation of a visual (white light) and auditory (tone generator, 70 dB, 2 kHz) stimulus combination, during which additional responses had no consequences. Responses on the inactive lever were recorded but had no programmed consequences. Animals were trained to self-administer cocaine or saline for 10 sessions. To prevent overdose, the number of cocaine infusions permitted during the first two training sessions were limited at 100 (session 1) and 150 (session 2) and animals failing to meet self-administration criterion (minimum of 50 infusions/6-hr session for the last 3 days of training) were excluded from the study.

Two saline control groups were included in the current immunoblotting study to replicate previous results showing no protein expression differences between extended (6-hr) with short (1-hr) access to IV saline and to extend these findings to glial-specific proteins (Ben-Shahar et al., 2013; Ben-Shahar, Ahmed, Koob, & Ettenberg, 2004). In Expts 1 and 2, cocaine 6-hr and saline 6-hr animals were compared for protein expression alterations at 3 and 30 days withdrawal following a cue-seeking test or untested, respectively. Expt 3a compared saline 1-hr and saline 6-hr at 3 and 30-days withdrawal following a cue-seeking test, and subsequent immunoblotting in Expt 3b only examined for 6-hr cocaine and 1-hr saline protein expression alterations during withdrawal, with or without cue-testing. All self-administration training and testing occurred during the dark phase of the light cycle. Following the 10 days of self-administration training, animals remained in the colony room for a minimum of 3 days of withdrawal.

2.2.5 Tests for Cue-elicited Cocaine-seeking

In Expts 1, 3a, and 3b, saline and cocaine self-administering animals were subjected to a 2-hr test for cue-elicited cocaine seeking under extinction conditions at either 3 or 30 days of withdrawal from the last day of cocaine self-administration (Rats in Expt 3b were only tested at the 3 day withdrawal time point). The duration of this test was chosen to capture potentially relevant changes in receptor protein expression from the Ben-Shahar et al., 2013 publication, however, results show this test does not significantly affect MBP expression, the main protein of interest to this dissertation (see results and Table 6). Animals were tethered as with self-administration training and lever-press responses were recorded. Responses on the active lever elicited the tone + light compound stimulus previously paired

with cocaine/saline infusions, but no infusions were administered during this test. Responses on the inactive lever had no programmed consequences, but were recorded.

2.2.6 Immunoblotting

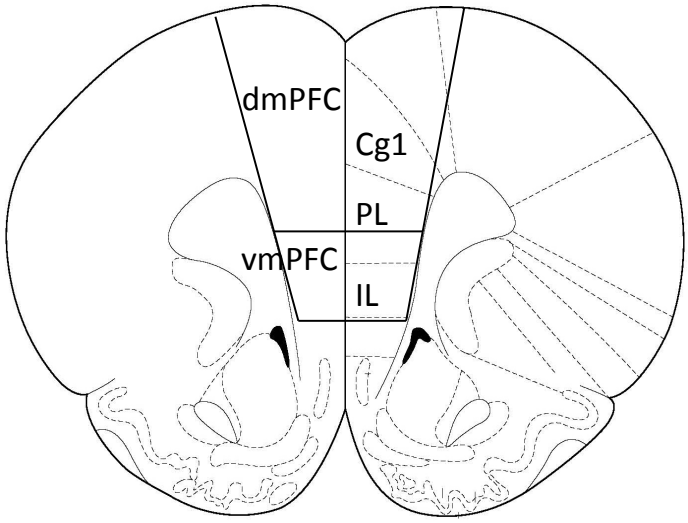
At 3 or 30 days of withdrawal or immediately upon completion of the 2-hr cue test under extinction conditions at these two time points, animals were killed by rapid decapitation (Expt 1-3a), or anesthetized with 4% isoflurane (Expt 3b) and, as conducted by others (see Ghasemzadeh, Vasudevan, Mueller, Seubert, & Mantsch, 2009; Obara et al., 2009) the dmPFC (anterior cingulate and dorsal PL cortices), vmPFC (ventral PL and IL cortices), NAc shell and core were dissected out over ice as indicated in Figure 1 (PFC and NAc).

As described previously by our group (Obara et al., 2009), the tissue for immunoblotting in Expts 1-3a was homogenized in a medium consisting of 0.32 M sucrose, 2mM EDTA, 1% w/v sodium dodecyl sulfate, 50 μM phenyl methyl sulfonyl fluoride, and 1 μg/ml leupeptin (pH=7.2) and 1 mM sodium fluoride, 50 mM sodium pyrophosphate, 20 mM 2-glycerol phosphate, 1 mM *p*-nitrophenyl phosphate, 1 mM orthovanadate, and 2 μM microcystin LR were included to inhibit phosphatases. Samples were then subjected to low-speed centrifugation at 10,000 g for 20 min. Protein determinations utilized the Bio-Rad DC protein assay (Bio-Rad, Hercules, CA), according to the manufacturer's instructions and homogenates were stored at -80 °C. For Expt 3b, which involved tissue processing for both immunoblotting and qRT-PCR techniques, the methods are described in *section 2.2.7* below.

For immunoblotting, protein samples (20 μg/lane) were subjected to SDS-polyacrylamide gel electrophoresis and non-reduced when run on Bis-Tris gradient gels (4-12%) and Tris-Glycine gradient gels (4-20%) (Invitrogen, Carlsbad, CA) or reduced when

run on Tris-Acetate gradient gels (3-8%) (Invitrogen). Bis-Tris gradient gels were used for separation of Cx30, Cx43, GS, MBP, MOG, and PLP, Tris-Glycine gradient gels for CLN, Cx32, and MAG and, Tris-Acetate gradient gels were used for separation of CD11b, CNP, Cx47, NF155, NG2+, and UGT8. Proteins were transferred to wet polyvinylidene difluoride (PVDF, BioRad) membranes, and were pre-blocked with phosphate-buffered saline containing 0.1% (v/v) Tween-20 and either 5% (w/v) bovine serum albumin (for CD11b,

A.



B.

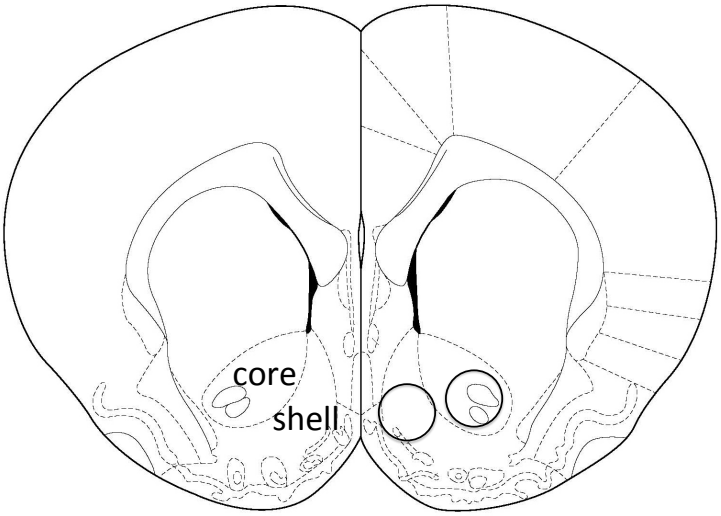


Figure 1. Pictogram depicting the loci of dissection to obtain tissue for immunoblotting and qPCR. Whole brains were sliced into 2 mm sections using a brain mold. Tissue was then collected by gross dissection from the vm- and dm-PFC (A) using small forceps. The NAc shell and core (B) were extracted using a 16 ¾ gauge punch.

Cx30, Cx43, Cx47, MBP, MOG, and NG2+), 1.5% normal horse serum or 5% (w/v) nonfat dried milk powder (for all other proteins) for no less than 2-hr at room temperature or overnight at 4 °C, followed by a 2-hr incubation with primary antibodies. The following primary antibodies were used: rabbit- anti-CD11b (Novus Biologicals, Littleton, CO; 1:500 dilution); mouse-anti-CNPase (Millipore, Temecula, CA, 1:500 dilution); rabbit-anti-Cx30 (Invitrogen, Carlsbad, CA; 1:500 dilution); mouse-anti-Cx32 (Chemicon International, Millipore); mouse-anti-Cx43 (Millipore; 1:1000 dilution); rabbit-anti-Cx47 (Invitrogen; 1:500 dilution); rabbit-anti-GS (Thermo Fisher Scientific, Rockford, IL; 1:10,000 dilution); mouse-anti-myelin associated glycoprotein (Millipore; 1:1000 dilution); rabbit-anti-MBP (GenWay Biotech, San Diego, CA; 1:1000 dilution); goat-anti-MOG (LifeSpan Biosciences, Seattle, WA; 1:1000 dilution); mouse-anti-Neurofascin (R&D Systems, Minneapolis, MN; 1:500 dilution); rabbit-anti-NG2 Chondroitin Sulfate Proteoglycan (Millipore; 1:1000 dilution); rabbit-anti-oligodendrocyte specific protein (claudin-11 or CLN; Abcam, Cambridge, MA; 1:1000 dilution); mouse-anti-PLP (Millipore; 1:1000 dilution); rabbit-anti-UGT8 (Proteintech Group Inc., Chicago, IL; 1:500 dilution). Membranes were washed, incubated with a horseradish peroxidase-conjugated anti-rabbit secondary antibody (Millipore; 1:40,000-1:100,000 dilution), anti-mouse secondary antibody (Jackson Immuno Research Laboratories, West Grove, PA; 1:40,000 dilution), or anti-goat secondary (Jackson Immuno Research Laboratories, West Grove, PA; 1:20,000 dilution) for 90 min at room temperature, washed again, and immunoreactive bands were detected by enhanced chemiluminescence using either ECL Plus (Amersham Biosciences, GE Healthcare Life Sciences, Pittsburgh, PA) or Pierce SuperSignal West Femto (Thermo Fisher Scientific, Rockford, IL). A rabbit anti-calnexin polyclonal primary antibody (Stressgen, Victoria, BC;

1:1000 dilution) was also used to index protein loading, transfer, and as a reference protein for comparison. The levels of immunoreactivity for all proteins were quantified using Image J (NIH, Bethesda, MD).

Each immunoblotting gel contained samples from each of the four treatments so comparisons could be made across drug treatment and withdrawal time-point or cue-testing experience. The goal of Expt 1 was to determine whether or not the incubation of cocaine craving phenomenon that occurs with the passage of time during withdrawal (WD) was correlated with altered expression of glial-associated proteins. Thus, all rats in Expt 1 were subjected to our cue test procedure to confirm the presence of incubated responding and the following four groups were compared for protein expression: SAL6h-3 days WD, SAL6h-30 days WD, COC6h-3 days WD and COC6h-30 days WD. Expt 2 was conducted to determine whether the effects of cocaine self-administration history upon protein expression reflected merely a pharmacodynamic response to cocaine. To address this question, rats were left undisturbed in their home cages during withdrawal and did not undergo cocaine-seeking test procedures. I compared protein expression across the following 4 groups: SAL6h-3 days WD, SAL6h-30 days WD, COC6h-3 days WD and COC6h-30 days WD. Expt 3a directly compared 1-hr and 6-hr saline self-administration at 3 and 30 days withdrawal with a 2-hr cue-seeking test prior to sacrifice on the same glial-specific proteins. I compared the protein expression across the following 4 groups: SAL1h-3 days WD, SAL1h-30 days WD, SAL6h-3 days WD, SAL6h-30 days WD. As the results from Expt 3a failed to indicate significant group differences in protein expression between rats allowed short vs. extended access to saline (see Results below), I opted to employ short-access saline self-administration procedures for Expt 3b to accommodate for the number of animals required for the direct

comparison of protein expression between cue-tested and test-naïve subjects. Secondly, in Expt 3b, I directly compared the tissue from animals subjected to our cue-testing procedure with tissue derived from animals left undisturbed in the home cage to confirm whether or not differences existed in the magnitude of saline-cocaine differences in protein expression revealed in Expts 1 & 2. Moreover, as the data from Expts 1 & 2 failed to indicate a main effect of, or interaction with, the Withdrawal factor (see Results below), all tissue was collected for Expt 3b at 3 days withdrawal only. Expt 3b compared protein expression in the following four groups at 3 days WD: SAL1h-cue-tested, SAL1h-untested, COC6h-cue-tested, and COC6h-untested.

The immunoreactivity of proteins bands of interest for each sample was first normalized to their respective calnexin immunoreactivity. Then the relative change in protein immunoreactivity was expressed as a percent of the average immunoreactivity of SAL1h-3 days WD (or SAL6h-3 days WD) group on the corresponding gel (n=3-4/gel). This latter procedure was conducted to reduce inter-membrane variability resulting from different chemiluminescence and developing procedures.

2.2.7 Quantitative Real-time Polymerase Chain Reaction (qRT-PCR)

In Expt 3b, animals were anesthetized with 2-3% isoflurane, decapitated, and the vmPFC was dissected out over ice as described for immunoblotting. This region was selected for study as it exhibited the most robust saline-cocaine differences in protein expression as determined by immunoblotting. Tissue samples were homogenized in QIAzol lysis reagent (Qiagen, Louisville, KY), using the Qiagen TissueRuptor, and separated into aqueous and organic phases by the addition of chloroform and centrifugation at 12,000 *g*. The aqueous phase was removed and purified for total RNA following the manufacturer's instructions

using the RNeasy Lipid Tissue Mini Kit (Qiagen), and was tested for quantity and purity using a Nanodrop 1000 spectrometer (Thermo Scientific). A total of 500 ng of RNA was used for cDNA synthesis via reverse transcription PCR (RT-PCR) using the iScript cDNA Synthesis Kit according to manufacturer's instructions (BioRad); aliquots of each sample for RT-PCR set were run without reverse transcriptase (aka NRT) as a negative control. The resulting cDNA was diluted first to 100 ng/μl for a stock solution, and then to 25 ng/μl to be used in qPCR analyses. qPCR was performed using the Sso Advanced Universal SYBR Green Supermix (BioRad) with a 20 μl reaction volume; this reaction mixture consisted of 10 μl of the SYBR Green supermix, 6 μl biological grade water, 2 μl of 25 ng/μl cDNA, and 2 μl of 0.01 nmol/μl forward and reverse primer. The reaction mixture was subjected to various cycling protocols in a BioRad CFX96 thermocycler depending on the target gene (Table 3). Oligonucleotide primer pair sequences were referenced from Padhi & Pelletier (2012), and generated by Eurofins MWG Operon (Huntsville, AL), except the reference gene Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which was developed by Integrated DNA Technologies (IDT, Coralville, IA); primer details and PCR conditions are presented in Table 3. All primer conditions were initially tested for specificity via a melting curve analysis (65 ^{o}C to 95 ^{o}C, in 0.5 ^{o}C increments every 3 sec) and were visualized on a 1% agarose gel with 50 μl of ethidium bromide. Each target sample was set to run in a technical duplicate for the target gene and with the GAPDH control gene. Negative controls consisted of NRT samples from each RT-PCR reaction, as well as a reaction devoid of cDNA (aka NTC). A serial dilution of 1:3 was done on 100 ng/μl control cDNA and run to measure the standard curve and efficiency of the reaction mixture; only plates with 90-110% efficiency were used for analysis.

Primer name	Accession	Primer sequence	Amplicon size (bp)	Cycles	Denature Temp & Time	Anneal Temp & Time	Extension/Read Temp & Time
MBP_isoform1	NM_001025291	F-TCCCGGGCTGTGCCACATGT	200	60	95C 5sec	62C 15sec	72C 25sec
		R-CCTCGGCCCCCCAGCTAAAT					
MBP_isoform2	NM_001025292	F-GGCTCTGGCAAGGACTCACA	182	60	95C 5sec	60C 15sec	72C 25sec
		R-GCCCCCCAGCTAAATCTGCT					
MBP_isoform3	NM_001025293	F-CTCTGGCAAGGTACCCTG	263	59-62 C did not yield specific amplification			
		R-CTCTTCCTCCCCAGCTAAAT					
MBP_isoform4	NM_001025294	F-GGCTCTGGCAAGGACTCACA	149	60	95C 5sec	61C 15sec	72C 25sec
		R-GCCCCCTTTCCTTGGGATGG					
MBP_isoform5	NM_001025295	F-CTCTGGCAAGGACTCACACA	185	60	95C 5sec	61C 15sec	72C 25sec
		R-CTCTTCCTCCCCAGCTAAAT					
GAPDH	NM_017008.4	F-AGAACATCATCCCTGCATCC	240	60	95C 5sec	60C, 61C, 62C, 15sec	72C 25sec
		R-AGGAGACAACCTGGTCCTCA					

Table 3. qRT-PCR primer details and running conditions. The five primer pairs relating to MBP isoforms 1-5 correspond to the 21.5 kDa, 18.5 kDa, 17 kDa, 17.3 kDa, and 14 kDa proteins, respectively. Each primer pair above contains a F-forward and R-reverse sequence to amplify RNA extracted from rat vmPFC tissue. GAPDH is used as the reference gene.

2.2.8 Immunoblotting for samples generated for RNA and protein determination

The organic phase of the tissue preparation with QIAzol lysis reagent was further processed for the extraction of total protein. Any remaining aqueous phase was removed and isopropanol added to precipitate protein followed by centrifugation at 12,000 *g.* The supernatant was removed and the protein pellet was incubated in 0.3 M guanidine-hydrochloride in 95% ethanol, followed by centrifugation at 7500 *g,* the supernatant removed and the process repeated twice. The protein pellet was air dried, re-suspended in 10 M urea and 50 mM DTT in water, broken up with a needle and incubated at room temperature for 1-hr. Samples were then incubated at 95 °C for 3 minutes, sonicated in short bursts, and centrifuged at 10,000 *g.* 5 μl of loading dye consisting of 20% glycerol, 0.004% bromophenol blue, and 0.125 M Tris HCl, was added to 20 μl of each sample, vortexed and loaded into a Bis-Tris gradient gel (4-12%; Invitrogen). Following electrophoresis, proteins were transferred to PVDF membranes and further processed via immunoblotting procedures as described earlier.

2.2.9 Statistical Analysis

Behavioral data statistical methods

Two-way ANOVAs were conducted to examine for IV Treatment (COC vs SAL) X Test (Cue-tested vs Untested) interactions regarding the number of infusions, active lever presses, and inactive lever presses. For the cue-tested animals, two-way ANOVAs for IV Treatment (COC vs SAL) X Lever (active vs inactive) were conducted. Fisher's LSD tests for multiple comparisons were used to deconstruct significant interaction effects. Significance was set at alpha = 0.05.

Immunoblotting statistical methods

Two-way ANOVAs were conducted on the data from the immunoblotting studies to examine for IV Treatment (COC 6h vs SAL 6h, or SAL 1h vs SAL 6h) X Withdrawal (3 or 30 days following the last self-administration session) interactions, or for IV Treatment X Test (Cue-tested vs Untested) interactions. Fisher's LSD tests were used to deconstruct significant interaction effects. Significance was set at alpha = 0.05.

qPCR statistical methods

Relative quantities of RNA transcripts were calculated, and normalized to SAL 1h-untested levels, then subsequently normalized to the reference gene GAPDH according to methods described in Hellemans, Mortier, De Paepe, Speleman, & Vandesompele (2007). Values were then compared in a two-way ANOVA of Treatment X Test.

All Data were analyzed with Prism 6 for Mac OS X, version 6 (GraphPad, Software Inc.).

2.3 Results

2.3.1 Expt 1: Examination of the effects of withdrawal from cocaine self-administration upon the expression of glial-related proteins in animals tested for cocaine craving during early and protracted withdrawal

As detailed in Ben-Shahar et al., 2013, rats with a 10-day history of 6-h access to IV cocaine exhibited a time-dependent intensification of responding on the active, cocaine-associated, lever when assessed under extinction conditions (i.e., an incubation of cocaine craving). Cocaine rats did not exhibit time-dependent changes in inactive lever responding, indicating that, the behavior of cocaine rats was selective for the cocaine-associated lever and therefore goal-directed.

I, thus, examined for saline-cocaine differences in the change in glial-specific protein expression in animals tested for cue-reinforced behavior at 3 versus 30 days withdrawal.

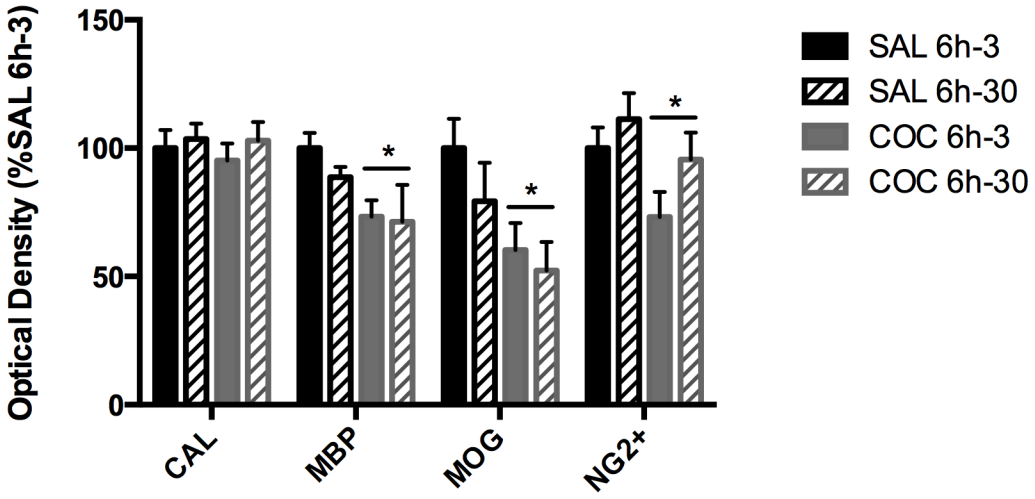
Relative to rats with extended access to saline, cocaine self-administration produced early and persistent reductions in glial-specific protein expression within distinct subregions of the PFC and NAc during withdrawal (vm- and dmPFC immunoblotting data is summarized in Table 4). A comparison of vmPFC protein levels between saline and cocaine animals at 3 or 30 days withdrawal revealed significant main effects of IV Treatment for MBP (Figure 2) [$F(1,32)=5.96$, $p=0.021$], MOG [$F(1,35)=7.67$, $p=0.009$], and NG2+ [$F(1,36)=4.52$, $p=0.040$]. The results of the ANOVA for these proteins did not indicate significant main effects of Withdrawal or significant IV Treatment X Withdrawal interactions (all p's>0.05). Analyses of the other proteins examined within the vmPFC failed to reveal any group differences in protein expression (two-way ANOVAs, all p's>0.05). Moreover, no statistically significant group differences in protein expression were found within the dmPFC for any of the proteins examined (two-way ANOVAs: all p's>0.05).

Within the NAc core, a comparison of protein levels between IV Treatment and Withdrawal revealed a significant main effect of IV Treatment for Cx43 expression only (Figure 3) [$F(1,48)=7.84$, $p=0.008$; 2-way ANOVAs, all other p's>0.05]. No statistically significant changes in protein expression were found within the NAc shell for either IV Treatment or Withdrawal for any of the proteins examined (two-way ANOVAs: p's >0.05).

2.3.2 Expt 2: Examination of the effects of withdrawal from cocaine self-administration upon the expression of glial-related proteins in the absence of behavioral testing

Due to the significant glial-protein expression changes observed within the vmPFC of Expt 1, I took a more focused approach in Expt 2 and only included the PFC subregions for this subsequent analysis. Relative to rats with extended access to saline, cocaine self-administration produced early and persistent augmentations in glial-specific protein expression within distinct subregions of the PFC. In the vmPFC, a significant interaction

A.



B.

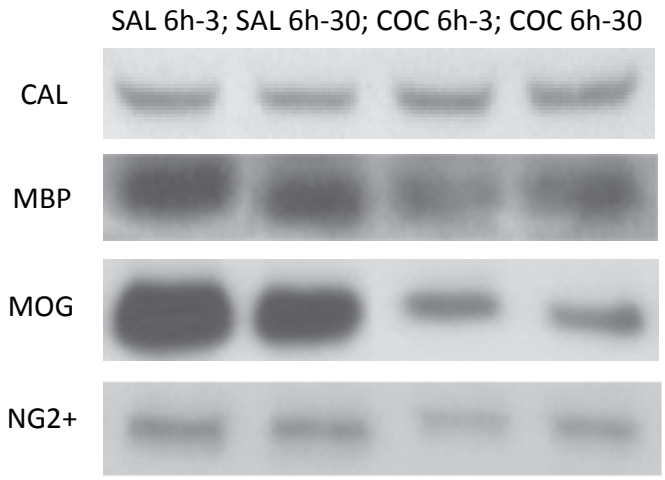


Figure 2. vmPFC immunoblotting results from Expt 1. Rats self-administering cocaine (COC) during extended access (6h) procedures and experiencing a 2h cocaine-seeking test prior to sacrifice exhibited decreased expression of MBP, MOG, and NG2+ within the vmPFC early in withdrawal (WD; 3 days), an effect that persisted into protracted withdrawal (30 days) compared to saline (SAL) self-administering controls. (A) The optical density for each protein within a treatment condition is represented as a percent of the SAL 6h-3 WD condition. (B) Representative immunoblots from SAL 6h-3 WD, SAL 6h-30 WD, COC 6h-3 WD and COC 6h-30 WD treatment conditions. Calnexin (CAL) is used as a reference protein to control for differences in gel loading, and is not significantly affected by IV Treatment or Withdrawal conditions. *$p<0.05$, IV Treatment main effect of COC versus SAL.

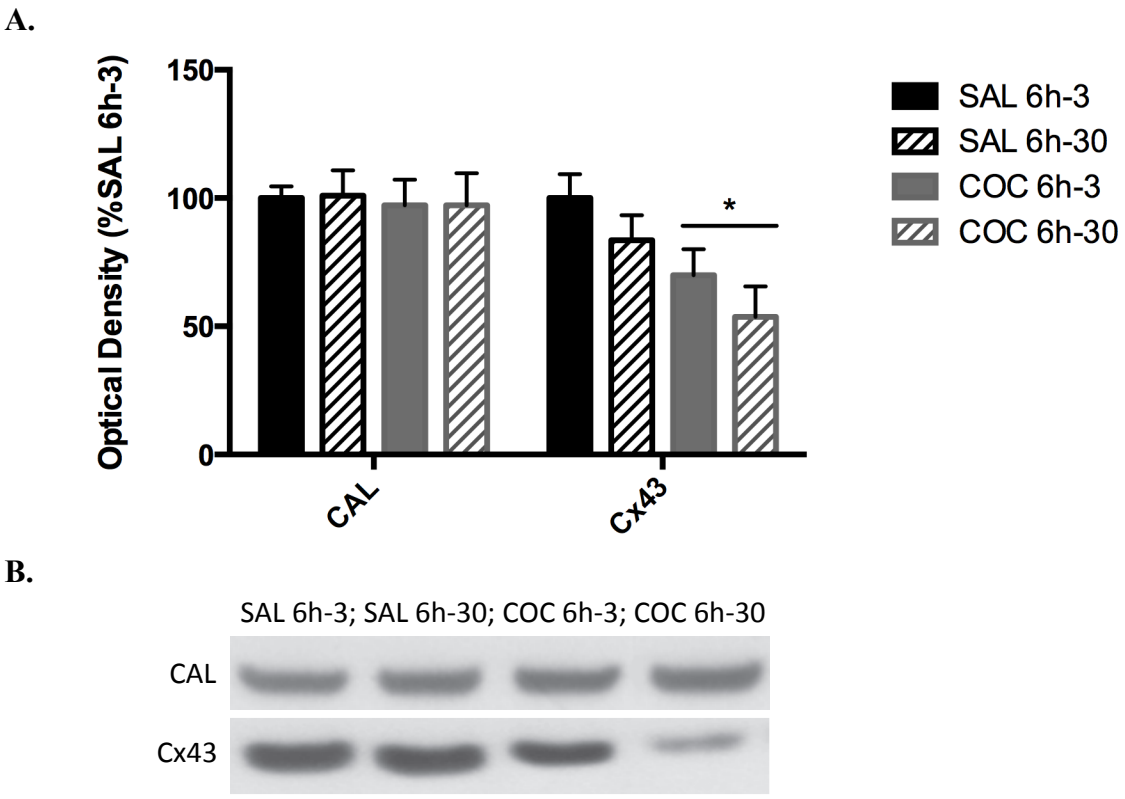
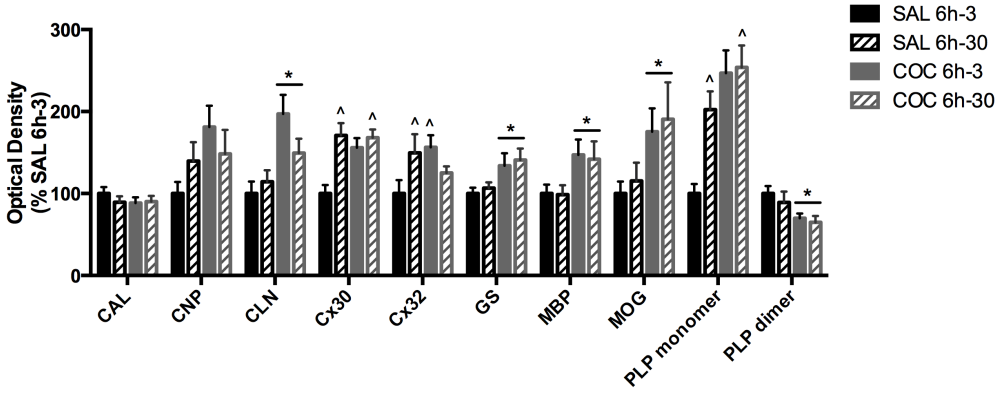


Figure 3. NAc core immunoblotting results from Expt 1. Rats self-administering cocaine (COC) during extended access (6h) procedures and experiencing a 2h cocaine-seeking test prior to sacrifice exhibited decreased expression of Cx43 within the NAc core early in withdrawal (WD; 3 days), an effect that persisted into protracted withdrawal (30 days) compared to saline (SAL) self-administering controls. (A) The optical density for each protein within a treatment condition are represented as a percent of the SAL 6h-3 days withdrawal condition. (B) Representative immunoblots from SAL 6h-3 WD, SAL 6h-30 WD, COC 6h-3 WD and COC 6h-30 WD treatment conditions. Calnexin (CAL) is used as a reference protein to control for differences in gel loading, and is not significantly affected by IV Treatment or Withdrawal conditions. *$p<0.05$, IV Treatment main effect of COC versus SAL.

between IV Treatment and Withdrawal was observed for Cx30 [F (1,51=6.01, p=0.018], Cx32 [F (1,49)=6.32, p=0.015], and PLP monomer [F (1,52)=3.96, p=0.052] (Figure 4). The PLP monomer levels exhibited by SAL 6h-3 were significantly lower than the other groups tested (post-hoc tests, p's<0.05). For both Cx30 and Cx32 expression, the SAL 6h-3 withdrawal group was significantly lower to COC 6h-3 and SAL 6h-30. For both PLP monomer and Cx30, the significant effects appear to be driven by a withdrawal effect within the SAL 6h groups, as the COC 6h groups are equivalently elevated compared to SAL 6h-3 rats at both withdrawal time-points. Cx32 expression within COC 6h groups, while not significant, appears to be elevated at the 3-day withdrawal time point and return to the levels expressed by SAL 6h-30 controls. However, Cx32 significantly increased across withdrawal within IV SAL 6h animals, indicating an interesting interaction between the normal elevation in expression seen in SAL 6h animals and cocaine experience.

In addition, I observed main effects of IV Treatment, but no main effects of Withdrawal or IV Treatment X Withdrawal interactions, for several other proteins within the vmPFC that reflected an elevation of protein expression in cocaine- versus saline-experienced animals. These included: CLN [F (1,48)=12.12, p=0.001], MBP [F (1,51)=6.79, p=0.012], MOG [F (1,50)=5.96, p=0.018], and GS [F (1,52)=7.81, p=0.007]. Interestingly, although the PLP monomer was elevated in cocaine versus saline animals, the PLP dimer expression was decreased, in a manner independent of time in withdrawal [F (1,48)=8.35, p=0.006]. These data indicate that a mere history of cocaine self-administration is sufficient to produce enduring changes in vmPFC expression of glial-specific proteins.

A.



B.

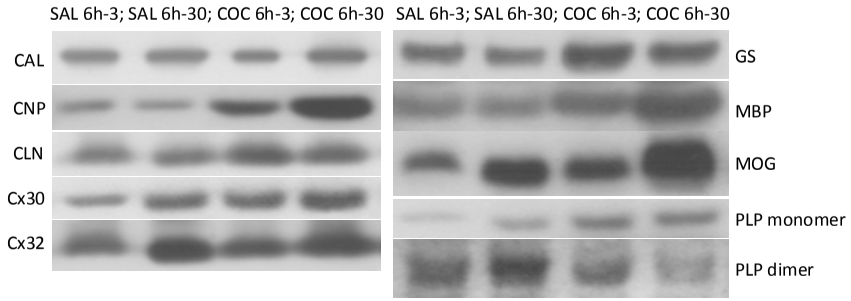
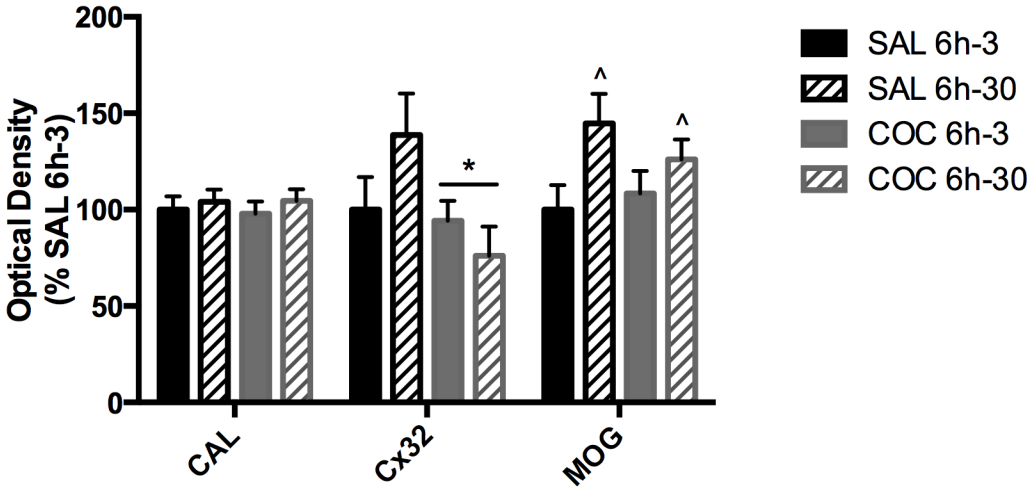


Figure 4. vmPFC immunoblotting results from Expt 2. Rats self-administering cocaine (COC) during extended access (6h) procedures and untested prior to sacrifice exhibited increased expression of: GS, CLN, MBP, and MOG within the vmPFC early in withdrawal (WD; 3 days), an effect that persisted into protracted withdrawal (30 days) compared to saline (SAL) self-administering controls. PLP dimer was found to be significantly decreased in COC animals compared to SAL. A significant interaction between IV Treatment and Withdrawal was found for Cx30, Cx32, and PLP monomer. (A) The optical density for each protein within a treatment condition is represented as a percent of the SAL 6h-3 WD condition. (B) Representative immunoblots from SAL 6h-3 WD, SAL 6h-30 WD, COC 6h-3 WD and COC 6h-30 WD treatment conditions. Calnexin (CAL) is used as a reference protein to control for differences in gel loading, and is not significantly affected by IV Treatment or Withdrawal conditions. *$p<0.05$, IV Treatment main effect of COC versus SAL; ^$p<0.05$, Interaction of IV Treatment and Withdrawal, values indicated are significantly increased compared to SAL 6h-3.

A.



B.

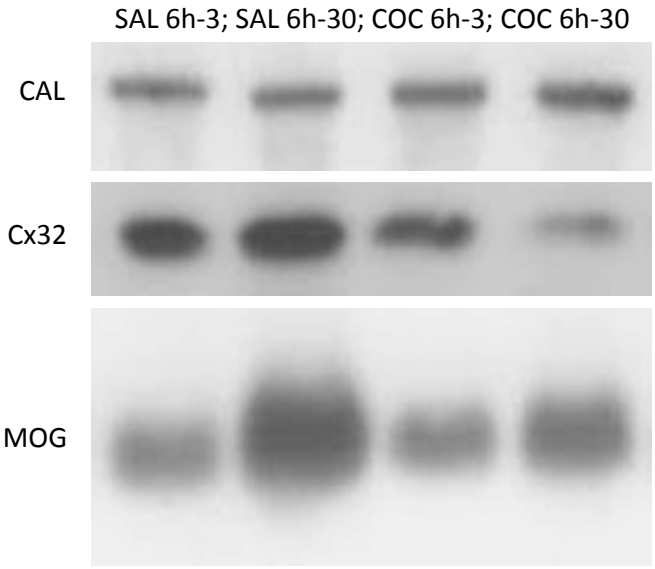


Figure 5. dmPFC immunoblotting results from Expt 2. Rats self-administering cocaine (COC) during extended access (6h) procedures and untested prior to sacrifice exhibited decreased expression of Cx32 within the dmPFC early in withdrawal (WD; 3 days), an effect that persisted into protracted withdrawal (30 days) compared to saline (SAL) self-administering controls. A significant increase in MOG expression was seen in protracted compared to early withdrawal. (A) The optical density for each protein within a treatment condition is represented as a percent of the SAL 6h-3 WD condition. (B) Representative immunoblots from SAL 6h-3 WD, SAL 6h-30 WD, COC 6h-3 WD and COC 6h-30 WD treatment conditions. Calnexin (CAL) is used as a reference protein to control for differences in gel loading, and is not significantly affected by IV Treatment or Withdrawal conditions. *$p<0.05$, IV Treatment main effect of COC versus SAL; ^$p<0.05$, Withdrawal main effect of 3 versus 30 days.

Within the dmPFC of rats left undisturbed in the home cage following self-administration experience, a few changes in protein expression were revealed by the two-way ANOVAs (Figure 5). A significant main effect of IV Treatment for Cx32 revealed a reduction in protein expression following cocaine experience [F (1,52)=4.51, p=0.039], but no main effect of Withdrawal or IV Treatment X Withdrawal interaction (p's>0.05)]. MOG levels were elevated at the 30d withdrawal time-point, regardless of self-administration experience [Withdrawal effect: F (1,49)=6.07, p=0.017; IV Treatment effect and IV Treatment X Withdrawal interaction, p's>0.05]. The findings for the dmPFC are summarized in Table 4. As the dmPFC exhibited only a few significant protein changes in Expt 2, all subsequent studies (with the exception of adolescent studies conducted in Chapter 3) focused exclusively on the vmPFC.

2.3.3 Expt 3a: Comparison of the effects of a history of short (1-h) versus extended (6-h) access to IV saline and behavioral testing upon protein expression.

In Ben-Shahar et al., 2013, rats allowed either short (1h) or long (6h) access to IV saline exhibited very similar levels of cue-reinforced responding in the absence of further IV saline administration. Surprisingly, both groups exhibited a time-dependent increase in active lever-pressing, suggestive of an incubation of responding. However, the increase in responding was not selective for the active lever and both groups exhibited a similar magnitude rise in responding on the inactive lever – an effect that may merely reflect behavioral hyper-reactivity to being returned to the operant chamber following a protracted period of absence.

In a manner consistent with behavior, no group differences were observed between short- and extended-access saline animals for expression of any of the OL-specific proteins within the vmPFC [2-way ANOVAs: no main IV Treatment effects or IV Treatment X

Withdrawal interactions, all p's>0.05]. However, we did observe a main effect of Withdrawal for the PLP-dimer, with increased protein expression at the 30-day, compared to the 3-day, withdrawal time point [PLP-dimer: $F(1,41)=12.00$, $p=0.001$]. Additionally, there was a significant decrease in CLN expression levels in SAL 1h and SAL 6h self-administering groups at the 30-day, compared to the 3-day, withdrawal time point (Figure 6) [$F(1,41)=6.01$, $p=0.019$]. No main effects of Withdrawal were significant for any of the other proteins ($p> 0.05$; Table 4).

2.3.4 Expt 3b: Replication study to test directly for an interaction between self-administration experience and behavioral testing upon protein expression

Expt 1 and 2 found opposite changes in OL-specific protein expression, and because these two experiments were conducted at different times and different investigators extracted tissue, Expt 3b sought to directly compare experimental procedures on animals run through self-administration at the same time with tissue extracted by a single investigator.

Self-administration behavior

During self-administration training, cocaine animals earned significantly greater infusions than saline controls, and the number of infusions earned were equivalent between the rats slated for cue-testing and those slated to remain in their home cage during withdrawal [IV Treatment effect: $F(1,44)=1142.00$, $p<0.0001$; no Testing effect or IV Treatment X Testing interaction, p's>0.05]. Average infusions were as follows: saline-untested: 5.03 ± 1.17, n=13; saline-tested: 4.15 ± 0.68, n=11; cocaine-untested: 94.97 ± 3.77, n=12; cocaine-tested: 97.64 ± 3.63, n=12. All behavioral data is listed in Table 5.

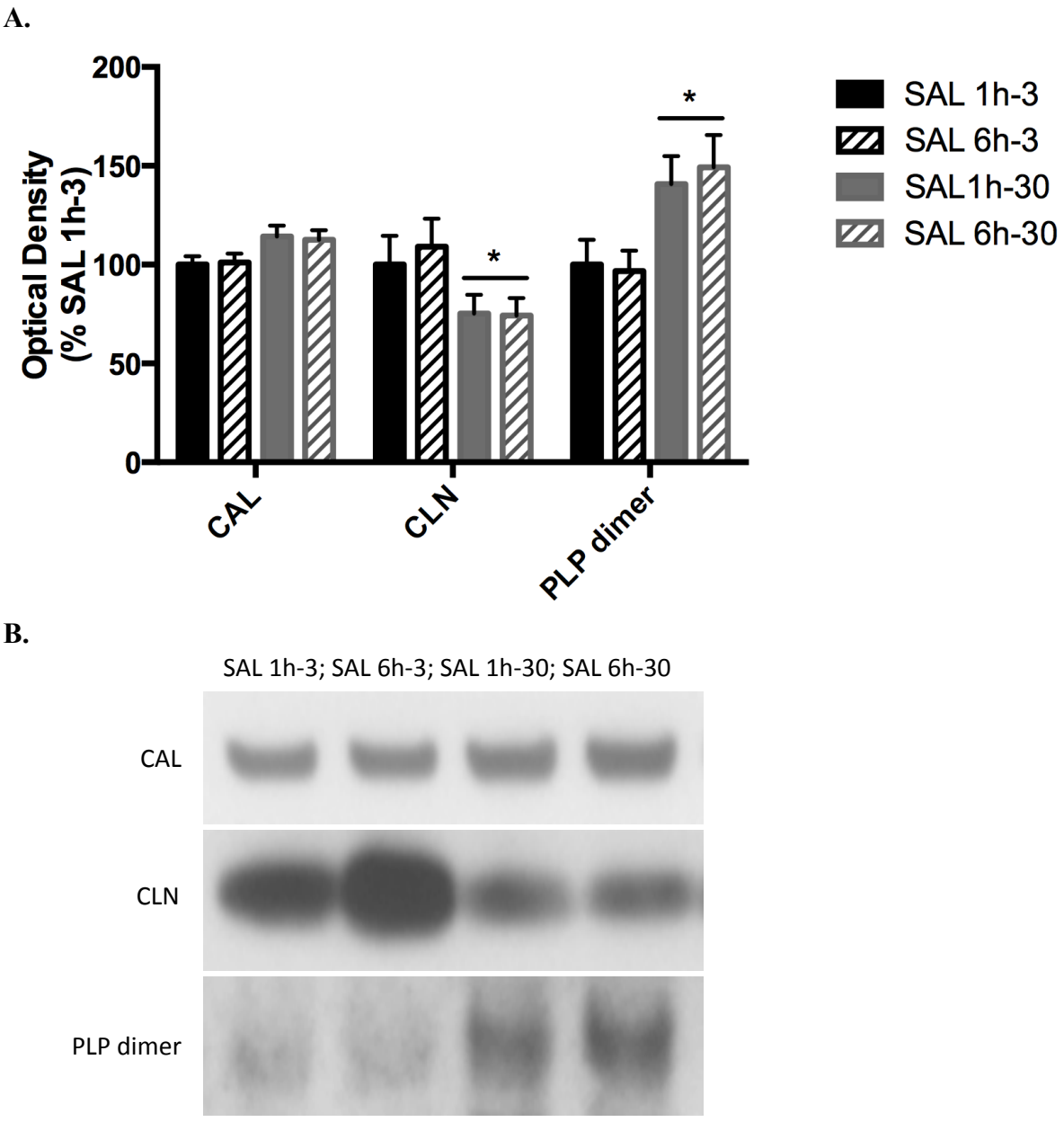


Figure 6. vmPFC immunoblotting results from Expt 3a. Rats self-administering saline (SAL) during short (1h) or extended access (6h) procedures and experiencing a 2h cocaine-seeking test prior to sacrifice exhibited decreased expression of CLN and increased expression of PLP dimer within the vmPFC at 30 days (30) withdrawal (WD) compared to the 3 day (3) withdrawal time point. (A) The optical density for each protein within a treatment condition are represented as a percent of the SAL 1h-3. (B) Representative immunoblots from SAL 1h-3 WD, SAL 6h-3 WD, SAL 1h-30 WD, and SAL 6h-30 WD treatment conditions. Calnexin (CAL) is used as a reference protein to control for differences in gel loading, and is not significantly affected by IV Treatment or Withdrawal conditions. *$p<0.05$, Withdrawal effect of 3 versus 30 days.

Self-administration Phase				
	Saline-Untested (n=13)	Saline-Tested (n=11)	Cocaine-Untested (n=12)	Cocaine-Tested (n=12)
Infusions	5. 03 ± 1.17	4.15 ± 0.68	94.97 ± 3.77	97.64 ± 3.63
Active Lever Presses	7.97 ± 1.90	6.58 ± 1.28	117.67 ± 8.04	125.00 ± 7.61
Inactive Lever Presses	4.08 ± 1.23	2.61 ± 0.35	1.21 ± 0.48	1.30 ± 0.70
Cue Test Phase				
Active Lever Presses		11.36 ± 2.53		96.58 ± 16.80
Inactive Lever Presses		7.417 ± 1.69		16.42 ± 3.21

Table 5. Behavioral outcomes obtained from Expt 3b, in which groups of animals were trained to self-administer saline (1 h/day) or cocaine (6 h/day) for 10 days. Half of the rats from each self-administration group were then tested for cue-reinforced responding at 3 days withdrawal (Tested), while the other half of the rats were left undisturbed in the home cage (Untested). Data represent the mean ± SEM of the number of animals indicated in parentheses.

Similarly, both COC 6h-cue-tested and COC 6h-untested groups pressed the active lever significantly more than SAL 1h-cue-tested and SAL 1h-untested controls as revealed by a significant main effect of IV treatment [$F(1,44)=404.80$, $p<0.0001$], but no Test effect or interaction between these variables (p's>0.05). Average active lever presses for SAL 1h-untested, 7.97 $\pm$ 1.90, n=13; SAL 1h-cue-tested, 6.58 $\pm$ 1.28, n=11; COC 6h-untested, 117.67 $\pm$ 8.04, n=12; and COC 6h-cue-tested, 125.00 $\pm$ 7.61, n=12.

COC 6h-cue-tested and COC 6h-untested groups also pressed the inactive lever significantly less than SAL 1h-cue-tested and SAL 1h-untested controls revealed by a significant main effect of IV treatment [$F(1,42)=6.35$, $p=0.016$). Average inactive lever presses for SAL 1h-untested, 4.08$\pm$ 1.23, n=13; SAL 1h-cue-tested, 2.61 $\pm$ 0.35, n=11; COC 6h-untested, 1.21 $\pm$ 0.48, n=11; and COC 6h-cue-tested, 1.30 $\pm$ 0.70, n=11.

Cue-test behavior

Animals in the COC 6h-cue-tested group responded on both active and inactive levers significantly more during the 2-hr cue-seeking test than did SAL 1h-cue-tested controls [Treatment X Lever interaction, $F(1, 43)=18.47$, $p<0.0001$]. The active lever presses for the COC 6h-cue-tested group was greater than the inactive lever presses for this same group, as well as both the active and inactive lever presses for the SAL 1h-cue-tested controls (p's<0.05). The inactive lever presses for cocaine and saline groups were not significantly different ($p>0.05$). Average active lever presses for the COC 6h-cue-tested and SAL 1h-cue-tested groups during the cue test are 96.58 $\pm$ 16.800, n=12 and 11.36 $\pm$ 2.531, n=11, respectively. Average inactive lever presses for the COC 6h-cue-tested and SAL 1h-cue-tested groups during the cue test are 16.42 $\pm$ 3.209, n=12 and 7.417 $\pm$ 1.694, n=12, respectively (Table 5).

Immunoblotting results

Protein and RNA extraction processes were optimized to enable detection of MBP, as future studies aimed to assess the functional relevance of this protein on behavior and cocaine addiction. Due to these differential extraction processes, only MBP was amenable to detection, thus, only this protein was assessed within the extracted vmPFC tissue. A two-way ANOVA of IV Treatment by Test revealed a significant main effect of IV Treatment for MBP, which reflected lower MBP in COC 6h-cue-tested versus SAL 1h-cue-tested controls (Figure 7) [$F(1,32)=6.13$, $p=0.019$; Test effect and IV Treatment X Test interaction, p's>0.05]. This Treatment effect replicates the decrease in MBP seen in Expt 1 (cue-tested animals) and was clearly independent of Test condition. Data is presented in Table 4.

2.3.5 Expt 4: Examination of an interaction between self-administration experience and behavioral testing upon mRNA expression

Analyses of the MBP-1 and -2 transcripts, which encode respectively for the 21.5 kDa and 18.5 kDa isoforms of MBP, failed to show a significant change in expression in either COC 6h-cue-tested and COC 6h-untested rats, compared to SAL 1h-cue-tested and SAL 1h-untested controls (Treatment x Test ANOVA, $p>0.05$). Although the 18.5 kDa MBP isoform was the only isoform measured in the immunoblotting studies, protein levels were reduced by cocaine experience (Table 4). Therefore, the changes in MBP expression due to cocaine, are not likely caused by preceding changes in gene transcription, and may reflect downstream protein degradation processes. MBP-3 primers were found to non-specifically amplify other transcripts in conjunction with the 17 kDa isoform of MBP and therefore were not analyzed for expression changes. MBP-4 and -5 were not run at the optimal cycling temperature and resulted in amplification of additional nonspecific transcripts as well.

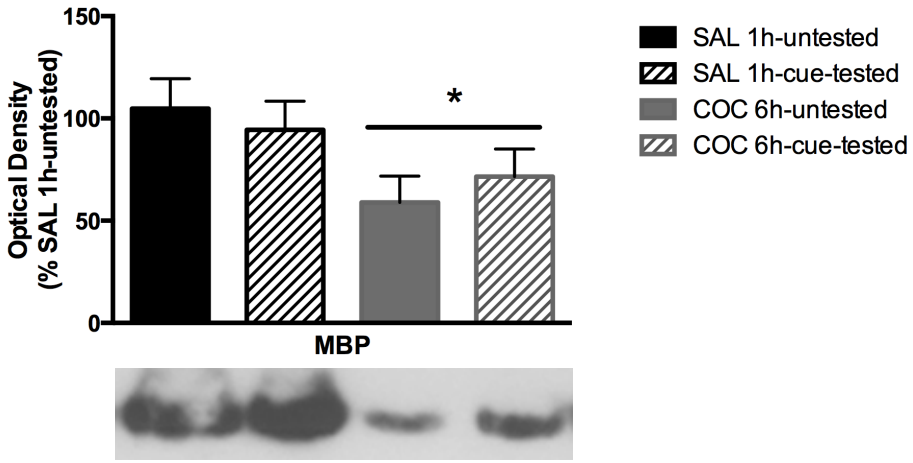


Figure 7. vmPFC immunoblotting results from replication study Expt 3b. Rats self-administering cocaine (COC) during extended access (6h) procedures exhibited decreased expression of MBP within the vmPFC compared to saline (SAL 1h) self-administering controls, regardless of experiencing a cocaine-seeking test (cue-tested) prior to sacrifice. Depicted data represent the optical densities for each protein within a treatment condition and are expressed as a percent of the SAL 1h-untested condition. A representative immunoblot from SAL 1h-untested, SAL 1h-cue-tested, COC 6h-untested and COC 6h-cue-tested treatment conditions lies below the graphical data. *$p<0.05$, IV Treatment main effect of COC versus SAL.

Table 4

vmPFC		**Chapter 2: Expt 1**	**Chapter 2: Expt 2**	**Chapter 2: Expt 3a**	**Chapter 2: Expt 3b**
Cell-type	**Protein**	**IV Treatment X Withdrawal in Cue-Tested Rats**	**IV Treatment X Withdrawal in Untested Rats**	**IV Saline Treatment X Withdrawal in Cue-Tested Rats**	**IV Treatment X Test (Replication)**
Astrocyte					
	Cx30	---	x: coc & sal 30> sal3		
	Cx43	---	---		
	GS	---	↑		
Oligodendrocyte					
	CLN		↑	▲t	
	CNP		---		
	Cx32		x: coc3>sal3 sal30>sal3		
	Cx47	---			
	MAG		---		
	MBP	↓	↑	---	↓
	MOG	↓	↑	---	
	NF155		---		
	NG2(+)	↓	---		
	PLP (monomer)	---	x: coc & sal 30> sal3	---	
	PLP (dimer)		↓	Δt	
	UGT8		---		
Microglia					
	CD11b	---	---		

dmPFC		Chapter 2: Expt 1	Chapter 2: Expt 2	Chapter 2: Expt 3a	Chapter 2: Expt 3b
Cell-type	**Protein**	**IV Treatment X Withdrawal in Cue-Tested Rats**	**IV Treatment X Withdrawal in Test-Naïve Rats**	**IV Saline Treatment X Withdrawal in Cue-Tested Rats**	**IV Treatment X Test (Replication)**
Astrocyte					
	Cx30	---	---		
	Cx43	---	---		
	GS	---	---		
Oligodendrocyte					
	CLN		---		
	CNP		---		
	Cx32		↓		
	Cx47	---			
	MAG		---		
	MBP	---	---		
	MOG	---	Δt		
	NF155		---		
	NG2(+)	---	---		
	PLP (monomer)	---	---		
	PLP (dimer)		---		
	UGT8		---		
Microglia					
	CD11b	---	---		

Table 4. Summary of the Immunoblotting Results for the vmPFC and dmPFC of the Studies Presented In Chapter 2.
↑ indicates cocaine>saline; ↓ indicates cocaine<saline; --- indicates cocaine=saline; Δt indicates 30 day>3 day; ▲t indicates 30 day<3 day; ---t indicates 30 day= 3day; x indicates interaction; indicates not examined

2.4 Discussion

Consistent with previous reports, a history of extended access to cocaine self-administration produced a time-dependent increase in cocaine-seeking behavior under extinction conditions (Ben-Shahar et al., 2013; Grimm et al., 2001; Tran-Nguyen et al., 1998). Although changes in glutamate receptors, and their downstream signaling cascades (e.g. AGS3), have been reported to occur during protracted withdrawal following extended cocaine access within the mPFC (Ben-Shahar et al., 2009, 2013; Bowers & Kalivas, 2003; Kalivas et al., 2005; Schmidt & Pierce, 2010), I failed to detect time-dependent changes in any of my glial specific markers that coincided with the incubation of cocaine-seeking behavior. Although correlative in nature, the temporal incongruence between protein/mRNA expression and behavior argue an unlikely role for cocaine-induced changes in the expression of glia-related proteins in the manifestation of the incubation of craving. However, the fact that glia-related protein changes were observed during early withdrawal and persisted for at least 30 days demonstrates clearly that a history of excessive cocaine-taking produces enduring "glioadaptations" that are likely to impact brain function and have behavioral consequences of relevance to other aspects of addiction

The results of Expt 1 indicated that a history of cocaine-taking produced a persistent decrease in Cx43 an astrocytic-specific gap junction protein within the NAc core. The present study is not the first to report an effect of cocaine experience upon the functional status of astrocytes. As discussed in the introduction to this chapter and Chapter 1, astrocyte specific GFAP expression is enhanced within the PFC, NAc core and shell from experimenter administered cocaine and 3 weeks withdrawal, suggesting cocaine can affect astrocytic process stability as GFAP provides intermediate filament scaffolding (Bowers &

Kalivas, 2003). Moreover, experimenter administered cocaine has been found to increase GFAP expression within the dentate gyrus, along with significant increases to cell numbers, size, and shape complexity (Fattore et al., 2002). Additionally, enhanced glutamate signaling within NAc core due to cocaine could interact with astrocytic ionotropic and metabotropic glutamate receptors (Porter & McCarthy, 1997). When stimulated, these receptors cause increases in cytoplasmic calcium concentrations within astrocytes; propagation of calcium waves is a form of intra and intercellular signaling and can affect gene transcription through calcium second messenger pathways (Araque, 2008). In astrocytes, Cx43, which forms gap junctions and hemichannels, mediates calcium wave propagation; therefore, an early and persistent reduction in Cx43 following extended cocaine access would be predicted to reduce calcium signaling both within and between astrocytes of the NAc core. Theoretically, impaired calcium signaling could result in immediate effects upon calcium-dependent enzyme activation, including those involved in regulating gene transcription, protein translation, synthesis and turn-over, which could be envisioned to have both short- and longer-term effects upon protein expression/function within astrocytes. A change in astrocytic calcium signaling could also result in alterations in astrocytic release of glutamate and ATP by calcium dependent exocytosis, leading to changes in neuronal excitability (Araque, 2008; Malarkey & Parpura, 2009). The functional relevance of altered astrocytic calcium signaling remains open as these findings are based on cell culture and brain slice studies (Araque, 2008; Malarkey & Parpura, 2009).

In Expt 1, I observed an early and persistent reduction in the OL proteins MBP, MOG and NG2+ within the vmPFC of cocaine self-administering animals. Such findings from an animal model of excessive cocaine intake are consistent with clinical reports of decreased

white matter integrity in chronic cocaine users (Bartzokis, Beckson, Lu, Edwards, & Bridge, 2002; Lim et al., 2002; Moeller et al., 2005, 2007). George et al. (2008) also reported decreases in NG2+ immunoreactive OLs within the PFC that correlated with deficits in working memory following long-access to cocaine. These authors suggested decreased labeling of NG2+ as indicative of decreased OL numbers; however, this particular protein is commonly used to identify OL progenitor cells (Armati & Mathey, 2010). Therefore, a reduction in NG2+ immunoreactivity could reflect a combined decrease in mature and progenitor OLs, or progenitors only. Alternatively, NG2+ positive cells have also been found to differentiate into gray matter astrocytes, neurons, or remain as a small population of mature NG2+ glial cells distinct from OLs (Mathey, Aurthur, & Armati, 2010). Therefore, changes in NG2+ levels are not specific to a particular cell type or a reflection of an effect of cocaine solely on OLs.

MOG is a minor component of compact myelin that is expressed during the later stages of OL development and has been proposed as a marker for maturity (Armati et al., 2010; Coffey & McDermott, 1997). The fact that both MOG and NG2+ expression is reduced following cocaine self-administration in Expt 1 would suggest a reduction in both mature and progenitor OLs and compliments the finding of George et al. (2008). This raises an interesting hypothesis that the stunted increase in white matter maturation of chronic cocaine addicts compared to normal, healthy controls could reflect, in part, a decrease in progenitor OLs reaching maturity and properly myelinating axons (Bartzokis et al., 2002). Unfortunately, as the tissue processing techniques conducted in Expt 3b were optimized for MBP protein detection and simultaneous RNA extraction, there was insufficient tissue to replicate the results from Expts 1 and 2 for protein other than MBP. Thus, the co-regulation

of MOG and NG2+ by cocaine requires replication in future studies in order to provide a more solid basis for the study of these proteins in addiction-related changes in brain and behavior. Towards this end, a follow-up study of adolescent rats is described in Chapter 3 that argues an independent regulation of NG2+ and MOG by cocaine.

MBP is a major protein component within the myelin sheath that is vital for the formation of myelin and the major dense line (Armati & Mathey, 2010; Atkins, Yon, Groome, & Sweatt, 1999). MBP is also reversibly phosphorylated at five locations mediated by protein kinase C (PKC) which can be activated following nerve impulse conduction, metabotropic receptor stimulation and rises in cytosolic calcium concentrations (Atkins et al., 1999; Murray & Steck, 1984; Vartanian, Dawson, Soliven, Nelson, & Szuchet, 1989). Alterations in MBP phosphorylation and expression levels impact myelin compaction and structural properties that affect the conduction velocity and efficiency of action potentials (Atkins et al., 1999; Murray & Steck, 1984). Therefore, a decrease in the levels of MBP expression within the vmPFC could reflect an early deficit resulting from cocaine that impacts myelin integrity and subsequently neuronal communication.

It is notable that the cocaine-induced reduction in vmPFC MBP expression observed originally in animals tested for cue-reinforced responding (Expt 1) was replicated in a separate cohort of animals in Expt 3b, conducted several years later. Moreover, the results of Expt 3b also indicated that the cocaine-induced reduction in vmPFC MBP was apparent in animals left undisturbed in the home cage prior to tissue collection, indicating that a history of extended cocaine access is sufficient to elicit this myelin-related protein change. These latter results from Expt 3b are at odds with the results of Expt 2, in which an increase in MBP was observed in test-naïve, cocaine-experienced animals. At this time it is unclear, why

significant opposite changes in MBP, MOG, and no change in NG2+ levels observed in Expt 2 were not consistent with Expt 1. It is unlikely, but possible, that the different times at which these experiments were conducted had unknown consequences on OL-specific protein expression and that tissue collection was not precisely consistent between the two studies. Given that decreased MBP expression seen in Expt 1 was replicated in Expt 3b when animals slated for cue testing or left untested were directly compared by a subsequent immunoblotting study, it will be important to replicate additional protein findings before conclusions can be drawn regarding a general effect of cocaine on myelin-specific protein expression.

The cocaine-induced reduction in MBP expression might reflect either decreased gene transcription or increased protein degradation. To begin to address these possibilities, we examined for changes in MBP mRNA expression within the same tissue sample employed in Expt 3b to detect changes in protein expression. In contrast to our hypothesis and despite the reduction in MBP protein expression, we did not detect any change in mRNA for two different MBP isoforms related to Treatment or Test. These data provide strong evidence against reduced gene transcription as underlying cocaine's effects upon MBP, leaving the possibility that the mechanism underpinning cocaine's ability to lower MBP expression reflects enhanced protein degradation. Unfortunately, this hypothesis is not easily addressed as the biogenesis and maintenance of the myelin sheath is poorly understood at present (Baron & Hoekstra, 2010).

Interestingly, despite observing rather robust cocaine-induced decreases in myelin-related protein expression within the vmPFC, the current immunoblotting study revealed few myelin protein changes within the dmPFC of cocaine-experienced animals. No changes in

protein expression were observed in Expt 1, although Expt 2 revealed a time-dependent increase in MOG in test-naïve rats. Intriguingly, a time-dependent increase in dmPFC MOG expression was replicated within the study of adolescent rats described in Chapter 3. As described earlier, MOG is often used as a molecular marker for mature myelin, a time-dependent increase, therefore, might reflect continued myelin maturation within the dmPFC of both adult and adolescent rats (Armati et al., 2010; Coffey & McDermott, 1997). However, as also discussed it remains unclear whether decreased dmPFC MOG in adult rats (Expt 2) is a reliable finding given the lack of change observed in Expt 1.

As reviewed in Chapter 1, cocaine exposure in animal models and in cell culture elevates inflammation-related transcripts and innate immune signaling. CD11b serves as a marker of microglial cells involved in innate immune signaling. While we did not see a change in CD11b expression following cocaine self-administration, other markers of microglial activation may be more sensitive indicators of an effect of cocaine on this glial cell type. For example, Ahmed et al. (2005) found increased IL-6, IL-4, and TNF mRNA within the PFC of rats with 18 days of short (1h) or extended access (6h) cocaine self-administration, and Piechota et al. (2010) found increased IL-6r and TNFα within the CP and NAc following a single injection of cocaine (25 mg/kg, IP) (further data analysis on microarray results from Ahmed et al. (2005) and Piechota et al. (2010) conducted by Clark et al., 2013). Administration of other drugs of abuse also result in enhanced immune gene expression including ethanol and methamphetamine (Loftis, Choi, Hoffman, & Huckans, 2011; Zou & Crews, 2010). Specifically, hippocampal slice cultures treated with ethanol for two-hours resulted in the elevation of several innate immune proinflammatory genes mediated by the transcription factor NF-κB, including the cytokines TNFα, IL-1β, and IL6

(Zou & Crews, 2010). Moreover, mice treated for seven days with 1 mg/kg methamphetamine (IP) expressed enhanced protein levels for IL-2, IL-6, IL-1β, and IL-10 within the HPC (Loftis et al., 2011). Thus, future studies, assessing an array of immune-related proteins are required to more accurately examine for cocaine-induced changes in microglial function as it relates to the manifestation of drug-elicited changes in addictive behavior.

In summary, the results of the studies presented in this chapter shows that extended access cocaine self-administration in rats followed by a 2-hr cue-seeking test leads to early and persistent reductions in glial subtype-specific proteins that may mediate the hypofunction within the vmPFC. Given the procedural differences between Expt 2 and 3b, it remains to be determined whether or not the capacity of cocaine to reduce glia-related protein expression within brain varies as a function of behavioral testing for drug-seeking. As conducted for MBP in Expt 3b, future studies need to directly examine the effect of cue-testing on expression levels of other glial-specific proteins, in order to better understand the role played by pharmacological and non-pharmacological factors in mediating the effects of cocaine self-administration upon the functional integrity of non-neuronal cells within brain regions exhibiting anomalous function in addiction. The present results for MBP clearly demonstrate that the cocaine-induced reduction in vmPFC protein expression is independent of behavioral testing, as well as changes in gene transcription. Thus, a reduction in vmPFC MBP expression is a pharmacodynamic response to excessive cocaine intake that occurs early in withdrawal, persists for at least 1 month following cessation of drug-taking and likely reflects impaired protein degradation. The functional relevance of reduced MBP expression for addiction-related behavior is examined directly in Chapter 4 and 5. The next chapter

examines the effect of cocaine on glial subtype-specific proteins within adolescent rats as adolescence is a period of enhanced impulsivity and risky drug-taking during which there is robust forebrain myelination (Bartzokis, Beckson, Lu, Edwards, & Bridge, 2002; Crews & Boettiger, 2009). Given the present results for cocaine-experienced adults, it is hypothesized that a history of extended cocaine access will have a very dramatic effect upon OL and myelin integrity, leading to greater deficits in frontal function.

Chapter 3 Effects of cocaine self-administration upon indices of myelin integrity in adolescents.

3.1 Introduction

As discussed in the general introduction, human adolescence is a key developmental period for the frontal cortex, with white matter maturation continuing through mid to late 40s (Bartzokis et al., 2002). Immature frontostriatal development in adolescence is associated with greater risk-taking, greater cocaine intake (which can last into adulthood), increased behavioral sensitization, and a decrease in the experience of the negative effects of drugs of abuse (Crews & Boettiger, 2009; Perry et al., 2007). Moreover, the majority of drug users are adolescents or young adults, and adolescent onset of drug use leads to a more rapid progression of abuse to dependency (Bernheim et al., 2013; Perry et al., 2007).

Animal models of adolescent drug use are in agreement with many of the features of human adolescent use and shed light on possible molecular mediators of these behaviors. For example, Wong, Ford, Pagels, McCutcheon, & Marinelli (2013) showed that adolescent male rats (PND 42) self-administered more cocaine at low to moderate doses (0.15-0.60 mg/kg) during daily short access sessions (1.5 hr/day, ~7-10 sessions total) and more cocaine when available at a moderate dose (0.60 mg/kg) during long-access sessions (6 hr/day, 10 sessions total, only dose tested) than adult (PND ~88) rats. Moreover, adolescent rats were shown to escalate their self-administration of cocaine under long-access conditions to a greater extent than adults, would "pay a higher price" for cocaine by responding more for drug at increased fixed-ratio schedules (FR6, FR12, and FR24), and exhibited higher firing rates of VTA DA neurons, compared to adults. By increasing or decreasing VTA DA neuron activity (infusion of eticlopride or quinpirole, respectively into the VTA), Wong et al. (2013) revealed that adults could behave more "adolescent-like" upon systemic eticlopride pretreatment or adolescents more "adult-like" upon systemic quinpirole pretreatment in the number of cocaine infusions administered during short-access conditions. However, the debate over

whether adolescent rats self-administer more cocaine is still open, as many labs using a variety of procedures have either supported an age difference (Anker & Carroll, 2010; Schramm-Sapyta et al., 2011) or failed to show a difference (Frantz, O'Dell, & Parsons, 2007; Harvey, Dembro, Rajagopalan, Mutebi, & Kantak, 2009; Kantak, Goodrich, & Uribe, 2007; Kerstetter & Kantak, 2007; Leslie et al., 2004; Li & Frantz, 2009). One key factor that may help explain this discrepancy is the dose of cocaine used, as age differences are more apparent at low to moderate doses, rather than higher doses (Anker & Carroll, 2010; Kantak et al., 2007; Kerstetter & Kantak, 2007; Wong et al., 2013).

In addition to evidence for higher drug-taking, adolescent rats also exhibit greater drug-craving than adults, when assessed in relevant animal models. For example, adolescents with a history of short-access cocaine self-administration (~15 days, 2hr/session, 2 sessions/day separated by 2 hrs, 0.4 mg/kg cocaine; 20 extinction sessions, 2 sessions/day separated by 2 hrs) exhibit greater lever-pressing under both extinction, as well as during cocaine- and stress-induced reinstatement procedures, compared to adult male rats (Anker & Carroll, 2010). Under these particular self-administration procedures, adolescents did self-administer more cocaine than adults during the maintenance phase of testing, again highlighting the point that different experimental procedures can reveal differential effects of age on cocaine-intake. Consistent with the observation that adolescent animals are more susceptible to stress-induced drug craving, other studies have indicated that cocaine-experienced adolescents are more prone than adults to exhibit anxiety in other behavioral paradigms. For example, a study by Alves et al. (2014), showed that adolescent cocaine exposure (15 days of IP treatment with 45 mg/kg cocaine/day, separated into 3 equal doses administered 1-hr apart + 10 days of withdrawal) results in abnormal behavior on the

elevated plus maze and Resident-Intruder paradigm when tested in adulthood. Therefore, not only do adolescents show heightened cocaine-seeking in response to stress, but adolescent cocaine exposure also results in altered stress responses that persist in adulthood.

The majority of evidence presented above supports greater cocaine sensitivity in adolescent versus adult rats. The data from Chapter 2 indicates that a history of cocaine-taking can produce enduring changes in the expression of glial-associated proteins in adult animals. Given that adolescence is a vulnerable period of neurodevelopment, the possibility existed that a history of cocaine-taking during adolescence would dramatically impact the developing PFC, and specifically glial cell protein expression. Adolescent rats were used as the subjects in similar cocaine self-administration and withdrawal procedures to Chapter 2, Expt 2. Based on the fact that adolescent rats are reported to take more cocaine than adults, we hypothesized that, relative to saline controls, the cocaine-induced changes in the expression of myelin-related proteins within the vmPFC would be more marked in adolescents than those observed in our study of adults (Chapter 2), and that adolescent cocaine experience might impact the expression of other proteins specific to other glial cell types, which we failed to detect in adult animals.

3.2 Methods

All methods are identical to those described in *Section 2.2*, with the following exceptions. Adolescent (PND 25, 100-125 g, at arrival) male Sprague-Dawley rats (Charles River Laboratories, Hollister, CA) were used as subjects for these self-administration experiments. Following surgical recovery, animals (n=10-15 per group at the start of each experiment) were trained to self-administer IV cocaine (0.15 mg/0.1 ml/infusion; National Institute on Drug Abuse, Bethesda, MD) during daily 6-hr sessions, or saline (0.1 ml/

infusion) during 1-hr sessions, on a FR1 schedule of reinforcement. Additionally, at either 3 or 30 days of withdrawal, animals were subjected to rapid decapitation procedures in the absence of behavioral testing in order to determine the effects of a history of cocaine-taking during adolescence upon protein expression, unconfounded by interactions with cue-testing procedures.

3.3 Results

3.3.1 Self-administration behavior

As observed in adult rats (see *section 2.3*), cocaine self-administering adolescents earned a significantly greater number of infusions [main IV Treatment effect, $F(1,43)=498.70$, $p<0.0001$] and pressed the active lever more than saline controls [main IV Treatment effect, $F(1,43)=22.04$, $p<0.0001$]. No group differences were observed regarding the number of inactive lever presses (2-way ANOVA, all p's>0.05; Table 6).

3.3.2 Immunoblotting results

Within the vmPFC, adolescent cocaine animals expressed significantly lower levels of NG2+ and lower CD11b, compared to saline controls [for NG2+, IV Treatment effect: $F(1,44)=5.54$, $p=0.023$; for CD11b, IV Treatment effect: $F(1,45)=4.00$, $p=0.05$], but the expression of neither protein varied with the duration of Withdrawal (Withdrawal effect or IV Treatment x Withdrawal interaction, p's>0.05). No other proteins showed significant IV Treatment, Withdrawal, or IV Treatment x Withdrawal Interaction effects (p's>0.05), Figure 8.

	SAL 1h-3 (12)	SAL 1h-30 (14)	COC 6h-3 (11)	COC 6h-30 (10)
Infusions	4.89 ± 0.77	5.53 ± 0.78	116.94 ± 9.03 *	123.37 ± 6.69 *
Active Lever	7.14 ± 1.03	8.26 ± 1.18	338.64 ± 139.57*	421.70 ± 104.82*
Inactive Lever	8.23 ± 1.58	7.83 ± 1.62	5.07 ± 1.81	10.53 ± 3.53

Table 6. Summary of the behavior exhibited by adolescent rats during the 10 days of self-administration training under long-access (6-h) procedures. Overall, cocaine (COC 6h) self-administering animals exhibited a greater number of active lever presses and infusions than did their saline (SAL 1h) self-administering counterparts. However, there were no differences in behavior between the COC rats slated for tissue collection at 3 versus 30 days withdrawal (respectively, 3 and 30), nor were there differences between the SAL groups slated for tissue collection at these time-points. Data represent the means ± SEMs of the last 3 days of training. The sample sizes are indicated in parentheses. *$p<0.05$ vs. SAL (main Treatment effect).

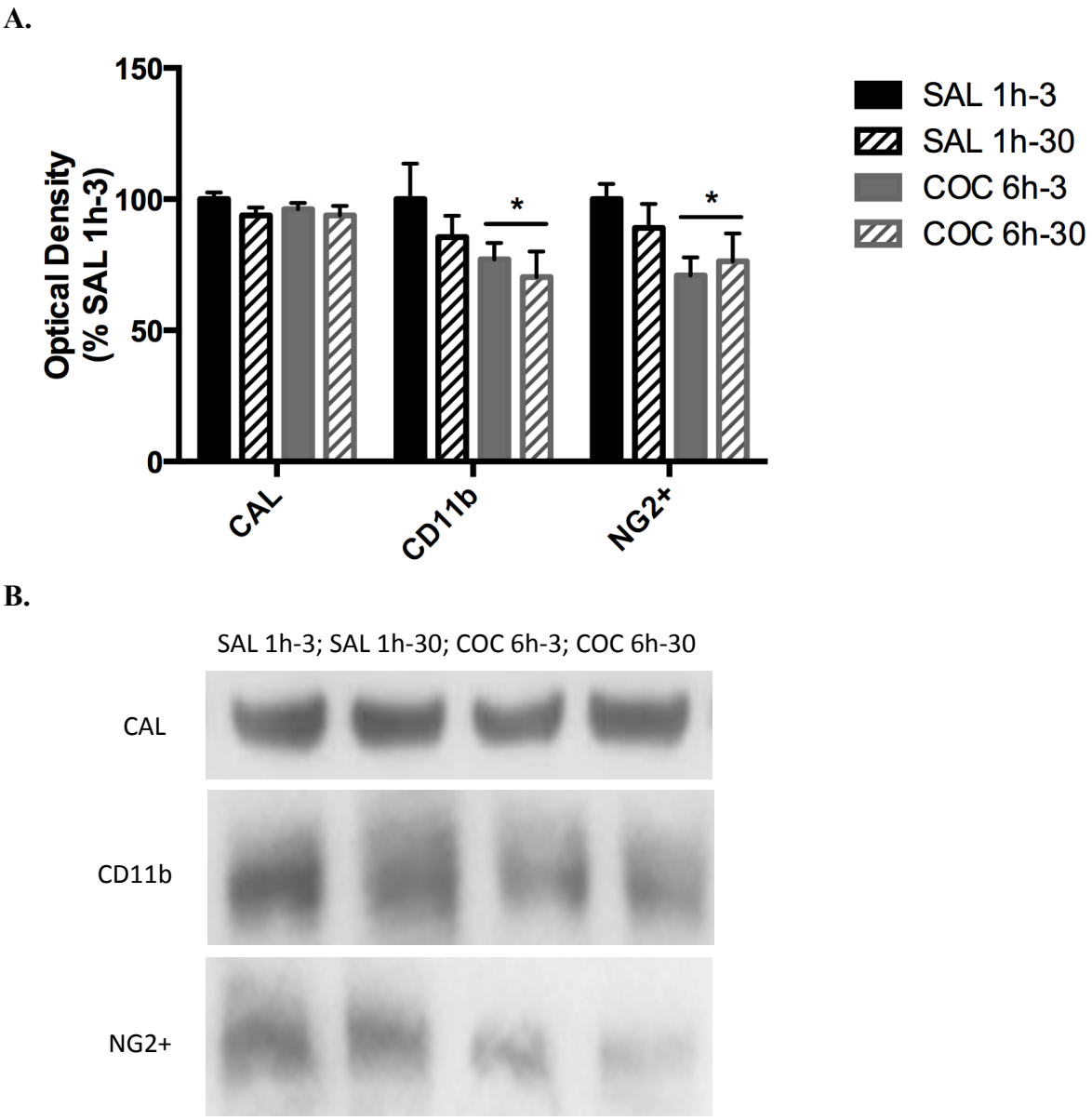
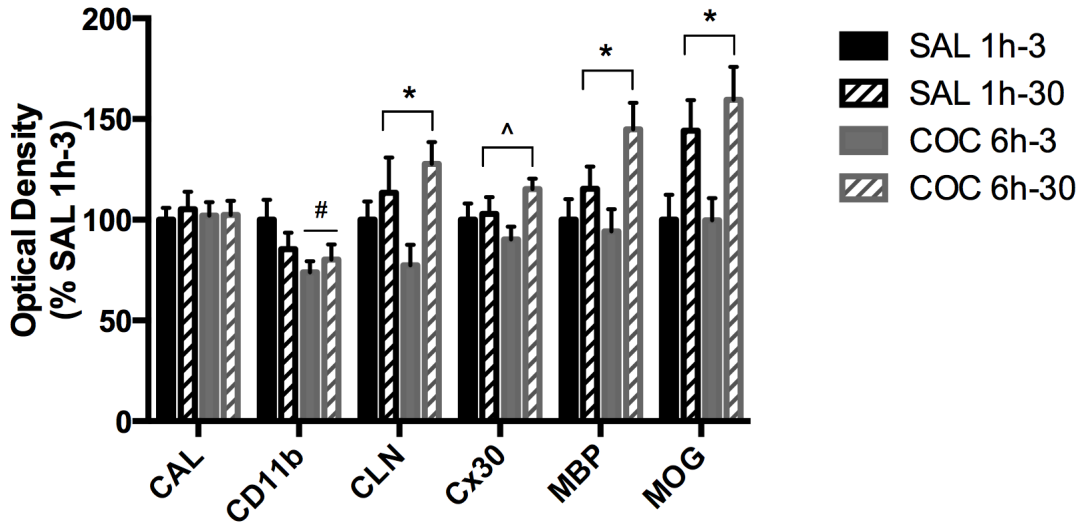


Figure 8. Adolescent vmPFC immunoblotting. Rats self-administering cocaine (COC) during extended access (6h) procedures and untested prior to sacrifice exhibited decreased expression of CD11b and NG2+ within the vmPFC early in withdrawal (WD; 3 days), an effect that persisted into protracted withdrawal (30 days) compared to saline (SAL 1h) self-administering controls. (A) The optical density for each protein within a treatment condition is represented as a percent of the SAL 1h-3 condition. (B) Representative immunoblots from SAL 1h-3 WD, SAL 1h-30 WD, COC 6h-3 WD and COC 6h-30 WD treatment conditions. Calnexin (CAL) is used as a reference protein to control for differences in gel loading, and is not significantly affected by IV Treatment or Withdrawal conditions. *$p<0.05$, IV Treatment main effect of COC versus SAL.

Within the dmPFC, both cocaine and saline animals showed time-dependent enhanced expression of CLN [Withdrawal effect: $F(1,46)=6.12$, $p=0.017$], MBP [Withdrawal effect: $F(1, 47)=8.22$, $p=0.006$], MOG [Withdrawal effect: $F(1,48)=13.76$, $p<0.001$], and a trend for Cx30 [Withdrawal effect: $F(1,48)=3.79$, $p=0.057$], but for none of these proteins did expression vary as a function of cocaine experience (IV Treatment and IV Treatment x Withdrawal Interaction, p's>0.05). Expression of the immune-cell marker CD11b showed a trend towards decreased expression following cocaine experience that just failed to meet statistical significance [IV Treatment effect: $F(1,48)=3.76$, $p=0.059$], but this trend did not vary as a function of withdrawal time (Withdrawal and IV Treatment x Withdrawal Interaction, $p>0.05$). None of the other proteins examined exhibited significant IV Treatment, Withdrawal, or IV Treatment x Withdrawal Interaction effects (p's>0.05), Figure 9.

A.



B.

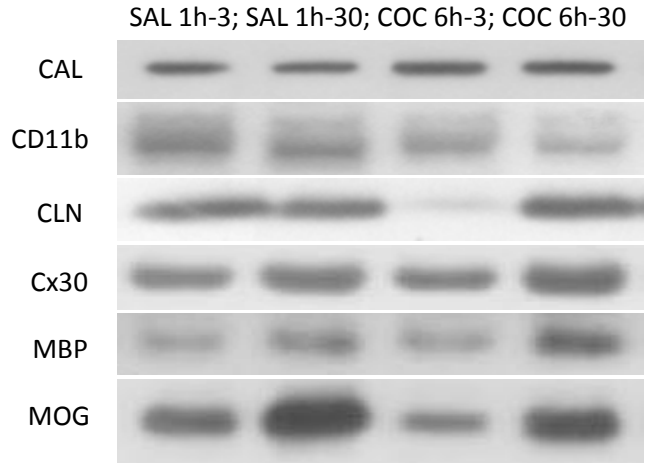


Figure 9. Adolescent dmPFC immunoblotting. Rats either self-administering cocaine (COC) during extended access (6h) procedures or saline (SAL 1h) untested prior to sacrifice exhibited increased expression of CLN, MBP, MOG, and a trend for Cx30 within the dmPFC in protracted (30) but not early withdrawal (WD; 3). CD11b showed decreased expression across early to protracted withdrawal, an effect that just failed to meet significance ($p=0.059$). (A) The optical density for each protein within a treatment condition is represented as a percent of the SAL 1h-3 condition. (B) Representative immunoblots from SAL 1h-3 WD, SAL 1h-30 WD, COC 6h-3 WD and COC 6h-30 WD treatment conditions. Calnexin (CAL) is used as a reference protein to control for differences in gel loading, and is not significantly affected by IV Treatment or Withdrawal conditions. *$p<0.05$, Withdrawal main effect of 3 versus 30, #$p=0.059$, trend to decreased expression over withdrawal, ^ $p=0.057$, trend to increased expression over withdrawal.

3.4 Discussion

It was hypothesized at the outset of this Chapter that cocaine experience during adolescence would have a marked impact upon the expression of glial-associated proteins within the PFC, owing to the facts that adolescents are reported to consume more cocaine than adults and adolescence is a period of robust neurodevelopment, which is presumed to render the brain more susceptible to environmental insults such as drug exposure. However, contrary to my hypothesis, I did not see the decrease in myelin-related proteins following extended access to cocaine in adolescent self-administering male rats as was seen in the adult vmPFC (Chapter 2), although the levels of NG2+ and CD11b were reduced in cocaine-experienced adolescents versus saline controls. However, while I observed very few protein changes within dmPFC of adult rats (Chapter 2), several significant *increases* in myelin proteins were observed across withdrawal in the dmPFC of both saline and cocaine administering animals, supporting this developmental period as one of enhanced myelination.

Myelin formation begins approximately PND 14 and accumulates rapidly over the next 30 days, followed by slow accumulation to the end of active myelination at PND 60 (Akiyama, Ichinose, Omori, Sakurai, & Asou, 2002). Enhanced cerebral expression of myelin-related transcripts and proteins have been documented in mice and rats to peak in early adolescence (PND 21) with high and stable expression lasting through adolescence into adulthood (~2 years) (Akiyama et al., 2002; Mitchell et al., 1992; Sorg, Smith, & Campagnoni, 1987; Wiktorowicz & Roach, 1991). Specifically, the 14 and 18.5 kDa isoforms of MBP (comprising 70% of the total MBP in adult brain) are detectable at PND21 and show increased expression up to 24 months in the cerebrum of rats (Akiyama et al., 2002). Moreover, all isoforms of MBP were found to increase within the PFC of male rats

between PND 28 and 49 (Kodama, Kikusui, Takeuchi, & Mori, 2007). Consistently, PLP and MBP mRNA and protein levels have been found to increase in mice from PND 10-40 with stable transcription continuing to PND 50 (Mitchell et al., 1992; Sorg et al., 1987; Wiktorowicz & Roach, 1991). MOG follows slightly behind MBP expression, emerging within the forebrain white matter between PND 15 and 21 and accumulating through PND 60 (Coffey & McDermott, 1997; Matthieu & Amiguet, 1990).

Therefore, the heightened level of expression in myelin-related proteins seen over withdrawal in adolescence likely reflects normal expression, an event that is no longer prominent in adult rats. Additionally, this enhanced expression may be buffering an effect of cocaine on myelin, as any decreases that occur in adult myelin is masked by the significant increase in transcription that translates to protein in adolescence. This would also be supported by the fact that cocaine does not seem to affect myelin-related transcription as neither the 21.5 nor 18.5 kDa MBP mRNA transcripts changed in response to cocaine, as discussed in Chapter 2. It is surprising we did not see a withdrawal-related increase in PLP expression given the increases in the other myelin-related proteins and enhanced PLP mRNA and protein expression during this developmental time period observed in other studies (Mitchell et al., 1992; Wang et al., 2008).

Contrary to adult rats, the vmPFC of adolescent rats showed very few changes in protein expression following cocaine or withdrawal. The decrease in NG2+ expression following cocaine is consistent with the data from adults from Chapter 2 (Expt 2), suggesting that cocaine may have an affect on OL precursor cells. As discussed in Chapter 1, I mentioned that this particular protein is not specific to OLs, but is also expressed in cells that mature into gray matter astrocytes, neurons and a small population of mature NG2+ glia

(Trotter, Karram, & Nishiyama, 2010). The majority of cells expressing NG2+ do develop into mature-myelinating OLs and a decrease in the expression of this protein following cocaine has now been replicated twice within my own work and that of George et al., 2008. NG2+ is a chondroitin sulfate proteoglycan, which suggests a role in the extracellular matrix and also contains sequences that would suggest binding partners with proteins relevant for cell migration and process movement (Trotter et al., 2010). It is not clear from the present immunoblotting findings whether the decrease in NG2+ is specific to this protein, represents a decline in the population of this cell/precursor cell group, or enhanced maturation of these cells to their mature phenotypes. Questions such as these would be better addressed with cell-fate studies in conjunction with cocaine administration, and experimental manipulation of this specific protein on animal behavior.

It is surprising that similar withdrawal-related increases in myelin-related proteins were not observed within the vmPFC of adolescent rats as those observed in adults. Several studies have examined a caudal-rostral, and one study also a ventral-dorsal, gradient in the appearance and maturation of myelin within both mice and rat central nervous systems (Coffey & McDermott, 1997; Mitchell et al., 1992). MOG, in particular, is seen to increase first in the brain stem at PND 7, spreading throughout the ventrolateral region of the pons before extending through the entirety of the pons by PND 21; cerebellar MOG staining was not evident at PND 14 but uniformly present in cerebellar white matter at PND 21, while forebrain MOG staining was not evident prior to PND 21 with ventral corpus callosum staining present prior to dorsal portions about PND 28. All regions exhibited a clear presence of MOG within the white matter before being detected within the gray matter (Coffey & McDermott, 1997). Moreover, PLP mRNA expression within the spinal cord peaks about

PND 12 before becoming stable, where brain expression does not peak till PND 30 before becoming stable (Mitchell et al., 1992). Therefore, it is possible that we did not detect age-related increases in vmPFC myelin-related proteins due to an earlier maturation of this region, though this seems unlikely and future studies will need to examine more systematically this ventral-dorsal difference in myelin protein expression specifically within the PFC, and perhaps also study how a history of cocaine impacts the caudal-rostral gradient of protein expression through studies of diencephalic and mesencephalic structures (e.g., NAc, VTA) that are also implicated in addiction.

Neither vm- nor dmPFC showed a difference in the expression of the microglial marker CD11b, which corresponds with the lack of cocaine effect on this protein within adult rats. To reiterate, this protein may not accurately reflect an effect of cocaine on microglial cells as other markers of inflammatory response and oxidative stress have been observed following cocaine both *in vivo* and *in vitro* (see Chapter 2, Discussion). Future studies will need to assess a wider array of proteins and/or microglial cellular responses to cocaine using the long-access cocaine self-administration model to glean a better understanding of the potential consequences of cocaine on this cell type.

The current findings suggest adolescence is not a "vulnerable" period for cocaine-induced impairment of OL maturation and myelination. However, future studies need to continue addressing the flexibility and vulnerability of the adolescent PFC and determine if transient or lasting effects of cocaine become apparent over time, specifically as it pertains to OL maturation and myelination and PFC function. The next chapter examines the behavioral consequences of experimentally decreasing MBP expression within the vmPFC of adult rats, given this protein was decreased following cocaine self-administration procedures in Chapter

2, Expt 1 and 3b. As was discussed in Chapter 1, the only studies examining the functional significance of MBP have been in mutant mice completely lacking expression of this protein, therefore the goal of the next chapter was to characterize the specific cognitive deficits associated with localized decreased MBP expression.

Chapter 4 Behavioral effects of reducing myelin basic protein within the vmPFC

4.1 Introduction

As shown in Expt 1 (Chapter 2), cocaine self-administration decreased myelin-specific protein expression within the vmPFC of adult male rats. For MBP, the cocaine-induced decrease in protein expression persisted for at least 4 weeks into drug withdrawal, occurred regardless of whether or not animals are tested for cocaine-seeking prior to sacrifice and did not reflect a change in the transcription of the major 21.5 or 18.5 kDa protein products of the gene encoding MBP. These data provide strong evidence that reduced MBP expression within the vmPFC is a cocaine-induced neuroadaptation that persists into protracted withdrawal, but the relevance of this particular neuroadaptation for behavior governed by this region is not yet known.

MBP is the only protein known to be essential to the formation of CNS myelin in mammals; its genetic deletion causes severe hypomyelination and premature death (Boggs, 2006). The MBP gene codes for five splice variants which are both developmentally regulated and localized to specific regions of the cell (Boggs, 2006; Padhi & Pelletier, 2012). The 18.5 kDa MBP isoform comes in 8 different charge isomers depending upon the kind of post-translational modifications and net effect on the charge of MBP (Boggs, 2006). MBP is also rapidly and reversibly phosphorylated, giving hint to a potential role in a dynamic cellular process (Atkins, Chen, Klann, & Sweatt, 1997; DesJardins & Morell, 1983; Murray & Steck, 1984; Ulmer, 1988). The most dominant isoform of MBP in humans is the 18.5 kDa protein, which is also expressed in rodents (Boggs, 2006). It is the expression of this protein isoform that was decreased following cocaine self-administration in Expts 1 and 3b (Chapter 2). MBP has a net positive charge that allows it to interact via electrostatic forces with the negative lipid head groups of the membrane, and also contains hydrophobic side chains that become embedded within the bilayer (Boggs, 2006). These are the main forces allowing

MBP to interact with the lipid membrane in order to adhere apposing sides to aid in the formation of the compact rings of myelin that surround axons (Boggs, 2006). Changes in the ratio of MBP to lipids, net increases or decreases in MBP protein levels, post-translational modifications that alter the net charge of MBP, and local ion concentrations all impact the ability and effectiveness of MBP to bind two lipid surfaces together to promote multi-bilayer formation (Boggs, 2006; Hu et al., 2004; Min et al., 2009).

MBP has additional interactions with the cytoskeleton, including actin and tubulin promoting polymerization and stabilization of fiber bundles; a process that is dynamically regulated by transmission of extracellular signals (Dyer, Philibotte, & Wolf, 1994; Dyer, 1993; Hill & Harauz, 2005; Hill, Libich, & Harauz, 2005). For example, anti-galactosylceramide antibody added to OL cultures mediates lipid and protein redistribution, changes in MBP phosphorylation, and depolymerization of microtubules. This latter effect is dependent on the expression of MBP, as these changes do not occur in OLs derived from *Shiverer* mice that lack this protein (Dyer, 1993). Therefore, changes in the expression of MBP could potentially impact the integrity of the myelin sheath, myelin process stabilization, OL response to nerve impulses, and ultimately affect neuronal communication due to a decrease in signaling efficiency from poor electrical insulation. As discussed in Chapter 1, few studies have examined the behavioral consequences of manipulating MBP; *Shiverer* mice with a deletion of the MBP gene exhibit severe motor deficits and a deficit in reversal learning on the T-maze (Inagawa et al., 1988). However, to date, there are no reports of specific experimental manipulation of MBP within distinct brain regions upon behavior. Therefore, the series of experiments described in this Chapter aim to characterize the effects of reduced MBP expression within the vmPFC on PFC-dependent behaviors in rats.

The PFC is critical for recognition memory. Lesions of the vmPFC in nonhuman primates impairs recognition memory resulting in significant learning impairments on object-in place and temporal order memory tasks (Warburton & Brown, 2010). Consistent with this, rats with mPFC lesions exhibit significantly impaired performance on the object-in-place task (Barker, Bird, Alexander, & Warburton, 2007). Recognition memory can also be assessed via a delayed non-matching to sample (DNMS) task. Rats with large aspiration lesions of the mPFC failed to choose the correct non-matching goal arm of a Y-maze when a 20 sec delay was instituted prior to being allowed to choose between the two goal arms (Kolb, Buhrmann, McDonald, & Sutherland, 1994). Similar results have been observed using DNMS tasks under operant conditions. When rats were trained to press a lever following a light presentation and omit a response with a tone presentation, rats with ibotenic acid lesions of the vmPFC exhibited delayed acquisition of the response contingencies when a 10 or 20 sec delay was instituted between cue presentation and availability of the lever to deliver food reward (light) or footshock (tone) (Delatour & Gisquet-Verrier, 1999). Moreover, rats with excitotoxic lesions of the mPFC exhibit reduced performance on the non-matching to sample T-maze, and distinct lesions of the PL/IL result in greater perseverative behavior in the matching-to-sample and reversal of the matching-to-sample T-maze than sham lesioned control rats (Dias & Aggleton, 2000).

Akin to the results of lesion studies, a history of psychomotor stimulant exposure also impairs performance on PFC-dependent recognition tasks. For example, extended access to methamphetamine self-administration in both male and females rats decreases the recognition index for both novel-object and object-in-place recognition tasks (Reichel, Chan, Ghee, & See, 2012). Also, extended access (6 hr/day) to cocaine self-administration in rats

results in significantly lower correct alternations with the DNMS T-maze when a 70s or 130s delay is instituted between a forced run and choice run compared to drug-naïve controls and rats with short access (1 hr/day) to cocaine (George et al., 2008). The deficit in DNMS T-maze performance was positively correlated with the density of neurons and NG2+ cells within the dmPFC and with NG2+ cells within the OFC. Therefore, experimental manipulation of MBP may alter the ability of the PFC to function properly in working-memory dependent tasks and it is hypothesized that experimentally decreased MBP within the PFC will result in reduced performance within the DNMS T-maze with increasing delay between forced and choice runs. Furthermore, we hypothesize experimentally reduced MBP expression will result in impaired performance on the object recognition tasks, as indicated by reduced recognition index scores.

Sensorimotor gating deficits are common among neuropsychiatric disorders including schizophrenia, Huntington's disease, and Tourette's syndrome, and are observed following both acute and chronic administration of cocaine in rats (Martinez, Ellison, Geyer, & Swerdlow, 1999). Female rats injected with either 20 or 30 mg/kg cocaine (IP) exhibited decreased pre-pulse inhibition to a 120 dB tone when preceded by 20ms bursts of 73, 76, or 82 dB pre-pulses. Moreover, rats implanted with subcutaneous pellets containing 120 or 240 mg cocaine and tested three days later also showed deficits in pre-pulse inhibition to the same startle pulses (Martinez et al., 1999). In an animal model of schizophrenia with a genetic deletion of the *Homer 1* gene, mice exhibit deficits in PPI and this sensorimotor getting deficit is associated with anomalies in PFC glutamate that include increased basal extracellular content and a failure to respond normally to an acute cocaine injection (Szumlinski et al., 2005). mPFC (ACC, PL, and IL) ibotenic acid lesions in rat result in

significantly higher PPI compared to control and lateral PFC lesions over startle pulses of 72, 76, 80, and 84 dB (Lacroix, Spinelli, White, & Feldon, 2000). Thus, the PFC also governs, at least in part, sensorimotor gating processes and cocaine-induced impairment in PFC function is likely to contribute to drug-induced impairments in sensorimotor gating. To examine for a relationship between decreased MBP expression within the vmPFC and sensorimotor gating, rats in the following series of experiments were also run through identical PPI procedures to those employed by Szumlinski et al., (2005). We hypothesize a decrease in MBP within the vmPFC will impair normal sensorimotor gating resulting in increased startle responses following pre-pulses, compared to rats with unaltered MBP expression.

Impulsivity can be broadly defined as decision-making or action without appropriate forethought and is one major risk factor for the development of drug addiction (Sun, Cocker, Zeeb, & Winstanley, 2012). Impulsivity can be further decomposed into cognitive forms of impulse control that can similarly be measured across humans and animal models using delay-discounting paradigms that reveal preference for a smaller-sooner versus larger-delayed reward (Sun et al., 2012). Delay-discounting procedures in rats have been used to show baseline impulsive choice differences that predict the acquisition and the rate of acquisition of cocaine self-administration (Perry, Larson, German, Madden, & Carroll, 2005). Distinct subregions of the PFC have been shown to play various roles in delay discounting. For instance, reversible inactivation of the orbitofrontal cortex (OFC) with baclofen/muscimol was found to increase impulsive choice in less impulsive rats (measured at baseline) when the delay between behavior and the large reward was cued with a light (Zeeb, Floresco, & Winstanley, 2010). However, in highly impulsive rats measured at baseline, baclofen/muscimol OFC microinjections decreased impulsive choice in rats when

the delay between behavior and large reward delivery was not cued. In human imaging studies, choices anticipating immediate rewards increased signal activity in the mPFC and mOFC, while choice of the delayed option was associated with increased activity in the lateral PFC and OFC (Dalley, Everitt, & Robbins, 2011). In a BOLD fMRI study, human participants showed greater activity in the vmPFC when deciding between objects to acquire the larger reward (Blair et al., 2006). Moreover, rats with excitotoxic lesions to the mPFC showed a preference for a low-reward arm entry in a T-maze when the high-reward arm required traversing a barrier to obtain the reward, an effect that was shown to be based on the amount of effort required to obtain the reward as lowering the barrier reinstated the preference for the high-reward arm, and indicating a role of the mPFC in determining the amount of work an animal will engage in to obtain a specific reward (Walton, Bannerman, & Rushworth, 2002). Given the role of the PFC in impulsive choice and decision-making for obtaining a reward, we hypothesize that decreased MBP within the vmPFC will promote impulsive choice possibly by decreasing normal processing within the brain region.

4.2 Methods

4.2.1 Animals

Subjects were identical to those described in Chapter 2, with the following exceptions. Animals were given *ad libitum* access to food and water, except during training and testing on the DNMS T-maze. Animals were allowed to acclimate to the colony room for two days following arrival and were handled daily for the duration of the experiment.

4.2.2 Surgical Procedures

Surgical procedures and lentiviral transduction vector delivery began 3 days following arrival of animals. Under ketamine/xylazine anesthesia (56.25 mg/kg, Henry

Schein, Dublin, OH, and 7.5 mg/kg, Lloyd, Shenandoah, IW, respectively, administered intramuscularly (IM)), animals' skulls were stabilized in a stereotaxic apparatus (Stoelting, Wood Dale, IL) and were implanted with a chronic 22 gauge bilateral guide cannula (1.5 mm spacing between cannula, Plastics One) above the vmPFC [anteriorposterior (AP), +3.1 mm; mediolateral (ML) +0.75 mm; dorsovental (DV) -3 mm; nose set at -3.5 mm]. Four small screws and cranioplastic cement secured the guide cannula to the skull. While still under anesthesia, animals were removed from the stereotaxic apparatus and microinjectors (28 gauge) were lowered bilaterally into the guide cannula and exited 2 mm beyond the end of the guide. Lentiviral transduction particles (LVV details described in subsequent section) containing short-hairpin RNA (shRNA) against MBP mRNA transcripts or control particles, were infused at a rate of 0.1 μl/min over 10 minutes (total volume is 1.0 μl/side) via a Harvard Apparatus PHD 2000 infusion pump (Harvard Apparatus, Holliston, MA), with a 5 minute waiting period to allow for diffusion of the virus from the tip of the microinjector. For post-operative pain, banamine (2 mg/kg, subcutaneous, Butler Schein Animal Health, Dublin, OH) was administered subcutaneously twice daily for two days following surgery. Behavioral testing procedures began 3 weeks following virus infusion and to allow animals to fully recover from surgery.

4.2.3 LVV information

Custom lentiviral transduction particles were ordered through Sigma-Aldrich to generate shRNA against MBP transcripts or empty vectors used as controls with a green fluorescent protein reporter expressed to verify transduction (LVV ctl, SHC003V; LVV1, product number: SCHLNV, clone ID TRCN0000090245; backbone pLKO.1-hPGK-Puro-

CMV-tGFP; LVV2, product number SCHLNV, clone ID TRCN0000376806, backbone pLKO.1-hPGK-Puro-CMV-tGFP; St. Louis, MO).

To verify transduction efficacy *in vivo* of the LVV's when microinjected into the rat vmPFC, animals (n=8-10/group) underwent identical surgical procedures as described earlier. Animals were allowed to recover and remained in the vivarium for 3 weeks, then they were anesthetized with 2-3% isoflurane and a microinjector filled with black India ink was inserted into the bilateral guide cannula (28 G, exited 2 mm beyond the bilateral guide) to mark where viral infusions had occurred. Animals were then decapitated and a punch of tissue around the ink spot was collected over ice for immunoblotting procedures identical to those described in *section 2.2.6*.

4.2.4 Object recognition tests

Apparatus

Object recognition tests occurred in a square open field (1m length, by 1m width, with 50cm high walls) constructed of white Plexiglas walls and a clear Plexiglas floor, situated on the laboratory floor. The location of the open field and position of "extrafield" cues was constant throughout object recognition tests. A soft, 60-watt light illuminated the room and a fan masked extraneous noise. A video camera mounted on the ceiling directly above the field was connected to AnyMaze^{TM} tracking software (Stoelting) and tracked the animals' movement and interaction with the inedible objects. The objects for the novel object test consisted of glass blocks (5 cm length, 5 cm width, 7 cm height) and a metal ring (8.5 cm in diameter with a center 3 cm diameter circle cut out), swabbed with either lemon or almond (McCormick, Sparks, MD) scent prior to each test; the object deemed "novel", the location of the novel object on the test, and the scent swabbed on the objects was counterbalanced

among subjects and duplicate identical objects were used to prevent both scents from being swabbed on the same object. The objects for the object-in-place test consisted of a light bulb, glass blocks (same as the novel object test), a 50ml centrifuge tube (Corning, Tewksbury, MA), and a glass jar (5.5 cm diameter, 5 cm high). The location of the four objects in the object-in-place test was constant for the exploration phase, but the two items with switched placements during the test phase was counterbalanced among subjects. The apparatus and objects were wiped down with 70% isopropyl alcohol between tests to mask odor.

Procedure

Prior to beginning testing, animals were placed individually into the open field apparatus for a 5-minute habituation session on two consecutive days and their total distance traveled, time spent immobile, and total number center entries (10 cm diameter circle in the center of the open field) were recorded. The animals were then split into two groups; half of the animals were tested in the novel object test followed by the object-in-place test, and the other half were tested in the opposite order. In the novel object test (methods similar to Reichel, Chan, Ghee, & See, 2012), animals were placed in the open field at the center of a wall, adjacent from the two identical objects with scent (either lemon or almond) and allowed to familiarize themselves with the objects for 3-minutes. Animals were returned to their home cages for a 90-minute waiting period before the animals were again placed in the open field and tested for their interaction time with one of the familiar objet and a novel object with a novel sent (i.e., one not employed during the familiarization test) scent over 3-minutes.

On the object-in-place test, familiarization occurred over a 5-minute duration where animals were placed in the center of the apparatus and allowed to freely explore four distinct

objects each placed in a distinct corner of the apparatus. The animals were again returned to their homecages for a 90-minute waiting period followed by a 3-minute test where the placement of two of the four objects was changed.

For both tests, object exploration was defined as sniffing or touching the object, but not climbing, sitting, leaning, or standing on the objects. For both tests, center entries were recorded and the time spent with the novel or repositioned objects was converted into a "recognition index" for statistical analysis: novel object test = (amount of time spent with novel object)/(time spent with both objects); object-in-place test = (amount of time spent with repositioned objects)/(time with all objects).

4.2.5 Delayed non-matching-to-sample (DNMS) T-maze

The T-maze apparatus consisted of white Plexiglas walls and floor with three guillotine doors constructed of the same material. One door enclosed the starting box and was raised upon initiation of a trial and two other doors could be lowered once an animal entered a particular arm of the maze. The dimensions of the maze were as follows: length of central start arm, 60 cm; length of start box, 30 cm; length of left/right arms, 50 cm; width, 15 cm; and height, 25 cm.

Rats were food deprived to 85% body weight and maintained on a restricted diet throughout T-maze testing. Habituation sessions to the T-maze started by placing the animal in the start box, raising the guillotine door and allowing the animal to freely explore the maze for 5-minutes. Habituation sessions were conducted twice daily till animals readily ate a food pellet at the end of each goal arm (45 mg pellets; Noyes, Lancaster, NH). Following habituation, animals were trained 10 trials/session on the DNMS task. Each trial began with a forced-choice run where the animal was only allowed entry to one rewarded goal arm

(randomly chosen) and one free-choice run where the animal had free access to both goal arms but only the opposite arm from the forced-choice run was rewarded. A "choice" required an animal to place two front paws and one back paw into one of the arms; no retracing was permitted. If the animal had alternated correctly and chosen the arm with a food reward, it was allowed to consume the food pellet before being returned to his cage. However, if the animal failed to alternate to the other arm from the forced-choice trial, they were confined to that arm without food reinforcement for 10-sec before being returned to their homecage. Between runs there was no delay, except to wipe the maze with 70% isopropyl alcohol to reduce olfactory cues. Training acquisition was set to ≥70% correct responses (animal correctly chose the opposite arm as the forced-choice run on the free-choice run) during two consecutive days. Rats were then moved to the testing phase, where the number of trials was increased to 16 and a delay (10, 70, or 130s) was introduced between the runs. Each animal experienced one delay period per day, and each delay period was run twice (total of 6 testing days, two for each delay period). The number of trials to reach acquisition criterion and the percentage of correct responses of the three delays were calculated. Training and testing in this behavioral test is very time and labor-intensive (>2 weeks/6 animals) and the laboratory is only equipped to run animals in cohorts of 6. Thus, the remaining animals were run through additional behavioral tests discussed below and animals slated for testing in the T-maze were run through these tests following completion of the paradigm.

4.2.6 Pre-pulse Inhibition

The apparatus and procedures are similar to those described previously (Szumlinski et al., 2005), except the apparatus was fitted with 3.5 inch (inner diameter) cylinder tubes (8

inches, length) for rats. Animals were placed in sound-attenuated startle chambers (SR-LAB, San Diego Instruments) and six different trial types were presented: no pulse (st0; only background noise of 70 dB), startle pulse (st110; 110dB/40 msec), low prepulse stimulus (st74, 74 dB/20 msec; given alone), high prepulse (st90, 90 dB/20 msec; given alone), and st74 or st90 given 100 msec prior to the onset of the st110 startle pulse (pp74 and pp90, respectively). St0, st110, pp74, and pp90 trials were applied 10 times, while st74 and st90 trials were applied five times, in random order with an average intertrial interval of 15 sec (range: 10-20 sec). The startle amplitude data were averaged across stimulus trials for each rat and prepulse inhibition was calculated as the percent decrease in response to a 110 dB startle tone when it is preceded by either a 74 dB or 90 dB prepulse tone.

4.2.7 Within-Session Delay Discounting (WSDD)

Food training

To encourage lever-pressing behavior for the WSDD procedure, animals were first trained to lever press for food reinforcement (45 mg pellets; Noyes, Lancaster, NH). Animals were trained in standard operant conditioning chambers (30 x 20 x 24 cm high; Med Associates Inc., St. Albans, VT) containing two retractable levers with a stimulus light located above each lever, a food pellet dispenser located outside the operant chamber, a food trough between the levers, a house light on the opposite wall to the levers, and a fan to provide ventilation and mask extraneous noise. At the beginning of each 30-min training session, one lever was extended into the chamber; depression of the lever delivered a single food pellet on a FR1 schedule of reinforcement without the presentation of additional cues. Animals were trained daily on both levers (levers were randomly chosen on which was presented first in a day) till they reached ≥50 responses in a single session for both levers, at

which time they progressed to lever-training procedures. All experimental data were recorded using MedPC-IV software (Med-Associates, St. Albans, VT, USA).

Lever-training procedures

Lever-training procedures have been adapted from a previous publication (Zeeb, Floresco, & Winstanley, 2010). Each session began with the illumination of the houselight, followed by the extension of one lever 3-sec later. If the rat depressed the lever within 10-sec, the lever was retracted and scored as a success, a food pellet was delivered into the food trough, the houselight extinguished 4-sec later, and the chamber was left in darkness for the remainder of the trial. If the animal failed to act on the lever within the 10-sec window, the lever was retracted and scored as an omission, the houselight extinguished 4-sec later, and the chamber left in darkness for the remainder of the trial. Each trial lasted 40-sec, with a total of 90 trials per testing session, and with no more than two consecutive presentations of the same lever. Lever-training acquisition criterion was set at 80 successful trial completions, and the total number of days to reach acquisition was recorded as well.

Delay-discounting

Delay-discounting procedures are nearly identical to those described in Zeeb et al., (2010) and will be described briefly here. Each trial began with the illumination of the houselight and the extension of the lever(s) 2-sec later. Similar to lever-training, the rat had 10-sec to respond on the lever to receive food reinforcement before it was retracted; otherwise, the trial was scored as an omission and in both instances the houselight was extinguished and the remainder of the trial was in darkness. Responding on one lever (lever A) always resulted in the delivery of one food pellet, while the opposite lever (lever B) always resulted in the delivery of three food pellets delivered 0.5-sec apart; the position of

lever A and B were counterbalanced between subjects. Each session consisted of 4 blocks (56-minutes total) of 12 trials each (70-sec duration), with two forced-choice trials at the start of each block. In the forced trials, one lever was presented per trial (lever A and B presented randomly) to indicate to the animal the reward available for that lever and whether there was a delay between lever depression and reward delivery. In the first three testing sessions, both levers resulted in immediate rewards for all trials. Session 4 began testing on the delay-discounting program, where a response in the first block of trials resulted in immediate reward for responses on both levers, followed by blocks 2-4 where a delay was introduced for the large reward lever (block 2, 15-sec; block 3, 30-sec; block 4, 45-sec) and resulted in a delay between lever responses and delivery of the food reward. For blocks 2-4, where a delay was present for lever B (large reward lever), a cue light located above lever B was illuminated immediately after lever depression and for the duration of the delay. Sessions continued 6 days per week until stable baseline responding was achieved for five consecutive days.

4.2.8 Statistical Analysis

For the 2 object recognition tests, one-way ANOVAs of Viral Treatment were conducted for total distance traveled, time spent immobile, and number of center entries on Habituation Days 1 and 2, and for familiarization and testing days for each recognition test. One-way ANOVAs were also conducted for the recognition indices generated for the novel object and object-in-place tests. A T-test was conducted during familiarization of objects for the novel object and a one-way ANOVA was conducted during familiarization for the object-in-place test to ensure no bias towards one object or location existed prior to testing. For the DNMS T-maze, days to reach training criterion were analyzed by one-way ANOVA of Viral

Treatment and two-way ANOVAs were used to analyze Viral Treatment x Delay for the percent correct alternations. For PPI, two-way ANOVAs of Viral Treatment (between subjects factor) x Startle Amplitude (repeated measures, within subjects factor) for baseline startle responses were conducted, and one-way ANOVAs of Viral Treatment on PPI to the 74 dB and 90dB pre-pulses were conducted to examine for differences in sensorimotor gating.

For WSDD, similar data analysis methods were used as conducted by Zeeb et al. (2010) in that an arcsine transformation was performed on the number of responses per block prior to statistical analysis in order to limit the effect of an artificially imposed ceiling (e.g. ten choices per block). Stable baseline behavior was assessed by a non-significant effect of Session x Delay when the large reward lever (delay lever) was analyzed across 5 sessions by two-way ANOVA. The last five days of stable responding were averaged for each animal at each delay period and used for subsequent analyses. Two-way ANOVAs of Viral Treatment x Delay were conducted for behavior on the large reward lever, small reward lever, and total number of omissions. Tukey's multiple comparisons test was used for post-hoc comparisons of significant two-way ANOVAs and Fisher's LSD tests were conducted for one-way ANOVAs. One-way ANOVAs of Viral Treatment on protein expression for MBP and MOG was used to analyze viral mediated changes in protein expression.

4.3 Results

4.3.1 Object recognition tests (novel object and object-in-place)

Viral treatment did not significantly alter the total distance traveled, time spent immobile, or the number of center entries during the habituation phase of testing on either day 1 or 2 (One-way ANOVA of Viral Treatment, all p's>0.05; figure 10a).

During novel object familiarization, there was no statistically significant bias towards either of the identical objects [$t(52)=0.85$, $p>0.05$]. Nor was there an effect of Viral

Treatment on the total distance traveled, time spent immobile, or total number of center entries (one-way ANOVAs, p's>0.05). No group differences were observed for the recognition index in the novel object test (one-way ANOVA, p>0.05; figure 10b), nor were significant effects observed for the total distance traveled, time spent immobile, or center entries during the test session (one-way ANOVAs, p>0.05).

Similar to the novel object test, there were no significant effects of Viral Treatment upon object recognition when assessed in the object-in-place test. No object bias was revealed during familiarization nor were group differences observed for the recognition index for displaced objects during the test (one-way ANOVAs, p's>0.05; figure 10c). Group differences were also not apparent for the total distance traveled, time spent immobile, or number of center entries during either the familiarization or the recognition test phases of the study (one-way ANOVAs, p's>0.05).

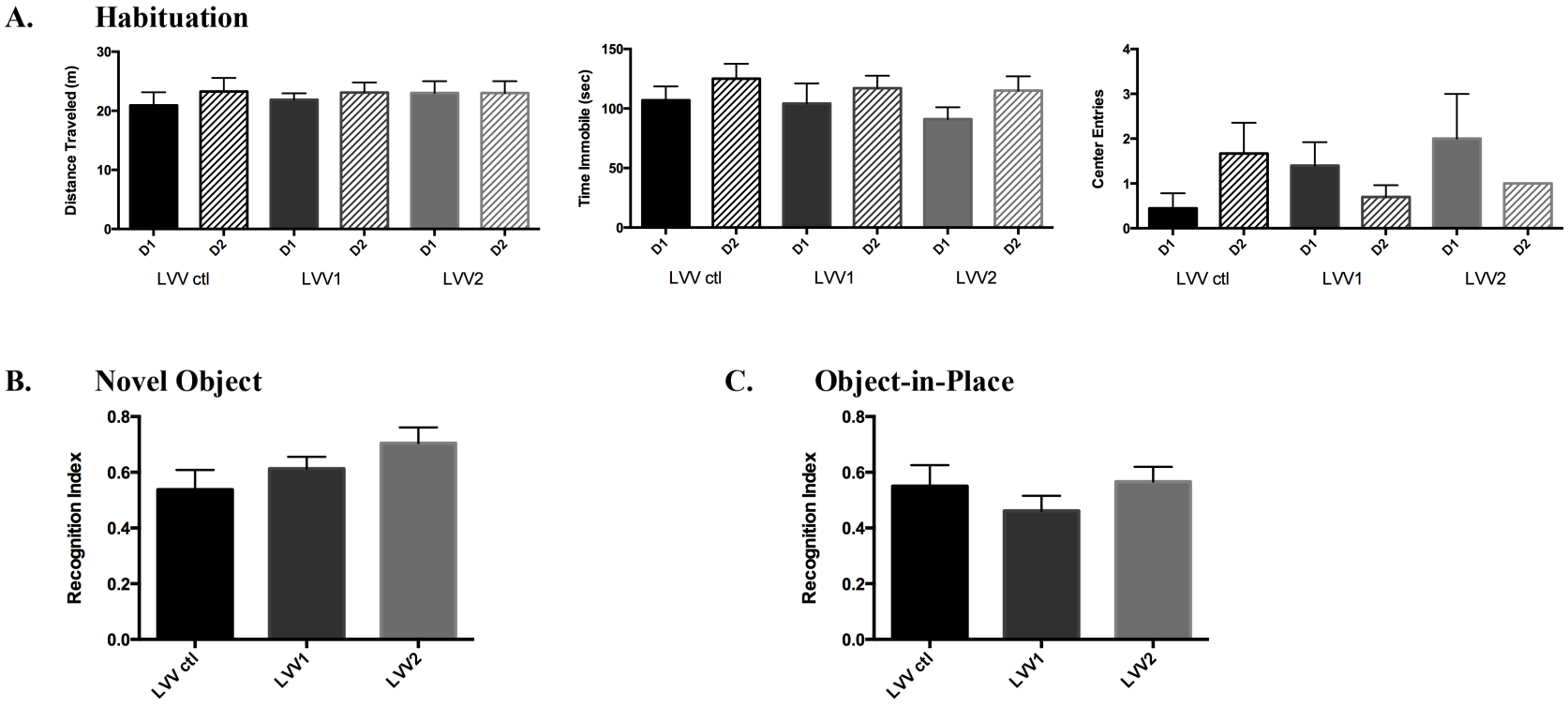


Figure 10. Summary of object recognition test results. (A) During habituation to the testing apparatus used for both the novel object and object-in-place tests, viral treatment (LVV ctl, LVV1, LVV2) failed to affect the total distance traveled, time spent immobile or the total number of center entries on either day 1 (D1) or day 2 (D2). (B) In the Novel Object recognition test, viral treatment failed to affect the amount of time animals spent with a novel object and scent following a 90 min delay between initial exposure and testing with a novel object and scent. (C) In the Object-in-Place test, viral treatment also failed to affect the amount of time animals spent with objects displaced during the test session after a 90 min delay from the initial exposure to objects in a particular orientation. Recognition Index: novel object test = (amount of time spent with novel object)/(time spent with both objects); Recognition Index: object-in-place test = (amount of time spent with repositioned objects)/(time with all objects). All p's >0.05.

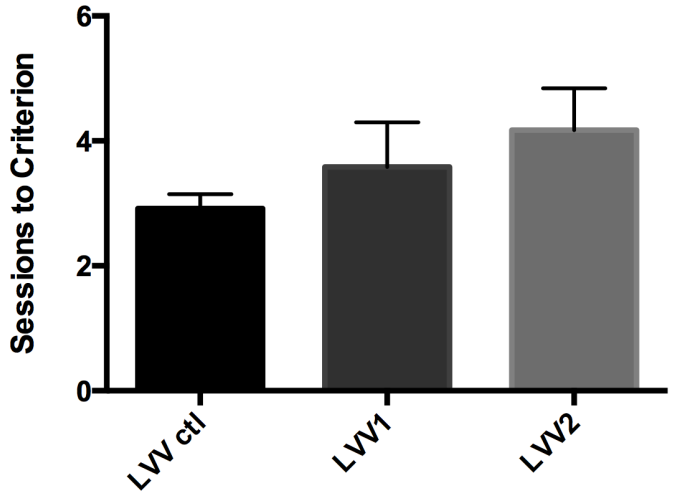
4.3.2 DNMS T-maze

Viral Treatment did not affect the total number of training sessions required to reach acquisition criterion (one-way ANOVA, $p>0.05$), nor was there an effect of Viral Treatment on the percent correct alternations in the T-maze (two-way ANOVA, $p's> 0.05$; figure 11a). Increasing the delay between the forced and choice trials (10sec, 70 sec, 130 sec) lead to a decrease in alternation accuracy for all groups (Figure 11b) [Delay effect: $F(2, 66)=4.51$, $p=0.015$; no Viral Treatment effect or Interaction: $p's>0.05$] with animals alternating less accurately at the 130 sec delay compared to the 10 sec delay [Tukey's multiple comparisons test, $p<0.05$).

4.3.3 Acoustic Startle and Pre-pulse Inhibition

Two-way ANOVAs of Viral Treatment x Startle Amplitude (repeated measures) failed to reveal a significant effect of Viral Treatment on sensorimotor gating either in basal activity in the chamber (0 dB) or in response to the 74, 90, or 110 dB tone intensities (all $p's>0.05$). One-way ANOVAs of Viral Treatment on the percent inhibition of the startle elicited by the 110 dB tone preceded by the 74 and 90 dB pre-pulses also failed to indicate group differences ($p>0.05$; figure 12).

A.



B.

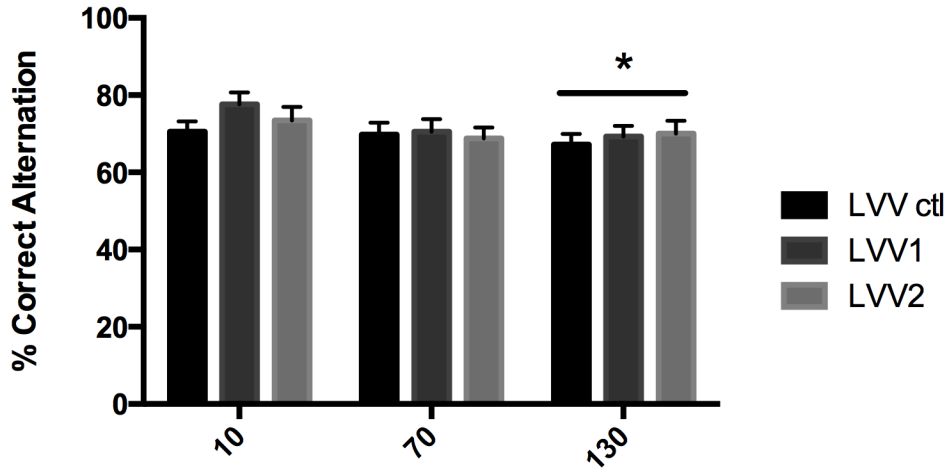


Figure 11. Summary of delayed non-matching to sample (DNMS) T-maze test results. (A) Viral treatment (LVV ctl, LVV1, LVV2) did not significantly affect the number of sessions required to meet the criterion of 2 consecutive days of $\geq$ 70% correct alternations between the forced run and choice runs ($p>0.05$). (B) Viral treatment also failed to affect the percent correct alternation (% Correct Alternation) when either a 10 sec (10), 70 sec (70) or 130 sec (130) delay was instituted between forced and choice runs (Viral Treatment, $p>0.05$). Rats did correctly alternate a greater proportion of choice runs when a 10 sec compared to 130 sec delay was instituted (* indicates a main effect of Delay, $p<0.05$).

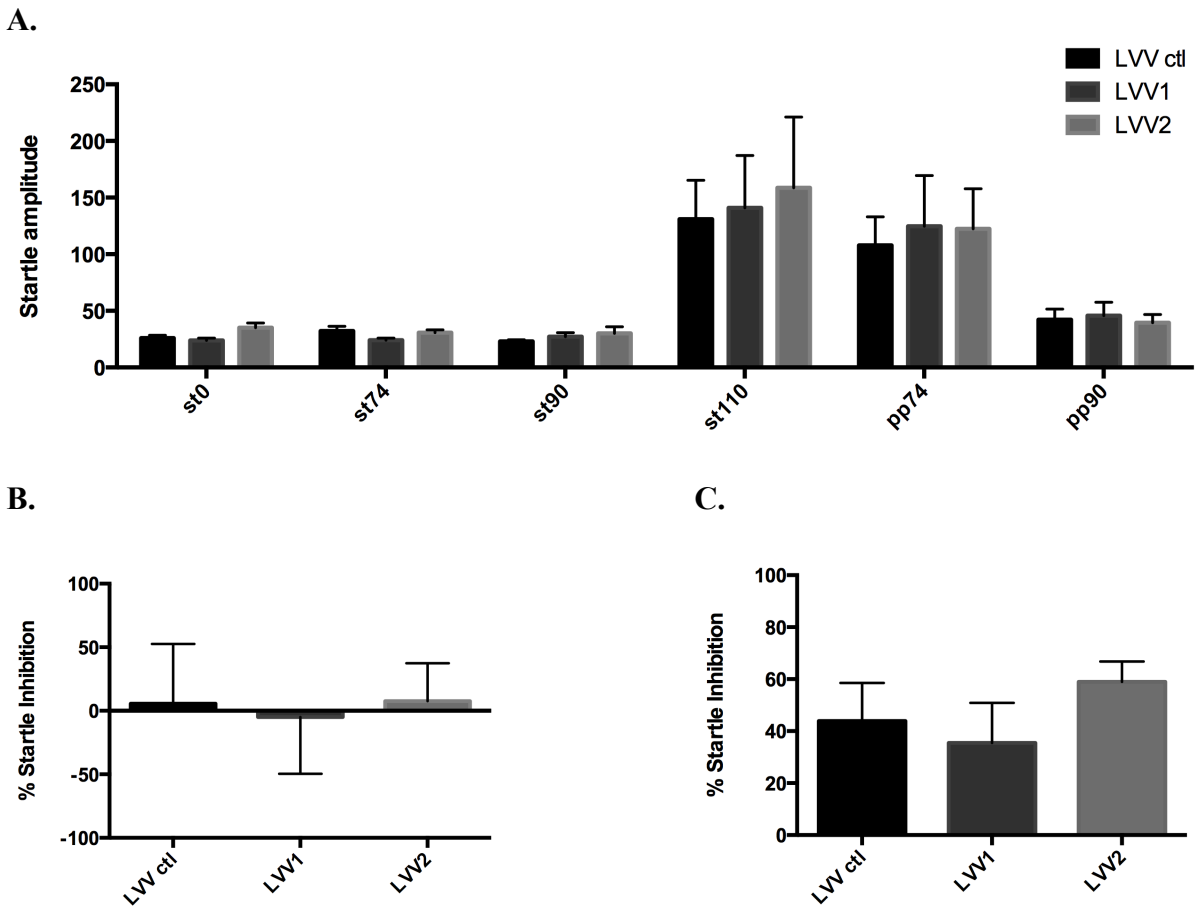


Figure 12. Summary of prepulse inhibition (PPI) test results. (A) Viral treatment (LVV ctl, LVV1, LVV2) failed to affect startle amplitudes in either basal activity in the chamber (0 dB) or in response to the 74, 90, or 110 dB tone intensities (all p's>0.05). (B) Viral treatment had no effect on percent inhibition of the startle elicited by the 110 dB tone when preceded by the 74 or (C) 90 dB pre-pulses (p's>0.05)

4.3.4 Within-session delayed discounting

The total number of sessions to reach food-training criterion for both right and left levers were combined to examine for differences in acquisition. One-way ANOVAs of Viral Treatment on sessions to food training criteria revealed a significant effect of Viral Treatment [$F(2, 33)=6.51$, $p=0.004$]. Fisher's LSD post-hoc tests revealed both LVV1- and LVV2-treated animals learned to self-administer food pellets faster than control virus-treated animals (figure 13a) (LVV1 vs. LVV ctl, $p=0.005$; LVV2 vs. LVV ctl, $p=0.003$).

The last 5 days of behavior on the large reward lever for each animal was determined to be stable as revealed by non-significant repeated measures two-way ANOVAs of Day x Delay (Day and Day x Delay, p's>0.05). However, animals did show a significant effect of Delay [$F(3, 144)=82.82$, $p<0.001$]. Post-hoc comparisons of delay time at each day revealed all delays to be significantly different from one another except the 30 sec and 45 sec delays (Tukey's multiple comparisons test, all days examined separately, $p<0.05$), indicating increased delay between behavior on the large reward lever and presentation of the reward resulted in animals responding less on that lever. Since behavior was stable over the last 5 days, each animal's responses over each delay for the last 5 days were averaged in order to compare Viral Treatment and Delay on lever responding behavior.

A two-way ANOVA of Viral Treatment x Delay for the average of the last five days of testing on the large reward lever, revealed significant main effects of Viral Treatment [$F(2, 136)=5.63$, $p=0.005$] and Delay [$F(3, 136)=85.99$, $p<0.001$], but no significant interaction (Viral Treatment x Delay, $p>0.05$; figure 13b). Overall, the LVV2 group exhibited the least impulsivity of the 3 groups tested, as indicated by greater total responses on the large reward lever across all delays (Fisher's LSD, LVV2 vs LVV ctl, $p=0.009$; LVV2 vs LVV1,

p=0.002). Post-hoc comparisons also revealed a delay-induced reduction in large reward lever responding, with behavior plateauing at the 30 sec delay (Fisher's LSD, p's≤0.05).

Consistently, the LVV2 treated group responded significantly less on the small reward lever than either LVV1 or LVV ctl animals revealed by an analysis of the small reward lever for the last five days of testing resulting in a main effect of Viral Treatment [F (2, 136)=8.51, p<0.001; Fisher's LSD, LVV2 vs LVV ctl, p<0.001; LVV2 vs LVV1, p<0.001]. Analysis of the small reward lever also revealed a significant main effect of Delay [F (3, 136)=38.35, p<0.001], but no interaction (Viral Treatment x Delay, p>0.05; figure 13c). Post-hoc comparisons revealed a delay-induced enhanced small reward lever responding, with behavior plateauing at the 30 sec delay (Fisher's LSD, p's≤0.05).

A two-way ANOVAs of Viral Treatment x Delay for the last five days of testing on omissions revealed a significant main effect of Delay [F (3, 136)=17.15, p<0.0001], but no effect of Viral Treatment or Interaction (p's >0.05; figure 13d). Post-hoc comparisons of Delay revealed a delay-induced enhancement in lever response omissions, with behavior plateauing at the 30 sec delay (Fisher's LSD, p's<0.05).

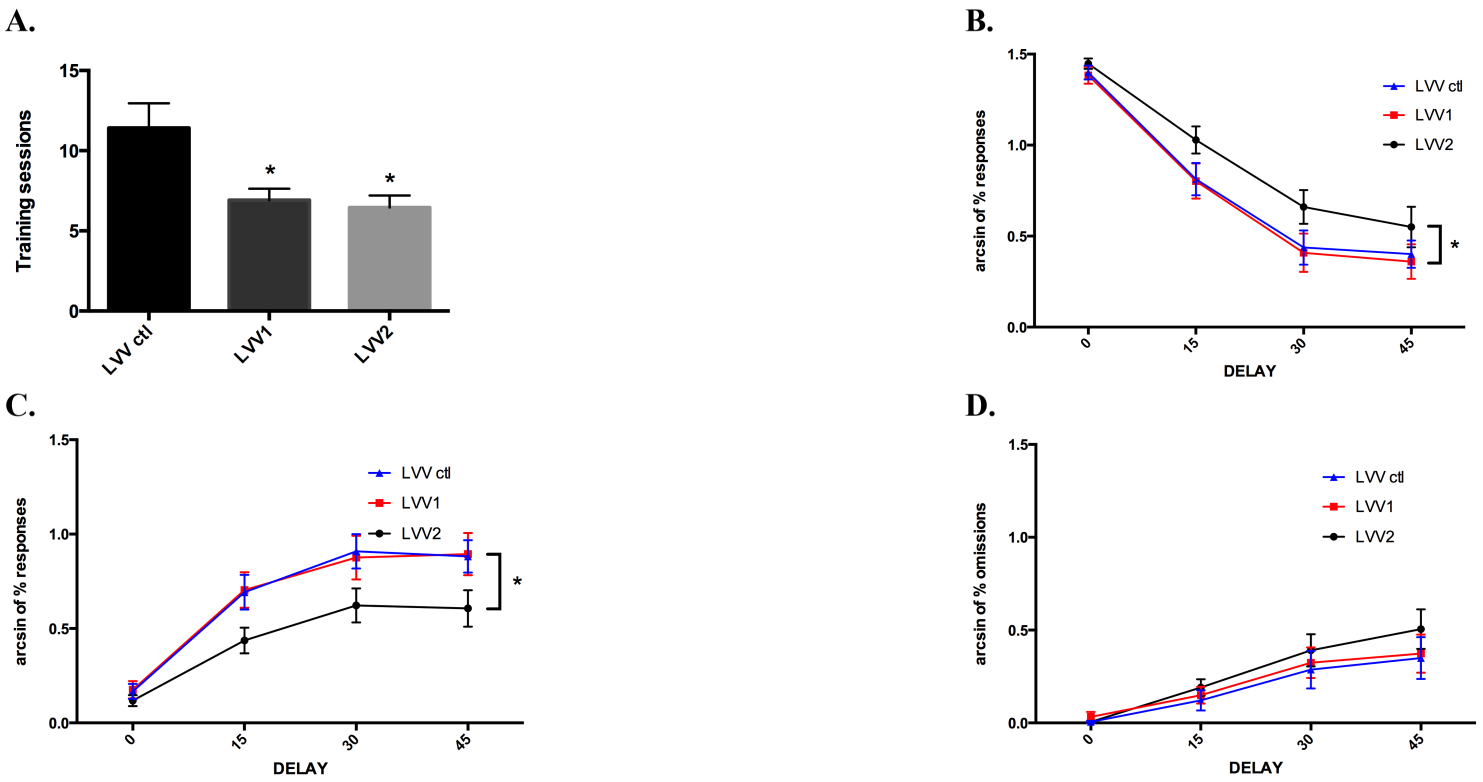


Figure 13. Summary of within-session delay discounting test results. (A) Viral treatment with either LVV1 or LVV2 significantly decreased the total number of training sessions required to meet food training acquisition criterion (>50 responses on the right and left levers, separately) versus rats infused with control virus (LVV ctl). (B) Animals treated with LVV2 pressed the large reward lever over all delays (in sec) more than LVV ctl and LVV1 animals. (C) Animals treated with LVV2 also pressed the small reward lever (no delay between behavior and food reinforcement) significantly less than LVV ctl and LVV1 animals. A shift upward on graph B and downward on graph C, indicate decreased impulsive choice in LVV 2 treated animals. (D) No difference was observed for the number of omitted responses across viral treatments. Across B, C, and D, there was a significant effect of Delay regardless of viral treatment (p's<0.05). Data used in statistical analysis for B, C, and D were arcsine transformed [arcsine (square root (% responses/10)], to limit the effect of an artificially imposed ceiling (maximum of 10 responses).

4.3.5 Verification of viral-mediated OL transduction by Western blotting

One-way ANOVA of Viral Treatment on MBP expression revealed a significant effect of Viral Treatment [$F(2, 41)=4.00$, $p=0.026$]. Fisher's LSD post-hoc analysis revealed significantly reduced MBP expression in LVV2 compared to LVV ctl ($p=0.015$), but no effect of LVV1 upon MBP expression ($p>0.05$). One-way ANOVA of Viral Treatment on MOG expression revealed a significant effect of Viral Treatment [$F(2, 25)=3.54$, $p=0.044$]. Fisher's LSD post-hoc analysis revealed significantly reduced MOG expression in LVV2 compared to LVV ctl (figure 14; $p=0.016$), but again, no effect of LVV1 on MOG levels.

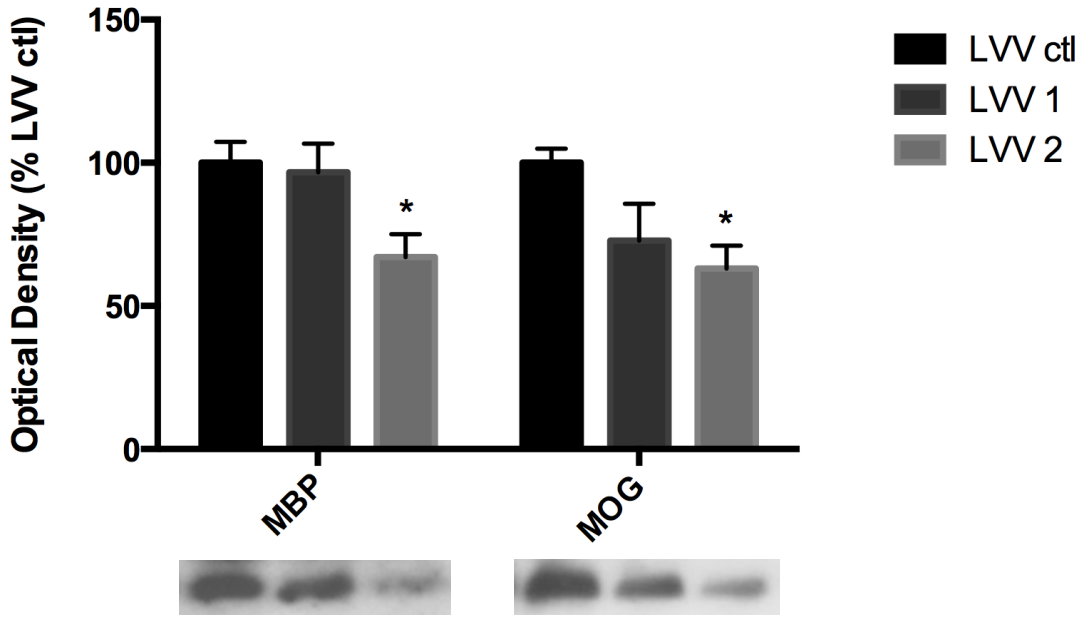


Figure 14. Immunoblotting to confirm MBP knock-down. LVV2 treated animals exhibited a significant decrease in MBP and MOG expression levels within the vmPFC approximately 4 months following virus microinjections, compared to LVV1 and control animals (LVV ctl). LVV1 animals did not show a significant decreased in either protein, compared to control animals. Data represent the optical density for each protein expressed as a percent of LVV ctl levels. Representative immunoblot images for each protein lay directly below the graphical representation of changes. *$p<0.05$.

4.4 Discussion

Contrary to the hypotheses proposed, virally mediated decreased MBP expression within the vmPFC did not impair working memory or learning within the object recognition tests or within the DNMS T-maze. Despite confirmation that our LVV2 construct significantly reduced MBP levels, LVV2 animals spent similar time interacting with new and displaced objects as familiar objects, did not differentially ambulate, and did not cross the center of the open field differently than controls. LVV2 animals also did not differ from controls in acquiring the T-maze task, nor were they differentially affected by an increase in delay between forced and choice trials. Surprisingly, decreased MBP expression lessened impulsive choice assessed in the WSDD task, opposite to the effect hypothesized for decreased myelin-integrity on vmPFC function and impulsive choice measures.

Distinct electrolytic lesions of the PL and IL subregions have been shown to decrease time spent in the center of an open field, and animals with IL lesions also showed a decrease in locomotor activity assessed via line crossings over a 10-minute test period in a circular open field apparatus (Jinks & McGregor, 2006). While we saw no differences in either of these dependent variables when MBP expression was decreased within the vmPFC, it is possible that this change in protein expression was not substantial enough to affect vmPFC function as an electrolytic lesion. Additionally, electrolytic lesions ablate fibers of passage, as well as local cellular populations, and would be predicted to have a greater impact than a specific OL manipulation. Moreover, given that we examined behavior in the open field over several sessions (two habituation sessions and two sessions per object recognition test), if increased anxiety-like behaviors had been a consequence of MBP manipulation, we would have expected this effect to be lasting and perhaps have been revealed between groups when the control animals habituated to the testing apparatus. Therefore, we do not believe that

decreased MBP expression within the vmPFC is affecting anxiety-like behavior mediated by this region that might confound performance in other memory-related tasks.

Our finding that MBP knock-down did not alter behavioral in object recognition tests is consistent with the results of excitotoxic lesion studies; large excitotoxic lesions centered on the PL (and encompassing the majority of the mPFC) failed to significantly alter rats' interaction with either a novel object or displaced objects in a study conducted by Ennaceur, Neave, & Aggleton, (1997). Though this study examined different delay periods than the current study (1 min or 15 min vs 90 min, respectively), the results are nonetheless consistent. In another type of object recognition task, in which different sensory modalities are examined in combination with PFC lesions, Reid, Jacklin, & Winters, (2014) observed a significant effect of impaired OFC function on object recognition, but failed to detect an effect of mPFC lesion. Specifically, animals were tested in a Y-maze where the exploration phase consisted of visual examination of the object (clear plastic barrier between rat and objects) or tactile examination (red light illumination to prevent accumulation of visual information), followed by a 1-hour delay prior to the choice interaction between the old and novel object. A cross-modal test was also conducted where rats were exposed to tactile information during the exploratory phase followed by visual information only during the choice phase. Rats with mPFC excitotoxic lesions showed the normal increase in exploratory behavior of novel objects across all conditions (a finding consistent with the present results), whereas, rats with OFC lesions failed to increase their exploratory behavior in the cross-modal condition when a 1-hr delay was instituted between exploratory and choice trial interactions. Together, these lesion data argue that the OFC, rather than the vmPFC, plays a more major role in recognition memory, arguing perhaps that the associations between

anomalies in vmPFC function and recognition memory observed in stimulant-experienced individuals might reflect a coincidence, rather than a cause-effect relation.

At the 10 sec delay of the DNMS T-maze, I observed no group differences on acquisition or percent correct alternation - a finding consistent with the results of lesion studies of the PL and IL at this delay time point (Delatour & Gisquet-Verrier, 2000; Delatour & Gisquet-Verrier, 1999; Delatour & Gisquet-Verrier, 1996; Dias & Aggleton, 2000; Li & Shao, 1998). However, ibotenic acid lesions of the vmPFC in rat have been shown to delay acquisition and impair performance on the T-maze when a 40 sec delay is incorporated between the sample or forced run and choice runs (Delatour & Gisquet-Verrier, 2000; Delatour & Gisquet-Verrier, 1996). When the last two training sessions in the Delatour & Gisquet-Verrier (2000) study were examined, no difference in percent correct choices were found between the sham and vmPFC lesioned groups, suggesting this deficit was overcome by the end of training. Given that more significant lesions induced by ibotenic acid result in performance deficits that are overcome with training, it is likely decreased MBP expression does not produce sufficient impairment of vmPFC function to reveal deficits in working memory, even when delays of 130 sec are instituted between forced and choice runs. Future studies could address this issue by infusing larger volumes or higher titers of virus to effect greater MBP knock-down than that produced in the study herein.

A consistent finding across studies of vmPFC damage is a deficit of reversal learning of new strategies once an original strategy has been established, reflected as behavioral inflexibility or attentional deficits within T-maze tests (Dias & Aggleton, 2000; Li & Shao, 1998). The failure to detect any effect of vmPFC knock-down upon behavior in the T-maze is a finding in line with the behavioral phenotype expressed by *Shiverer* and *mld* mice (which

do not express MBP or contain only one copy of the gene, respectively) that also fail to show deficits in learning the location of a food reward in the T-maze. However, these mutant mice do show deficits in reversal learning when the location of the food reward is changed and repeatedly revisit the originally rewarded arm (Inagawa et al., 1988). I did not conduct reversal learning procedures in my study and therefore, the possibility still exists that we may have detected an effect of decreased MBP expression had we further assessed the animals on a matching-to-sample task, following the non-matching to sample task. This issue can be explored in future research. Taken together, both the current findings and previous studies of do not reveal a role of MBP in the vmPFC for anxiety-related or working-memory related tasks.

Contrary to our hypothesis, we did not detect an effect of decreased MBP expression on sensorimotor gating. Consistent with the current studies, both ibotenic acid and excitotoxic lesions of the vmPFC within rats failed to affect startle responses or prepulse inhibition (Lacroix, Broersen, Weiner, & Feldon, 1998; Sullivan & Gratton, 2002). Both of these findings contrast with specific dopaminergic manipulations of the rat vmPFC, including dopamine receptor antagonist (SCH31966 or sulpiride) or agonist (apomorphine) microinfusions result in disrupted prepulse inhibition without affecting startle responses (Broersen, Feldon, & Weiner, 1999; Ellenbroek, Budde, & Cools, 1996). Interestingly, local mPFC infusions of amphetamine (indirect DA agonist) or cis-flupenthixol (DA antagoinst) failed to have an effect on PPI. It is unclear why specific pharmacological manipulations of the vmPFC would result in PPI deficits without all DA modulators affecting PPI and without also seeing an effect of lesioning this brain area, and it remains to be determined exactly how the vmPFC is functioning to mediate this behavioral phenomenon.

The only task in which we detected a significant effect of our MBP knockdown was in the WSDD task, in which the LVV2 group exhibited behavioral indices of decreased impulsive choice. While this finding is contrary to our hypothesis, a survey of the extant literature pertaining specifically to the role for the vmPFC in impulsive choice, as assessed with delay discounting, reveals inconsistent results. Administration of the D2 agonist quinpirole, or antagonist eticlopride, into the vmPFC (PL and IL) results in increased impulsive choice for the small, immediate reward over the larger delayed reward, but administration of the D1-like antagonist SCH23390, microinjected into the mPFC failed to have a significant effect on impulsive choice (Yates et al., 2014). However, Loos et al. (2010) found SCH23390 infusion into the vmPFC to increase impulsive choice. Excitotoxic lesions of the mPFC failed to increase impulsive choice for the smaller, immediate reward, however the lesions did produce a significant flattening of the within-session preference shift from large to small rewards (Cardinal, Pennicott, Sugathapala, Robbins, & Everitt, 2001). This latter lesion finding is similar to the current result that LVV2 animals with reduced MBP expression did not shift as readily to the smaller, immediate reward as did control animals. However, in other studies, reversible inactivation of the vmPFC, via muscimol microinjections, failed to effect impulsive choice and also did not "flatten" the preference shift in rats assessed in WSDD (Feja & Koch, 2014). Interestingly, muscimol microinjections into the vmPFC increased premature responses, perseverative responses, omissions and decreased accuracy on the 5-choice serial reaction time task (5-CSRTT) - effects consistent with those produced by NMDA receptor antagonism within the IL (Feja & Koch, 2014; Murphy, Dalley, & Robbins, 2005). This indicates the vmPFC, and specifically the IL subregion, may play a more important role in impulsive action (e.g. motor impulsivity

resulting in premature responding), than in impulsive choice or decision-making. Unfortunately, however, impulsive actions such as premature responding are not assessed in the WSDD paradigm. Moreover, heightened impulsive choice has been associated with poor working memory capabilities (Winstanley et al., 2010), and my LVV-infused animals exhibited neither behavioral anomaly. Therefore, future examination of the role of MBP within the vmPFC and specifically the IL, should focus on tasks that measure impulsive action such as the 5-CSRTT, continuous performance test, and go/no-go paradigms, instead of tasks that assess impulsive choice and perseveration (e.g. WSDD, DNMS T-maze) (Winstanley et al., 2010). Examination of impulsive action tasks may shed light on the current finding of increased food training acquisition seen in animals with decreased MBP expression and may relate to learning ability.

An alternative explanation to enhanced impulsive action underlying increased acquisition of food-training could relate to the role of the vmPFC (specifically, the IL) in consolidation of new behavioral-outcome contingencies. As reviewed in the General Introduction, enhanced glutamate signaling within the vmPFC (via AMPA or mGluR1/5 receptors) facilitates extinction learning while decreased signaling (via mGluR1/5 receptor antagonism) impairs extinction learning in cocaine-experienced animals (Ben-Shahar et al., 2013; Fontanez-Nuin et al., 2011; LaLumiere et al., 2010). Moreover, enhanced GABA signaling within the vmPFC impairs extinction learning (LaLumiere et al., 2010). Both glutamatergic and GABAergic effects on extinction are seen on a subsequent test day, suggesting the activity of this region is involved in the learning of new contingencies, consolidation of the memory of this new contingency and guiding subsequent behavioral output. Local infusions of the active viruses generating shRNA against MBP transcripts

would affect OLs around the site of infusion and the corresponding local axons which they myelinate. I would hypothesize this local change in myelin would mainly effect local inhibitory interneurons, as excitatory neuronal axons project out of the area of transfected OLs and the majority of their myelin would remain undisturbed. It is possible that the signaling efficiency of these local inhibitory neurons is reduced, ultimately reducing the GABAergic signal on pyramidal cells, and thereby, increasing the output of the vmPFC. As stated previously, increased IL activity results in enhanced extinction learning and may therefore explain the enhanced learning of food training seen in animals with decreased MBP expression. Along similar lines, decreased IL activity is associated with behavioral disinhibition (Feja & Koch, 2014; Ji & Neugebauer, 2012). Therefore, enhancing the activity of the vmPFC (via disrupting local inhibitory neurons) may partially explain the decreased impulsive choice exhibited by LVV2 rats in the within-session delay discounting procedure. Additionally, pharmacological manipulations of the PL and IL have shown these regions to differentially affect drug-seeking and impulsive-like behavior, and electrophysiological experiments have shown enhanced IL activity to decrease PL activity (Ji & Neugebauer, 2012). Given the above, future assessment of the cognitive effects of decreased MBP expression might be more fruitful if the knock-down is localized to the IL specifically, as this subregion appears to be more consistently implicated in behavioral inhibition of relevance to impulsive action.

A molecular target of particular interest for future study is PLP, as this protein also exhibited decreased expression following extended access cocaine self-administration and whose genetic deletion results in a variety of cognitive deficits. As reviewed in the General Introduction, mice lacking PLP exhibit abnormal anxiety-like behaviors in the elevated-plus

maze, light/dark box, and social interaction test, along with mildly impaired PPI and robust memory deficits exhibited on the T-maze (Tanaka et al., 2009). Although these mice do not express PLP throughout their life, these robust deficits, coupled with my evidence for an enduring cocaine-induced reduction in vmPFC PLP expression, warrant a more systematic examination of cognitive deficits when PLP is experimentally manipulated within distinct brain regions in adult animals. Moreover, a combination of decreased MBP and PLP within the vmPFC may better model the cocaine-induced decreased expression and potentially highlight associated cognitive deficits.

Overall, the present results do not suggest a deficit of vmPFC function following decreased MBP expression, but perhaps still suggest a change in signaling efficiency locally. Future studies will need to focus on tests where vmPFC damage (e.g. lesion studies) have been consistently shown to affect behavioral output, instead of PFC-dependent tests that are not specific to the PL or IL. Additionally, future studies will need define what the observed decreased impulsive choice means, for example, are the animals perseverating on the large reward lever, are they less sensitive to the effect of delay on devaluing the incentive motivational properties of the reward, or perhaps these animals exhibit a deficit in reversal learning impairing them from withholding a response on the large reward lever. Further characterization of the structural consequences of decreased MBP expression in adult rats, along with electrophysiological approaches and comprehensive behavioral testing is needed before we have a clearer understanding of the role for MBP in cognitive, emotional, sensorimotor and motivational processing.

Chapter 5 Decreased MBP expression within the adult rat vmPFC does not affect cocaine self-administration, extinction, or reinstatement behavior.

5.1 Introduction

As reviewed in Chapter 1, chronic cocaine use is associated with deficits in executive function mediated by the PFC. In humans, disruptions in vmPFC function by physical assault or cocaine use results in deficits in working memory, decision-making tasks (e.g. gambling tasks), and measures of impulsivity (e.g. delay discounting, go/no-go tasks). Moreover, imaging studies of chronic cocaine addicts demonstrate altered PFC neural activity during these tasks that predict relapse following a period of abstinence. The PFC functional anomalies might relate to changes in myelination as there is evidence for both reduced white matter within the PFC of chronic cocaine users and decreased expression of myelin-related proteins in postmortem tissue from cocaine addicts (Albertson et al., 2004; Bartzokis, Beckson, Lu, Edwards, & Bridge, 2002; Kristiansen et al., 2009; Lehrmann et al., 2003; Lim et al., 2008, 2002; Moeller et al., 2005, 2007). Due the limitations of imaging methodology and the interpretational confounds inherent in studying humans, it is difficult to determine the distinct contribution of particular PFC subregions to the cognitive deficits associated with chronic cocaine use and impossible to decipher the cellular and molecular underpinnings of perturbed PFC function.

Animal models of cocaine addiction enable the delineation of cause-effect relations between cocaine-taking and the functional status of particular brain regions, as well as the ability to systemically examine the underpinnings of cocaine's effects upon brain function. As reviewed in Chapters 1 and 4, cocaine produces also anomalies in PFC function in rodent models of cocaine addiction. Consistent with reduced PFC function following cocaine experience, I observed decreased expression of myelin-related proteins within the vmPFC in rats with a 10-day history of extended access to IV cocaine (Expt 1 & 3b) that may impart abnormal neuronal signaling. While the results of Chapter 4 fail to indicate marked effects of

MBP knock-down upon PFC-dependent behaviors in cocaine-naïve animals, those negative data do not preclude the possibility that the lower MBP expression observed within the vmPFC of cocaine-experienced rats might underpin an aspect of drug-taking or –seeking behavior. Indeed, there is behavioral neuropharmacological evidence indicating that manipulations of the vmPFC influences cocaine acquisition, extinction and reinstatement (Gass & Chandler, 2013; Schenk et al., 1991; Weissenborn et al., 1997). Thus, I hypothesize that a reduction in vmPFC MBP levels will facilitate addiction-related behaviors in rats as manifested by increased drug intake, as well as increased drug-seeking during abstinence and in response to stimuli that trigger relapse.

To address this hypothesis, I employed the well-characterized extinction-reinstatement model of drug relapse, which enabled a within-subjects approach to studying the effects of vmPFC MBP knock-down upon: 1) the acquisition and maintenance of drug-intake; 2) the dose-response function for drug-intake; 3) the extinction of drug-seeking; and 4) the capacity of cocaine-associated cues and non-contingent cocaine priming injections to reinstate drug-seeking behavior, following extinction.

5.2 Methods

5.2.1 Animals and Surgical Procedures

Animals were identical to those described in Chapter 2 with the following exceptions. Animals were allowed to acclimate to the colony room for two days following arrival before surgical procedures began, were handled daily for the duration of the experiment, had *ad libitum* access to food and water, and were surgically implanted with IV catheters. Surgical implantation of guide cannulae and LVV infusion was identical to that described in Chapter 4.

Two weeks were allowed for recovery from intracranial surgeries and to allow the LVV to transduce before IV catheter implantation. Under 2-3% isoflurane anesthesia, animals were implanted with a chronic silastic catheter as previously described in Chapter 2.

5.2.2 Self-Administration Training

Three-weeks post-LVV infusions (and 1 week following jugular catheter implantation), animals (n=10-15 per group at the start of each experiment) were trained to self-administer IV cocaine (0.25 mg/0.1 ml/ infusion; National Institute on Drug Abuse, Bethesda, MD) during daily 2-hr sessions, on a FR1 schedule of reinforcement. Animals were trained in identical standard operant conditioning chambers as described in Chapter 2. At the beginning of each session, the animal's catheter was connected to a motorized pump via a liquid swivel as previously described and the self-administration program was identical to that described in Chapter 2. One exception was the session length, which was shortened to 2 hrs/day to increase the feasibility of the study. Self-administration training was conducted daily until animals reached stable self-administration levels (<10% variation in the number of infusions earned across 3 days), at which time, the dose of cocaine was varied in order to generate a within-subjects dose-response function to determine the effect of MBP knock-down upon cocaine sensitivity. For this, rats were allowed to self-administer a lower cocaine dose (0.125 mg/0.1ml/infusion) for 5 consecutive days and then the dose was raised to 0.50 mg/0.1 ml/infusion for an additional 5 days.

5.2.3 Extinction and Reinstatement procedures

Following the dose-response phase of study, I next determined whether or not MBP knock-down influenced an animal's ability to learn to suppress responding on the cocaine-associated lever in the absence of cocaine availability. Responding under such extinction conditions is considered to reflect drug-seeking behavior and is interpreted as drug-craving.

To extinguish responding, the animals entered 2-hr, daily, extinction training sessions during which the animals were tethered, but responding on the active lever resulted in no programmed consequences (i.e., no cocaine infusion or presentation of the cocaine-paired cues). Behavior was considered extinguished when animals emitted $\leq$25 active lever presses within a session, across 2 consecutive days. Once animals had met this criterion, they underwent a cue-primed reinstatement of cocaine-seeking test. At the beginning of the cue-prime test, a 20-sec presentation of the tone and light compound stimulus previously paired with cocaine-delivery was presented. Active lever presses resulted in additional cue-presentations on an FR1 schedule of reinforcement, but no cocaine delivery. Following this cue-primed test, lever-pressing behavior was again extinguished by daily testing in the absence of cocaine or cocaine-paired cues and then animals were subjected to tests for cocaine-primed reinstatement. For cocaine-primed reinstatement, rats were injected IP with 5 mg/kg and 15 mg/kg cocaine (tests separated by a phase of extinction training), immediately before tethering the animals in the operant chambers for 2 hrs. During cocaine-primed reinstatement testing, active lever presses resulted in neither cocaine delivery nor the presentation of the cocaine-paired cues. Throughout training, maintenance, dose-response testing, extinction and reinstatement testing, inactive lever presses were recorded, but had no programmed consequences.

5.2.4 Imaging of LVV transduction

To verify transduction and placement of microinjections within the vmPFC, animals undergoing behavioral testing were anesthetized with 2-3% isoflurane, decapitated and brain tissue removed and stored in a solution of 4% paraformaldehyde and 30% sucrose for a minimum of 3 days. Brain tissue encompassing the vmPFC was sectioned at 50 μm and

examined at 4X, 40X, and 60X magnification under a fluorescent microscope to visualize the location and extent of cellular transduction (figure 15).

5.2.5. Statistical Analysis

To examine for differences in LVV treatment on acquisition and maintenance of cocaine self-administration, a two-way ANOVA of Viral Treatment x Day was conducted on the proportion of active lever presses (Active lever presses/Total lever presses, expressed as a percent) and the total number of infusions across the first 9 days of self-administration. To examine the cocaine dose-response sensitivity for each Viral treatment, a two-way ANOVA of Viral Treatment x Dose was conducted on the proportion of active lever presses and infusions. A one-way ANOVA of Viral Treatment on the total number of days to initially extinguish lever response behavior was conducted for the proportion of active lever presses. The last two days of each extinction phase prior to reinstatement procedures were averaged for each animal and used to compare against behavior during the reinstatement tests. A two-way ANOVA of Viral Treatment x Phase was conducted for the proportion of active lever presses to assess for differences in LVV on reinstatement of cocaine-seeking.

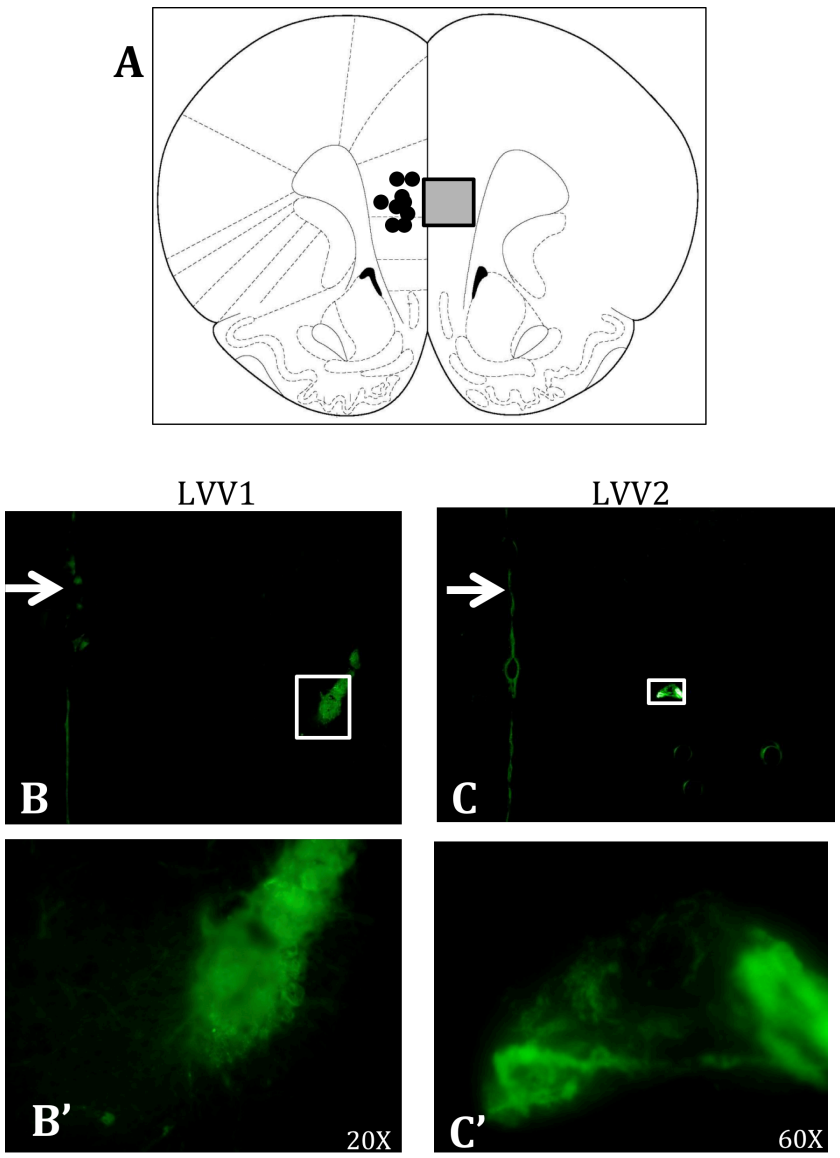


Figure 15. Summary of histological findings. A) Cresyl violet-stained tissue sections (50 uM) through the PFC were examined from a subset of LVV-infused rats as an index of microinjector tip location. The majority of the microinjector tips (black dots) were localized within the PL, with no evidence for localization within the IL. (B and C) In vivo transduction efficacy by, and spread of, the LVV constructs within the PL were verified by immunofluorescence for the GFP reporter. Representative micrographs (4× magnification) of tissue sections from 2 different rats revealed very localized transduction around the point of microinjection with little to no spread. Arrows point to the midline of the brain. (B') 40× magnification of the GFP immunofluorescence from the rat in panel B. (C') 60X magnification of the GFP immunofluorescence from the rat in panel C. In all cases, the GFP immunofluorescence was diffuse and it was difficult to discern discrete cellular anatomy.

5.3 Results

5.3.1 Self-administration acquisition and cocaine dose-response function

A two-way, repeated measures ANOVA of infusions earning during the first 9 days of self-administration training failed to demonstrate a change in intake across days or a difference in intake among virally treated animals (no Main effects or Interaction effects, p's>0.05). Thus, MBP knock-down within the vmPFC did not alter the stable levels of cocaine-taking observed during the acquisition phase of cocaine self-administration (figure 16a). However, analysis of the proportion of total lever-presses directed at the active lever (an index of reinforcement) over the first 9 days of training revealed a significant progressive shift in response allocation [Day effect: $F(8,176)=9.24$, $p<0.001$], which varied as a function of viral treatment [Viral Treatment x Day, $F(16,176)=1.88$, $p=0.025$], (figure 16b). Deconstruction of the significant interaction along the Day factor revealed significant virus differences on Days 1 and 3-5, with Fisher's LSD post-hoc tests indicating lower reinforcement in LVV2 vs. LVV clt on Day 1 ($p=0.048$), and greater reinforcement on Day 4 ($p=0.041$) and Day 5 ($p=0.040$) and in LVV2 vs. LVV1 on Day 3 ($p=0.010$). Thus, although MBP knock-down did not influence the total amount of cocaine administered at the 0.25 mg/inf training dose, LVV2 treated animals exhibited signs of greater cocaine reinforcement during self-administration training.

To examine whether or not MBP knock-down influenced the dose-response functions for cocaine-directed behavior and intake, two-way ANOVAs of Viral Treatment x Dose were conducted on the ratio of active vs. total lever-presses and on the number of infusions earned during the dose-response phase of study. The number of cocaine infusions earned decreased as a function of cocaine dose [$F(2,70)=463.50$, $p<0.001$; all doses significantly different from one another, LSD, p's <0.05], but we observed no significant main effect of Viral

Treatment or an interaction effect (p's>0.05; figure 16d). Group differences were not observed regarding the allocation of lever-pressing behavior during dose-response testing, with rats in all groups emitting nearly 100% of their responses on the active lever, regardless of dose and regardless of LVV infusions (figure 16d; all p's>0.05). Thus, MBP knock-down within the vmPFC does not appear to influence sensitivity to the reinforcing properties of cocaine nor does it alter the dose-response function for cocaine intake, at least at the doses assessed in this study.

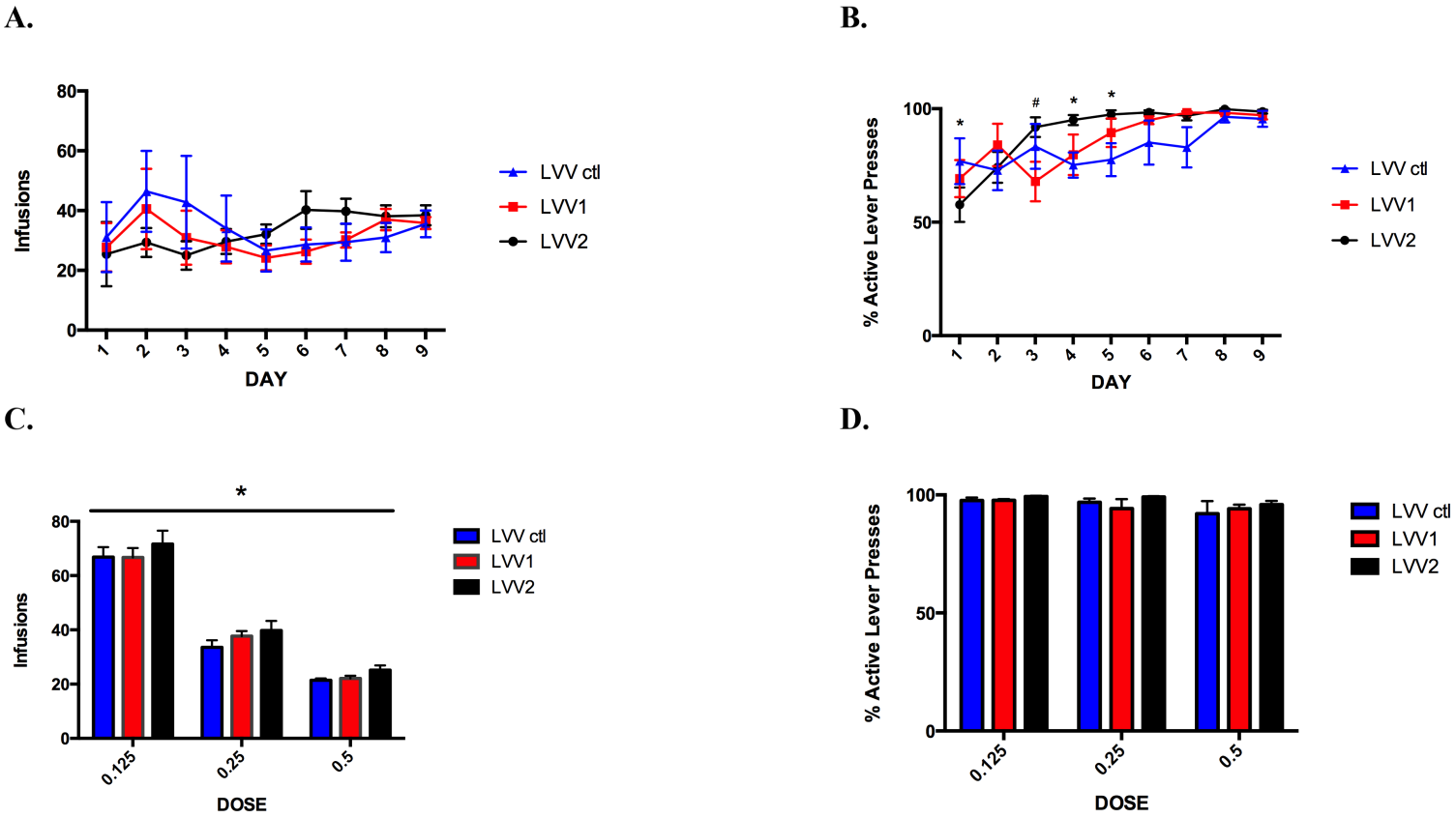


Figure 16. Summary of the effects of viral treatment on the acquisition and maintenance of cocaine self-administration behavior. (A) The total number of cocaine infusions (0.25 mg/inf) was stable during the first 9 days of cocaine self-administration training and no group differences were noted for cocaine intake. (B) All groups exhibited a progressive increase in the proportion of total lever presses directed at the active lever during self-administration training (%Active Lever Presses). However, LVV2 rats exhibited the lowest goal-directed behavior on day 1 of training, but the highest goal-directed behavior on days 4-5 (*$p<0.05$ vs. LVV ctl; #$p<0.05$ vs. LLV1), (C) The total number of infusions administered decreased with ascending dose of cocaine and this dose-dependent effect was similar in all treatments. (D) The proportion of active:total lever presses was neither dose- nor virus-dependent.

5.3.2 Extinction and Reinstatement

To examine whether or not MBP knock-down influenced the ability to learn to suppress drug-seeking behavior in the absence of cocaine and cocaine-paired cues, we examined for group differences in the rate of extinction learning as indicated by the number of days to reach extinction criterion. However, group differences were not observed for the number of days to initially extinguish lever responding (one-way ANOVA, $p>0.05$); LVV ctl, 5.92 $\pm$ 0.79; LVV1, 4.71$\pm$ 0.45; LVV2, 5.91 $\pm$ 1.12). Thus, MBP knock-down does not appear to influence the ability to learn to suppress drug-seeking behavior in the absence of cocaine delivery or the presentation of the cocaine-paired cues.

I next examined whether or not vmPFC MBP knock-down influenced drug-seeking behavior in response to cocaine-paired cues or cocaine priming injections. A two-way ANOVA of Viral Treatment x Phase revealed the expected main effect of Phase (figure 17) [$F(3,127)=16.36$, $p<0.001$], with rats in all groups exhibiting greater active lever responding during the Cue- and 15 mg/kg cocaine-primed reinstatement tests versus that exhibited by the rats during the last 2 days of extinction training, indicating that these procedures reinstated cocaine-seeking behavior. In contrast, the level of responding exhibited following the 5 mg/kg cocaine priming injection was not significantly different from that exhibited during extinction (figure 17; $p>0.05$), indicating that this cocaine dose was not sufficient to reinstate responding. Viral Treatment did not significantly impact behavior during any phase of testing (p's>0.05; figure 17). Thus, MBP knock-down within the vmPFC does not influence the capacity of either cocaine or cocaine-associated cues to reinstate drug-seeking behavior, following extinction.

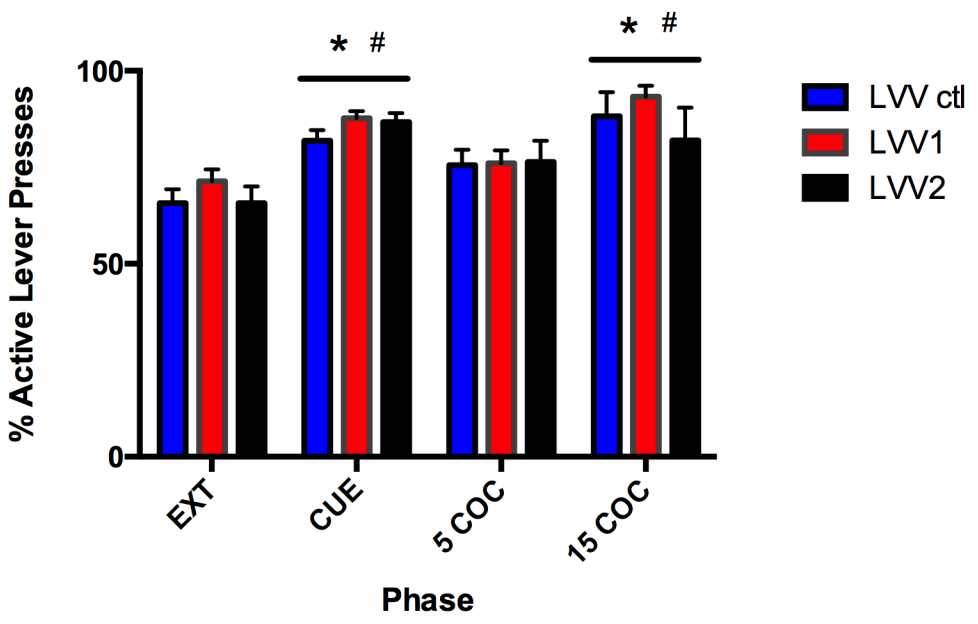


Figure 17. Summary of viral treatment on extinction and reinstatement. Viral treatment did not significantly affect goal-directed behavior across extinction or reinstatement tests as indicated by no group differences at any time during testing. . The total lever-presses directed at the active lever (% Active lever presses) during reinstatement tests elicited by presentation of the cocaine-paired cue (CUE) and a 15 mg/kg cocaine injection (15 COC) was higher than both that exhibited during the last 2 days of extinction training (EXT) and that elicited by a 5 mg/kg cocaine priming injection (5 COC), *$p<0.05$ to EXT; #$p<0.05$ to 5 COC.

5.4 Discussion

In the following series of experiments, I failed to find a significant effect of MBP knock-down within the adult rat vmPFC on cocaine intake during the acquisition and maintenance of cocaine self-administration when 0.25 mg/inf cocaine was available, nor did I observe any knock-down effect upon cocaine intake across a range of doses. MBP knock-down within the vmPFC influenced cocaine-directed behavior (indexed by the % total lever-presses directed at the active lever) only during the first 5 days of self-administration training, but no effect of knock-down was apparent during later training, dose-response testing, extinction training or testing for cue- or cocaine-primed reinstatement. These data argue that the reduction in vmPFC MBP expression observed in cocaine self-administering rats (Chapter 2) does not appear to contribute significantly to the manifestation of addiction-related behaviors in a well-validated animal model of cocaine-taking and relapse. The failure to observe robust and consistent effects of shRNA infusion upon addiction-related behavior does not reflect a failure of our shRNA constructs to transduce OLs within the vmPFC as immunohistochemical evidence supported OL transduction within the brains of the cocaine self-administering animals and the immunoblotting results of Chapter 4 indicated the effectiveness of the LVV2 construct in reducing MBP expression in cocaine-naïve animals. However, the volume of tissue showing viral transduction is small and only encompasses a minor portion of the PL. Therefore, the mostly negative findings herein may likely reflect an insufficiency of knock-down to disrupt vmPFC activity as it relates to cocaine-self-administration, extinction and reinstatement procedures.

In Chapter 4, I provided immunoblotting evidence that LVV2 infusions caused a roughly 30% reduction in MBP within the vmPFC, a decrease that closely mirrors that produced by a history of cocaine-taking under long-access procedures in Chapter 2 (Expt 1

and 3b). However, the data in Chapter 4 clearly indicate that a 30% knockdown of MBP was insufficient to cause obvious cognitive disruption in drug-naïve animals, and only moderately decreased impulsive choice and enhanced food acquisition training. Consistent with this latter result for food-induced responding, MBP knock-down facilitated cocaine reinforcement early during the acquisition of cocaine self-administration, as indexed by an increased proportion of total lever responses directed at the active lever in LVV2 rats, versus one or the other groups tested. Immunoblotting was not conducted in cocaine-experienced animals to avoid interpretational confounds associated with drug-induced changes in MBP expression. While the detection of the GFP reporter by immunofluorescence in cocaine-experienced animals argues that both shRNA constructs transduced cells within the PL, the relative *in vivo* efficacy of the two shRNA constructs cannot be ascertained by immunohistochemical procedures. Given that identical LVV infusion procedures were employed in this study of cocaine-experienced animals as those employed in the drug-naïve rats described in Chapter 4, it is presumed that LVV2 elicited an ~ 30% knock-down of MBP protein expression in cocaine-experienced animals, while LVV1 and the control construct had no effect on MBP expression. The observation that only LVV2 influenced, albeit moderately and transiently, cocaine reinforcement during early training is consistent with the presumption that only LVV2 impacted MBP protein expression. However, the fact that cellular transduction was observed within vmPFC of all 3 virus groups upon completion of behavioral testing but the effect of LVV2 upon behavior was transient in both this series of cocaine experiments, as well as during behavioral testing in cocaine-naïve animals (Chapter 4), demonstrates that if the ~30% reduction in MBP expression produced by LVV2 was sufficient to impair vmPFC function to influence certain behaviors (e.g., augment instrumental responding for positive

reinforcers), this functional impairment could be over-come by subsequent training. However, it is important to highlight again the discrete location of LVV transduction, as a more pronounced effect of LVV2 may be seen if a larger area had been transduced by the virus.

The studies outlined in Chapters 4 and 5 are the first to ascertain the effects of local knock-down of MBP and this assumption and the extent of protein knock-down induced by either shRNA construct cannot be known. There currently exists only one publication examining the effect of decreased MBP on cognitive function of which mice lacking complete expression of MBP RNA (*Shiverer*) or significantly reduced levels (*mld*) exhibit a deficit in reversal learning that is proportionate to the amount of MBP expressed (less MBP corresponds with greater learning deficits), but maintain the ability to learn the original location of a food reward in a T-maze (Inagawa et al., 1988). *Mld* mice express 2% MBP RNA (compared to wild-type control mice), but exhibit a significant improvement in reversal learning compared to *Shiverer* mice that produce 0% MBP RNA (Inagawa et al., 1988; Popko et al., 1987). Moreover, as low as 25% MBP RNA of normal is sufficient to produce intermediate levels of myelination assessed by electron microscopy (Popko et al., 1987). Therefore, only a ~30% decrease in protein expression (~70% expression of normal levels) in the current study is unlikely to cause a robust deficit in vmPFC function and may explain the lack of effect on cocaine self-administration, extinction and reinstatement behaviors.

Several other myelin specific proteins were also affected by cocaine administration (e.g. MOG, NG2+, PLP) in conjunction with MBP, raising the possibility of a cumulative effect of decreased myelin-related protein expression on vmPFC function as it relates to cocaine self-administration, extinction and reinstatement procedures. One promising

candidate is PLP as discussed in Chapter 4, as mice lacking PLP exhibit abnormal anxiety-like behaviors, mildly impaired sensorimotor gating, and robust memory deficits on the T-maze (Tanaka et al., 2009). Given the limited number of publications regarding behavioral deficits following complete loss of MBP and PLP, and that changes in neuronal conduction have only been studied in cultures completely lacking expression of myelin specific proteins (e.g. OMgp, PLP, & MBP), it is difficult at this time to interpret what a partial loss of one protein (e.g. MBP) or the cumulative effect of multiple protein decreases may mean for the function of a brain region (Boiko et al., 2001; Inagawa et al., 1988; Nie et al., 2006; Tanaka et al., 2009, 2006).

Targeting MBP expression within the vmPFC of the current experiment was based on the cocaine-induced decreased expression discovered in Chapter 2. However, specifically targeting this brain region may not fully elucidate the behavioral ramifications of a loss of this protein. In the few experiments that have examined for cocaine-related decreased myelin-protein expression, the focus was on the white matter within the anterior striatal level of rhesus monkeys, the splenium of the CC and the NAc (tissue punches often containing the anterior commissure), where the decrease in MBP and myelin-related mRNA transcripts represents myelin abnormalities within fiber tracts containing the axons from many brain areas (Kovalevich et al., 2012; Narayana et al., 2009; Smith et al., 2014). It is possible that the current local manipulation of MBP within the vmPFC does not create substantial enough loss of myelin integrity to promote vmPFC dysfunction, and by targeting larger fiber tracts (e.g. CC) to reduce neuronal signaling efficiency affecting input from a variety of brain regions, a deficit in MBP may become more apparent. As fiber tracts were not specifically sampled in the original immunoblotting procedures of Chapter 2, additional experiments

sampling the rostrum, genu, and splenium of the CC would need to be conducted to determine whether or not a history of long-access cocaine intake produce similar changes in MBP mRNA and protein expression as those found in the previous references discussed.

Chapter 6 General Discussion

Summary of Studies and Findings

The data presented in this dissertation are the first to examine for an effect of long-access (6h/day) cocaine self-administration on glial-related protein expression within the PFC of adult and adolescent male rats. Adult rats tested for cue-elicited cocaine-seeking during a 2-hr test were found to have decreased myelin-specific protein expression within the vmPFC (IL and ventral PL); this effect was present at 3 days withdrawal and persisted for at least 30 days. These myelin-specific changes were not found within the dmPFC (dorsal PL and ACC), nor were they observed within the NAc shell or core subregions. A follow-up study was designed to ascertain whether or not the reduction in myelin-specific protein expression exhibited by adult cocaine-experienced animals reflected a pharmacodynamic response to cocaine or some interaction between cocaine and the test for drug-seeking in which rats were not subjected to the cocaine-seeking test procedures. Interestingly, adults not experiencing a cocaine-seeking test prior to sacrifice exhibited increased myelin-specific protein expression within the vmPFC, as well as enhanced expression of the astrocytic proteins that regulate glutamate recycling and internal communication of signaling molecules (GS and Cx30, respectively). As the possibility existed that differences in the localization of the tissue punches between the 2 immunoblotting experiments and the different times at which the experiments were conducted may have underpinned the discrepancies in results, I subsequently ran a replication study to directly compare the expression of one particular protein, MBP, in rats subjected to the cocaine-seeking test versus those that were test-naïve. To determine whether or not cocaine-induced changes in MBP protein expression might reflect alterations in gene transcription, MBP mRNA levels were also examined. MBP was specifically chosen based on consistent findings in both the preclinical animal and clinical

literature indicating decreased MBP expression following a history of cocaine (Albertson et al., 2004; Kovalevich et al., 2012; Narayana et al., 2009; Smith et al., 2014). While I did not find an effect of cocaine on MBP transcription, I did replicate my initial finding of decreased protein expression following cocaine experience. Consistent with the available literature (Albertson et al., 2004; Kovalevich et al., 2012; Narayana et al., 2009; Smith et al., 2014), the cocaine-induced decrease in MBP expression was observed regardless of whether the animals were tested for drug-seeking prior to sacrifice. From the results obtained in Chapter 2, I concluded that a history of excessive IV cocaine administration is sufficient to produce an enduring reduction in the total protein expression of MBP, specifically within the vmPFC. Moreover, I concluded that this pharmacodynamic response to cocaine does not reflect a reduction in gene transcription and thus, likely reflects increased protein turn-over, the mechanism(s) of which still require elucidation. I also concluded that the cocaine-elicited reduction in vmPFC MBP expression was temporally dissociated from the manifestation of "incubated" cocaine-seeking and thus, was unlikely to underpin the development of this behavioral phenomenon.

Adolescence is a period of robust forebrain myelination that is characterized by a high prevalence of risk-taking, including drug-taking (Crews & Boettiger, 2009; Perry et al., 2007). Adolescents typically consume more drug than adults and this greater drug consumption is theorized to relate to their neural immaturity, but likely also reflects glial immaturity. Thus, studies in Chapter 3 determined whether or not the more immature adolescent brain might be more susceptible to the effects of cocaine-taking on glial-related proteins. Remarkably, I did not find any effect of cocaine on myelin-related proteins within the vmPFC of adolescent male rats, but a decrease in the microglial marker CD11b, and the

non-specific OL precursor cell marker NG2+. The failure to observe changes in myelin-specific proteins was unexpected given that adolescence is a time of major PFC maturation and myelination; however, this was not the case. In support of the relative immaturity of the PFC during cocaine self-administration testing of adolescents, I observed increases to occur over the course of the 30-day withdrawal period for many myelin-specific proteins within the dmPFC of both cocaine- and saline-self-administering rats. While these latter data highlight the adolescent period as an important time for increased myelin protein expression within dmPFC that may reflect enhanced myelination, the present results clearly do not support a greater sensitivity of adolescents to the effects of cocaine upon protein indices of myelin integrity and if anything, argue that the relative immaturity of the adolescent PFC may render it less susceptible to cocaine-associated anomalies in myelin-specific proteins and perhaps also myelination. This latter conclusion is cautionary, however, as I only examined for cocaine-induced changes in protein expression within mPFC subregions at 3 and 30 days withdrawal (i.e., in young adulthood). As myelination in humans continues into the 40's, it remains to be determined whether or not adolescent cocaine experience might perturb the later developmental trajectory of myelin-related protein expression/myelination within frontal cortex to impact the function of this structure and behavior in later adulthood.

To determine the functional relevance of reduced MBP expression within the vmPFC for behaviors associated with the function of this region, I next experimentally manipulated MBP protein levels by microinjecting LVV particles capable of generating shRNAs against MBP mRNA. While the ultimate goal was to assess the effect of decreased MBP levels on cocaine addiction-related behaviors, I deemed it important at the outset of behavioral testing to examine for LVV effects upon general cognitive, motivational, emotional and

sensorimotor processing to facilitate interpretation of the results of subsequent cocaine studies. Pilot immunoblotting data indicated that of the 5 commercially available LVV particles, the LVV1 and LVV2 particles were the only particles with any *in vivo* knock-down efficiency (at least under our microinfusion procedures), although LVV1 was less efficacious than LVV2 at reducing MBP expression in this pilot study. Nevertheless, I tested adult rats for effects of infusing either shRNA construct into the vmPFC on working memory, anxiety-like behavior, learning and impulsive choice measures. Post-mortem determination of *in vivo* knock-down efficiency within a subset of rats in this study using immunoblotting confirmed significant knock-down of MBP by LVV2 within micropunches of tissue from the vmPFC (~30% reduction in total protein expression of the major 18.5 kDa MBP isoform), while knock-down of this isoform was not detected in LVV1-infused animals. Despite confirming a ~30% reduction in MBP expression within vmPFC of LVV2 rats, LVV2 infusion failed to affect a majority of behaviors tested, with the exceptions of facilitated acquisition of instrumental responding for food and decreased impulsive choice within the WSDD paradigm. Thus, I concluded from the results of Chapter 4 that LVV2 is more efficacious than LVV1 at reducing MBP expression *in vivo*, however, a 30% reduction in MBP within the vmPFC is likely not sufficient to impact robustly the function of this region, at least in drug-naïve subjects. Nevertheless, I clearly established that my intra-vmPFC LVV infusions procedures impacted little the primary reinforcing properties of food/the ability to acquire an operant response, motor activity, working spatial memory, long-term spatial memory, sensorimotor or emotional processing, all of which might influence drug-taking or –seeking behavior.

As a history of cocaine reduced MBP expression within the vmPFC by ~30% (Chapter 2), in the final experiment, I examined the possibility that MBP knock-down within the vmPFC might facilitate cocaine addiction-related behavior. For this, I employed identical LVV infusion procedures as conducted in Chapter 4, presuming that LVV2 would elicit a ~30% reduction in MBP expression akin to that produced by a history of cocaine-taking and impact behavior, while LVV1 would likely be without effect upon MBP expression or behavior. As the results of Chapter 2 already demonstrated that the manifestation of "incubated" drug-craving produced by a history of long-access cocaine self-administration could be temporally dissociated from drug-elicited changes in vmPFC MBP expression, I opted to study the effects of vmPFC MBP knock-down upon the acquisition and maintenance of cocaine self-administration under short-access self-administration procedures, which also facilitated the study of the effects of virus infusion upon cocaine dose sensitivity, as well as the extinction and reinstatement of cocaine-seeking. Immunohistochemical evidence from a subset of rats supported transduction within discrete areas around the microinjector tip within the vmPFC (mainly PL localization) by all 3 LVVs in cocaine-experienced animals. However, akin to the results of Chapter 4, the effect of vmPFC knock-down of MBP on cocaine reinforcement was modest at best and only manifested as a transient increase in cocaine-directed responding during early self-administration training. I observed absolutely no effect of LVV infusion upon drug-taking across a range of cocaine doses, nor did I observe any effect of LVV infusion upon measures of drug-seeking in the absence of IV cocaine. Presuming that the *in vivo* knock-down efficiencies of the LVVs were equivalent in this cocaine study as in the study of cocaine-naïve animals, these data indicate that a ~ 30% reduction in MBP expression is insufficient to promote higher drug-taking or relapse.

However, the small zone of transduction left the majority of the PL intact and may account for the lack of significant behavioral findings.

Extrapolating these findings back to the immunoblotting results from Chapter 2, it is not likely that the ~30% reduction in vmPFC MBP expression observed in highly cocaine-experienced rats contributes majorly to any aspect of their "addicted" phenotype and may represent an epiphenomenon of cocaine experience with no causal relation. While the present results do not support a critical role for MBP in regulating various aspects of cocaine addiction-related behavior, the present results by no means preclude the possibilities that (1) a reduction in vmPFC MBP expression might influence drug-taking behavior under procedures that engender very high drug consumption (e.g., long-access models), (2) the possibility that cocaine-elicited perturbations in MBP, in conjunction with other anomalies in myelin-specific protein expression, might impact more robustly drug-taking and –seeking behaviors, and/or (3) the use of larger infusion volumes of viral treatment might transduce a larger volume of tissue resulting in changes in cocaine addiction-related behavior. Given that cocaine-induced reductions in myelin-specific protein expression persist into protracted withdrawal, future study of the potential relevance of this glioadaptation and accompanying protein changes are warranted.

A Reflection Upon Past Knowledge

As discussed in the General Introduction, the current state of our knowledge regarding the effects of cocaine on OLs, and specifically, on myelin-related protein expression, is very limited. At the outset of this dissertation, only three post-mortem clinical studies existed reporting on the expression of myelin-related proteins in the brains of cocaine users. The results of these studies revealed decreased MBP, MOBP, PLP, and CLN within

the striatum and changes in PLP expression within the dlPFC, the direction of which appeared to depend upon whether or not the individual was dependent upon cocaine or upon crack/cocaine (Albertson et al., 2004; Kristiansen et al., 2009; Lehrmann et al., 2003). Moreover, *in vivo* imaging studies of chronic cocaine addicted humans consistently reveal decreased white matter within the inferior frontal white matter and CC (Bartzokis et al., 2002; Lim et al., 2008, 2002; Moeller et al., 2005, 2007), which is consistent with the post-mortem results for myelin-related protein expression. Although this collection of clinical data supports the notion that a history of cocaine-taking perturbs myelin, particularly in forebrain, the reliance on self-reported cocaine and other drug use, in conjunction with confounding subject factors of age of first drug use, other environmental insults, poverty, mental/physical abuse, comorbid mental disorders, etc., render it impossible to derive cause-effect relations between cocaine use in humans and OL physiology. Therefore, the use of animal models of cocaine addiction is essential to the investigation of cocaine on OLs.

To my knowledge, there are five publications regarding the effects of cocaine administration (either experimenter- or self-administered) on changes to OL-related gene transcription and protein levels in animal models (George et al., 2008; Kovalevich et al., 2012; Narayana et al., 2009; Nielsen et al., 2012; Smith et al., 2014). Consistent with the immunoblotting results presented in Chapter 2 for rats with a 10-day history of extended access cocaine self-administration, decreased MBP protein and/or mRNA expression following cocaine has been reported within the white matter of the NAc and CC in a study of cocaine self-administering non-human primates (300 days of cocaine experience), or in mice injected with cocaine for 14-28 days, (Kovalevich et al., 2012; Narayana et al., 2009; Smith et al., 2014). The present results extend these earlier findings, as well as the results from

post-mortem studies of humans, by demonstrating that a relatively short history of cocaine-taking (10 days) is sufficient to reduce protein expression of myelin-specfic proteins at least within the vmPFC. Moreover, my findings demonstrate that the cocaine-induced reduction in myelin-specific protein expression persist into protracted withdrawal in adult cocaine-experienced animals. Lastly, my results demonstrate that cocaine-induced changes in myelin-specific proteins can be observed from tissue sampled from grey matter, indicating that a history of cocaine-taking is capable of also influencing myelin integrity in points of synaptic contact that is presumed to influence local circuitry (see discussion below).

In addition to the 3 animal studies highlighted above, George et al. (2008) reported a decrease in NG2+ immunoreactive cells within tissue containing the ACC, PL and IL regions from the PFC of rats with a more extensive IV cocaine self-administration history than that employed herein (6-20, 1h sessions + a minimum of 85, 6h sessions; 0.5 mg/kg/inf cocaine). My results indicating that a 10-day history of IV cocaine (0.25 mg/inf; 6 hrs/day) decreased NG2+ protein expression within vmPFC (PL/IL) extend these latter findings of George et al. (2008) by demonstrating that (1) months of cocaine self-administration experience are not required in order to observe a cocaine-induced reduction in NG2+ protein expression; (2) the cocaine-induced reduction in NG2+ protein expression persists into protracted withdrawal; and (3) NG2+ protein expression responds to cocaine in a similar manner in adolescents as in adults. NG2+ protein serves a marker for OL precursors, astrocytes, mature NG2+ cells, and neurons (though, not commonly found colocalized with neuronal markers.). While the results of the present series of immunoblotting experiments cannot discern the origin of the NG2+ signal, the capacity of self-administered cocaine to lower NG2+ levels within the PFC of both adults (George et al., 2008; Chapter 2) and adolescents (Chapter 3) could reflect a drug-

induced reduction in NG2+-type cells, a reduction in immature OLs, astrocytes, or neurons, and future studies could seek to clarify the nature of this protein adaptation using double- or triple-labeling of proteins and immunohistochemical approaches in order to have a deeper understanding of how cocaine self-administration history influences cellular integrity within PFC subregions.

Of the human and rodent research to date, only two studies have drawn correlations between decreased changes in indices of myelin integrity due to cocaine experience and cognitive deficits. Moeller et al. (2005) found increased impulsivity measured on the Barratt Impulsiveness Scale-II in cocaine users with decreased fractional anisotropy measured by DTI within the anterior CC. Moreover, George et al. (2008) found working memory function positively correlated with the number of NG2+ immunoreactive cells within their tissue derived from the entire PFC (e.g., ACC, PL, and IL), as well as within the OFC of rats. These correlations indicate a relation between the functional status of glia within forebrain and cognitive function and this relation supports cocaine-elicited changes in glial function within forebrain in the cognitive deficits reported in cocaine-experienced individuals. Chapters 4 and 5 are the first studies, in my knowledge, to examine for a direct effect of manipulating OL-specific protein expression within the vmPFC on cognitive function and cocaine addiction-related behaviors. Although a reduction of MBP produced few significant findings, I did see a decrease in impulsive choice, enhanced acquisition of food training, and a slight increase in lever preference during acquisition of cocaine self-administration. Decreased impulsive choice seen in Chapter 4 is similar to the flattening of the large reward preference in animals with mPFC excitotoxic lesions (Cardinal et al., 2001), but contrasts with the effects of pharmacological manipulations of DA and GABA systems that either enhance or

do not significantly alter impulsive-like behavior (Feja & Koch, 2014; Loos et al., 2010; Yates et al., 2014). Electrophysiological experiments have shown an inhibitory influence of the IL on the PL (Ji & Neugebauer, 2012), therefore some of the discrepancy regarding whether the vmPFC has a role in impulsive choice may stem from manipulations that encompass both regions instead of refining manipulations to distinct subregions. As my viral infusions were localized to a small portion of the PL, decreasing MBP levels within the PL (specifically) appears to account for the decreased impulsive choice also noted in rats with larger excitotoxic lesions of the mPFC (Cardinal et al., 2001).

Although the effects were few, it is exciting to demonstrate a behavioral change by the manipulation of a single OL protein within a defined brain region in rats. These findings further highlight the importance of understanding how cocaine-related changes in OL protein expression contribute to the etiology of cocaine addiction, and normal cognitive function. As has been discussed, there are very few published papers examining for non-neuronal changes following cocaine, and even fewer looking at the functional significance glial-specific protein expression changes on behavior, making the interpretation of the data presented herein difficult and mostly conjecture. Advances in biotechnology have revealed how the extensive network of processes from a single OL can myelinate up to 60 internodal segments, impact axonal diameter via OL-specific proteins, promote normal node of Ranvier formation, and partially determine action potential propagation speed (Boiko et al., 2001; Mathey, Arthur, & Armati, 2010; Nie et al., 2007; Sadahiro et al., 2000; Tanaka et al., 2009). Given their widespread reach, it is likely that a loss of or damage to OLs could produce a significant affect on neuronal processing and the overall output of a brain region, ultimately affecting behavior. Moreover, limiting the study of addiction to neuronal mechanisms neither

facilitates a complete understanding of the disease nor reveals the full array of targets for therapeutic treatment. Therefore, not only is it imperative for research to continue elucidating the functions of OLs in normal physiology and their dynamic interactions with neurons and other glial cell types, but also how these cells are impacted during disease and the functional significance of those changes.

Future Directions

The data presented in this dissertation just begins to uncover the effects of cocaine on nonneuronal cells, and OLs specifically. Several inconsistencies from the immunoblotting results in Chapters 2 and 3 will need to be addressed prior to further testing of the functional significance of glial-specific protein changes. First, although I replicated the results in Expt 1 showing that both cue-tested and test-naïve animals exhibit decreased MBP levels following long-access cocaine self-administration, there was not sufficient tissue to reexamine MOG, NG2+, PLP or CLN levels to verify a specific effect of cocaine. It is interesting that NG2+ was decreased within the vmPFC of both adults (cue-tested) and adolescents (test-naïve) within Chapters 2 and 3, respectively, and NG2+ immunoreactive cells were decreased within the ACC, PL, and IL combined regions and OFC of the George et al. (2008) study. As discussed previously, a decrease in NG2+ does not necessarily implicate a change in OLs, however, this consistent finding warrants further study as to whether this is evidence of OL precursor cell loss or enhanced maturation of these cells to mature OLs. NG2+ positive cells represent a unique target of investigation as they have been shown to differentiate into OLs, astrocytes and neurons, form synaptic specializations with neurons, express GABA and glutamate receptors and can generate action potentials (Fields, 2008; Trotter et al., 2010);

their role in normal brain function and their impact on cocaine-related behaviors remain unresolved questions.

Future investigation into the OL-specific protein changes following cocaine will need to address whether myelin protein expression is affected in the absence of withdrawal and whether these changes persist beyond 30 days. Though I did not see an effect of withdrawal on protein expression, which argues against reduced OL-specific protein changes as underpinning the "incubation of craving" phenomenon, the persistence of these changes may underlie the enduring anomalies of frontal activity observed in chronic cocaine users and the propensity for relapse even after substantial periods of abstinence (Bechara & Damasio, 2002). Moreover, correlational studies examining the impact of cocaine on OL proteins and cognitive function (such as that of George et al., 2008) may help define the specific behaviors and tests to examine for the functional significance of changes when experimentally manipulating single proteins or combinations of proteins within distinct brain regions in the future.

While we examined changes in OL proteins, whose decrease would be hypothesized to cause a loss of myelin integrity, there are currently no studies specifically examining for general structural changes to myelin following cocaine. The composition of lipids and proportion of MBP to lipid content determines the strength of the adhesive properties and stability of synthetic membrane preparations (Hu et al., 2004; Min et al., 2009). Moreover, animals with complete knockout of PLP, MBP, CNP, and myelin associated glycoprotein have regions of loosely stacked and uncompact myelin, reduced spiraling, and distorted and inconsistent periodicity (Edgar et al., 2009; Inoue, Nakamura, Mikoshiba, & Tsukada, 1981, 1982; Uschkureit, Sporkel, Stracke, Bussow, & Stoffel, 2000). Therefore, the fact that long-

access cocaine self-administration was shown in this dissertation to disrupt the normal levels of myelin structural proteins highlights the importance of looking directly at the myelin for obvious cocaine-induced abnormalities. Though, MBP only decreased by ~30%, and this is not likely to greatly impact the integrity of myelin given even 25% mRNA expression of normal is sufficient to produce intermediate levels of myelination, a combination of myelin-related protein changes may lead to greater structural deficits. These basic characterization studies need to be conducted prior to the generation of hypotheses related to the potential impact on neuronal signaling efficiency and ultimately the activity of brain regions that affect behavioral output.

The expression levels of proteins OMgp, and PLP have been shown to affect the formation of nodes of Ranvier and conduction velocity of nerve impulses (Boiko et al., 2001; Nie et al., 2007). It remains to be seen whether a partial loss of protein expression also produces deficiencies in neuronal conduction. Though the initial experiments would need to be conducted in cell culture preparations, similar LVV microinfusion experiments could be used in the future to assess the electrophysiological properties of brain regions connected to the LVV transduced structure and examine for alterations in neurotransmission via microdialysis procedures. As rats withdrawn from extended cocaine administration produces an increased resting membrane potential and lack of the characteristic bistable neuronal membrane properties within the mPFC (Trantham et al., 2002), it would be intriguing to see if LVV2 infusion into the vmPFC mimicked any of these cocaine-induced properties. Changes in basal glutamate and DA tone within structures such as the PFC and NAc may not only reflect cocaine-induced alterations in receptor composition, but may also reflect changes in the signaling efficiency of afferents. While this is highly speculative, it could be possible

that inefficient neuronal conduction due to myelin anomalies may partially underlie decreased release of neurotransmitters. To provide preliminary evidence for such a mechanism, infusion of LVVs against MBP into the CC (containing a large proportion of axonal fibers) may produce a substantial enough decrease in overall myelin integrity to examine for changes in the electrophysiological properties of brain regions receiving afferents via these tracts and specific changes in neurotransmission by looking at basal neurotransmitter tone. Moreover, examining a combination of decreased myelin-specific proteins found to decrease following cocaine on these measures might highlight a general impact of OLs on brain function as it relates to cocaine addiction. Of course, it would be prudent to examine for cocaine-induced changes in myelin-related proteins within the CC prior to looking at the general consequences of changing myelin-specific protein expression.

Specifically decreasing MBP within the vmPFC had few behavioral consequences, while this likely reflects a minor contribution of specific MBP decreases on vmPFC function, the use of additional behavioral tests may expose further effects. For example, inactivation (muscimol) or NMDA receptor antagonism of the IL has been shown to increase impulsive action assessed on the 5-CSRTT task revealed by premature and perseverative responding and decreased accuracy (Feja & Koch, 2014; Murphy et al., 2005). Moreover, knockout of MBP (*Shiverer* and *mld*) has been shown to negatively affect reversal learning on the T-maze (Inagawa et al., 1988). Therefore, behavioral tasks that examine for deficits in specific brain loci versus general PFC dysfunction, may indicate a stronger effect of MBP expression changes.

The small zone of transduction following bilateral infusion of 1 ul/hemisphere into the PL, begs the question of whether a larger region of transduced cells would promote more

robust behavioral changes. Future studies that systematically test how infusion volume influences the extent of transduction, could define an optimal dose to decrease MBP throughout the extent of the PL and/or IL. Following, such viral transduction characterization, the studies presented herein could be replicated to examine for more pronounced effects on impulsive choice and cocaine-paired lever responses during acquisition. Defining the appropriate volume of virus to infuse for the size of area targeted, would promote a subregional analysis of decreased MBP on behavior that may help in elucidating the functional significance of MBP and specific brain loci.

Conclusion

In summary, the data within this dissertation represent an initial investigation into the consequences of cocaine on non-neuronal cells and specifically on proteins expressed within OLs. The results indicate that cocaine decreases myelin-related proteins within the vmPFC of adult male rats undergoing long-access cocaine self-administration and withdrawal procedures, although the reliability of these findings need to be replicated in future experiments. This dissertation also represents a first query to the functional consequences of reduced OL protein expression following cocaine exposure. While specifically decreasing MBP within the vmPFC (mainly PL) produced few behavioral consequences, I did show enhanced food training and responding during cocaine acquisition and a decrease in impulsive choice. Although this work is preliminary, it is expected to inspire future investigation into the role nonneuronal cells have on cocaine addiction and as potential targets for the generation of therapeutic agents for the prolonged and debilitating consequences of chronic cocaine use.

References

Ahmed, S. H., & Koob, G. F. (1998). Transition from moderate to excessive drug intake: change in hedonic set point. *Science*, *282*(5387), 298–300. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9765157

Ahmed, S. H., Lutjens, R., van der Stap, L. D., Lekic, D., Romano-Spica, V., Morales, M., … Sanna, P. P. (2005). Gene expression evidence for remodeling of lateral hypothalamic circuitry in cocaine addiction. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(32), 11533–8. doi:10.1073/pnas.0504438102

Akiyama, K., Ichinose, S., Omori, A., Sakurai, Y., & Asou, H. (2002). Study of expression of myelin basic proteins (MBPs) in developing rat brain using a novel antibody reacting with four major isoforms of MBP. *Journal of Neuroscience Research*, *68*(1), 19–28. doi:10.1002/jnr.10188

Albertson, D. N., Pruetz, B., Schmidt, C. J., Kuhn, D. M., Kapatos, G., & Bannon, M. J. (2004). Gene expression profile of the nucleus accumbens of human cocaine abusers: evidence for dysregulation of myelin. *Journal of Neurochemistry*, *88*(5), 1211–1219. doi:10.1046/j.1471-4159.2003.02247.x

Alves, C. J., Magalhães, a, Melo, P., de Sousa, L., Tavares, M. a, Monteiro, P. R. R., & Summavielle, T. (2014). Long-term effects of chronic cocaine exposure throughout adolescence on anxiety and stress responsivity in a Wistar rat model. *Neuroscience*, *277*, 343–55. doi:10.1016/j.neuroscience.2014.07.008

Anker, J. J., & Carroll, M. E. (2010). Reinstatement of cocaine seeking induced by drugs, cues, and stress in adolescent and adult rats. *Psychopharmacology*, *208*(2), 211–22. doi:10.1007/s00213-009-1721-2

Araque, A. (2008). Astrocytes process synaptic information. *Neuron Glia Biology*, *4*(1), 3–10. doi:10.1017/S1740925X09000064

Atkins, C. M., Chen, S. J., Klann, E., & Sweatt, J. D. (1997). Increased phosphorylation of myelin basic protein during hippocampal long-term potentiation. *Journal of Neurochemistry*, *68*(5), 1960–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9109522

Atkins, C. M., Yon, M., Groome, N. P., & Sweatt, J. D. (1999). Regulation of Myelin Basic Protein Phosphorylation by Mitogen-Activated Protein Kinase During Increased Action Potential Firing in the Hippocampus, *Journal of Neurochemistry, 73*(3), 1090–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10461899

Baker, D. a, Shen, H., & Kalivas, P. W. (2002). Cystine/glutamate exchange serves as the source for extracellular glutamate: modifications by repeated cocaine administration. *Amino Acids*, *23*(1-3), 161–2. doi:10.1007/s00726-001-0122-6

Baker, D. A., Mcfarland, K., Lake, R. W., Shen, H., Tang, X., Toda, S., & Kalivas, P. W. (2003). Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse, *Nature Neuroscience, 6*(7), 743–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12778052

Baker, D. A., Xi, Z., Shen, H., Swanson, C. J., & Kalivas, P. W. (2002). The Origin and Neuronal Function of In Vivo Nonsynaptic Glutamate, *The Journal of Neuroscience:*

The Official Journal of the Society for Neuroscience, 22(20), 9134–41. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12388621

Barbas, H., & Pandya, D. N. (1989). Architecture and intrinsic connections of the prefrontal cortex in the rhesus monkey. *The Journal of Comparative Neurology*, *286*(3), 353–75. doi:10.1002/cne.902860306

Barker, G. R. I., Bird, F., Alexander, V., & Warburton, E. C. (2007). Recognition memory for objects, place, and temporal order: a disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *27*(11), 2948–57. doi:10.1523/JNEUROSCI.5289-06.2007

Baron, W., & Hoekstra, D. (2010). On the biogenesis of myelin membranes: sorting, trafficking and cell polarity. *FEBS Letters*, *584*(9), 1760–70. doi:10.1016/j.febslet.2009.10.085

Bartzokis, G., Beckson, M., Lu, P. H., Edwards, N., Bridge, P., & Mintz, J. (2002). Brain maturation may be arrested in chronic cocaine addicts. *Biological Psychiatry*, *51*(8), 605–11. doi:10.1016/S0006-3223(02)01315-X

Bartzokis, G., Beckson, M., Lu, P. H., Nuechterlein, K. H., Edwards, N., & Mintz, J. (2001). Age-related changes in frontal and temporal lobe volumes in men: a magnetic resonance imaging study. *Archives of General Psychiatry*, *58*(5), 461–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11343525

Bear, M., Connors, B., & Paradiso, M. (2007). *Neuroscience* (pp. 210, 373). *Sunderland (MA): Sinauer Associates.* doi:978-0878937257

Bechara, A., & Damasio, H. (2002). Decision-making and addiction (part I): impaired activation of somatic states in substance dependent individuals when pondering decisions with negative future consequences. *Neuropsychologia*, *40*(10), 1675–89. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11992656

Bechara, A., Damasio, H., Tranel, D., & Anderson, S. W. (1998). Dissociation Of working memory from decision making within the human prefrontal cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *18*(1), 428–37. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9412519

Bechara, A., Dolan, S., Denburg, N., Hindes, A., Anderson, S. W., & Nathan, P. E. (2001). Decision-making deficits, linked to a dysfunctional ventromedial prefrontal cortex, revealed in alcohol and stimulant abusers. *Neuropsychologia*, *39*(4), 376–89. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11164876

Bechara, A., & Martin, E. M. (2004). Impaired decision making related to working memory deficits in individuals with substance addictions. *Neuropsychology*, *18*(1), 152–62. doi:10.1037/0894-4105.18.1.152

Bedi, G., Preston, K. L., Epstein, D. H., Heishman, S. J., Marrone, G. F., Shaham, Y., & de Wit, H. (2011). Incubation of cue-induced cigarette craving during abstinence in human smokers. *Biological Psychiatry*, *69*(7), 708–11. doi:10.1016/j.biopsych.2010.07.014

Ben-Shahar, O., Ahmed, S. H., Koob, G. F., & Ettenberg, A. (2004). The transition from controlled to compulsive drug use is associated with a loss of sensitization. *Brain Research*, *995*(1), 46–54. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14644470

Ben-Shahar, O., Keeley, P., Cook, M., Brake, W., Joyce, M., Nyffeler, M., … Ettenberg, A. (2007). Changes in levels of D1, D2, or NMDA receptors during withdrawal from brief or extended daily access to IV cocaine. *Brain Research*, *1131*(1), 220–8. doi:10.1016/j.brainres.2006.10.069

Ben-Shahar, O., Obara, I., Ary, A. W., Ma, N., Mangiardi, M. a, Medina, R. L., & Szumlinski, K. K. (2009). Extended daily access to cocaine results in distinct alterations in Homer 1b/c and NMDA receptor subunit expression within the medial prefrontal cortex. *Synapse*, *63*(7), 598–609. doi:10.1002/syn.20640

Ben-Shahar, O., Sacramento, A. D., Miller, B. W., Webb, S. M., Wroten, M. G., Silva, H. E., … Szumlinski, K. K. (2013). Deficits in ventromedial prefrontal cortex group 1 metabotropic glutamate receptor function mediate resistance to extinction during protracted withdrawal from an extensive history of cocaine self-administration. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *33*(2), 495–506. doi:10.1523/JNEUROSCI.3710-12.2013

Bernard, R., Kerman, I. A., Thompson, R. C., Jones, E. G., Bunney, W. E., Barchas, J. D., … Watson, S. J. (2011). Altered expression of glutamate signaling, growth factor, and glia genes in the locus coeruleus of patients with major depression. *Molecular Psychiatry*, *16*(6), 634–46. doi:10.1038/mp.2010.44

Bernheim, A., Halfon, O., & Boutrel, B. (2013). Controversies about the enhanced vulnerability of the adolescent brain to develop addiction. *Frontiers in Pharmacology*, *4*(November), 118. doi:10.3389/fphar.2013.00118

Bhat, M. A., Rios, J. C., Lu, Y., Garcia-fresco, G. P., Ching, W., Martin, M. S., … Bellen, H. J. (2001). Axon-Glia Interactions and the Domain Organization of Myelinated Axons

Requires Neurexin IV / Caspr / Paranodin, *Neuron, 30*, 369–383. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11395000

Binder, D. K. & Steinhauser, C. (2009). Role of astrocytes in epilepsy. In V. Parpura & P. G. Haydon (Eds.), *Astrocytes in (Patho)Physiology of the Nervous System* (pp.649-672). New York, NY: Springer Science+Business Media, LLC.

Blair, K., Marsh, A. A., Morton, J., Vythilingam, M., Jones, M., Mondillo, K., … Blair, J. R. (2006). Choosing the lesser of two evils, the better of two goods: specifying the roles of ventromedial prefrontal cortex and dorsal anterior cingulate in object choice. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *26*(44), 11379–86. doi:10.1523/JNEUROSCI.1640-06.2006

Block, R. I., Erwin, W. J., & Ghoneim, M. M. (2002). Chronic drug use and cognitive impairments. *Pharmacology, Biochemistry, and Behavior*, *73*(3), 491–504. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12151022

Boggs, J. M. (2006). Myelin basic protein: a multifunctional protein. *Cellular and Molecular Life Sciences : CMLS*, *63*(17), 1945–61. doi:10.1007/s00018-006-6094-7

Boiko, T., Rasband, M. N., Rock, S., Caldwell, J. H., Mandel, G., Trimmer, J. S., … Caldwell, R. (2001). Compact Myelin Dictates the Differential Targeting of Two Sodium Channel Isoforms in the Same Axon. *Neuron, 30*, 91-104. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11343647

Bolla, K., Ph, D., Ernst, M., Kiehl, K., Mouratidis, M., Psy, D., … London, E. (2004). Prefrontal Cortical Dysfunction in Abstinent Cocaine Abusers. *The Journal of Neuropsychiatry and Clinical Neurosciences,16,* 456–64. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15616172

Bossert, J. M., Marchant, N. J., Calu, D. J., & Shaham, Y. (2013). The reinstatement model of drug relapse: recent neurobiological findings, emerging research topics, and translational research. *Psychopharmacology*, *229*(3), 453–76. doi:10.1007/s00213-013-3120-y

Bowers, M. S., & Kalivas, P. W. (2003). Forebrain astroglial plasticity is induced following withdrawal from repeated cocaine administration. *European Journal of Neuroscience*, *17*(6), 1273–1278. doi:10.1046/j.1460-9568.2003.02537.x

Bowers, M. S., Mcfarland, K., Lake, R. W., Peterson, Y. K., Lapish, C. C., Gregory, M. L., … Carolina, S. (2004). Activator of G Protein Signaling 3: A Gatekeeper of Cocaine Sensitization and Drug Seeking. *Neuron, 42*, 269–81. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15091342

Brabant, C., Quertemont, E., & Tirelli, E. (2005). Influence of the dose and the number of drug-context pairings on the magnitude and the long-lasting retention of cocaine-induced conditioned place preference in C57BL/6J mice. *Psychopharmacology*, *180*(1), 33–40. doi:10.1007/s00213-004-2138-6

Breiter, H. C., Gollub, R. L., Weisskoff, R. M., Kennedy, D. N., Makris, N., Berke, J. D., … Hyman, S. E. (1997). Acute Effects of Cocaine on Human Brain Activity and Emotion. *Neuron*, *19*, 591–611. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9331351

Brenner, M., Goldman, J.E., Quinan, R.A., & Messing, A. (2009). Alexander disease: A genetic disorder of astrocytes. In V. Parpura & P. G. Haydon (Eds.), *Astrocytes in (Patho)Physiology of the Nervous System* (pp.591-648). New York, NY: Springer Science+Business Media, LLC.

Broersen, L. M., Feldon, J., & Weiner, I. (1999). Dissociative effects of apomorphine infusions into the medial prefrontal cortex of rats on latent inhibition, prepulse inhibition and amphetamine-induced locomotion. *Neuroscience*, *94*(1), 39–46. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10613495

Camí, J., & Farré, M. (2003). Drug addiction. *The New England Journal of Medicine*, *349*(10), 975–86. doi:10.1056/NEJMra023160

Campbell, C. B., & Hodos, W. (1970). The concept of homology and the evolution of the nervous system. *Brain, Behavior and Evolution*, *3*(5), 353–67. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/5522599

Capriles, N., Rodaros, D., Sorge, R. E., & Stewart, J. (2003). A role for the prefrontal cortex in stress- and cocaine-induced reinstatement of cocaine seeking in rats. *Psychopharmacology*, *168*(1-2), 66–74. doi:10.1007/s00213-002-1283-z

Cardinal, R. N., Pennicott, D. R., Sugathapala, C. L., Robbins, T. W., & Everitt, B. J. (2001). Impulsive choice induced in rats by lesions of the nucleus accumbens core. *Science*, *292*(5526), 2499–501. doi:10.1126/science.1060818

Carr, D. B., & Sesack, S. R. (2000). Projections from the Rat Prefrontal Cortex to the Ventral Tegmental Area: Target Specificity in the Synaptic Associations with Mesoaccumbens and Mesocortical Neurons. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *20*(10), 3864–73. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10804226

Castelluccio, B. C., Meda, S. a, Muska, C. E., Stevens, M. C., & Pearlson, G. D. (2014). Error processing in current and former cocaine users. *Brain Imaging and Behavior*, *8*(1), 87–96. doi:10.1007/s11682-013-9247-y

Chang, J., Janak, P. H., & Woodward, D. J. (1998). Comparison of Mesocorticolimbic Neuronal Responses During Cocaine and Heroin Self-Administration in Freely Moving Rats. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *18*(8), 3098–115. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9526026

Chang, J., Sawyer, S. F., Paris, J. M., & Kirillov, A. (1997). Single Neuronal Responses in Medial Prefrontal Cortex During Cocaine Self-Administration in Freely Moving Rats. *Synapse (New York, N.Y.)*, *26*(1), 22–35. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9097402

Chernoff, G. F. (1981). Shiverer: an autosomal recessive mutant mouse with myelin deficiency. *The Journal of Heredity*, *72*(2), 128. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6168677

Childress, A. R., Mozley, P. D., McElgin, W., Fitzgerald, J., Reivich, M., & O'Brien, C. P. (1999). Limbic activation during cue-induced cocaine craving. *The American Journal of Psychiatry*, *156*(1), 11–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9892292

Clark, K. H., Wiley, C. A, & Bradberry, C. W. (2013). Psychostimulant abuse and neuroinflammation: emerging evidence of their interconnection. *Neurotoxicity Research*, *23*(2), 174–88. doi:10.1007/s12640-012-9334-7

Coffey, J. C., & McDermott, K. W. (1997). The regional distribution of myelin oligodendrocyte glycoprotein (MOG) in the developing rat CNS: an in vivo immunohistochemical study. *Journal of Neurocytology*, *26*(3), 149–61. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9192283

Conde, F., Maire-lepoivre, E., Audinat, E., & Crepel, F. (1995). Afferent connections of the medial frontal cortex of the rat. II. Cortical and subcortical afferents. *The Journal of Comparative Neurology, 352*, 567–93. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7722001

Costa, B. M., Yao, H., Yang, L., & Buch, S. (2013). Role of endoplasmic reticulum (ER) stress in cocaine-induced microglial cell death. *Journal of Neuroimmune Pharmacology : The Official Journal of the Society on NeuroImmune Pharmacology*, *8*(3), 705–14. doi:10.1007/s11481-013-9438-8

Crews, F. T., & Boettiger, C. A. (2009). Impulsivity, frontal lobes and risk for addiction. *Pharmacology, Biochemistry, and Behavior*, *93*(3), 237–47. doi:10.1016/j.pbb.2009.04.018

Crews, F. T., Zou, J., & Qin, L. (2011). Induction of innate immune genes in brain create the neurobiology of addiction. *Brain, Behavior, and Immunity*, *25 Suppl 1*, S4–S12. doi:10.1016/j.bbi.2011.03.003

Dalley, J. W., Everitt, B. J., & Robbins, T. W. (2011). Impulsivity, compulsivity, and top-down cognitive control. *Neuron*, *69*(4), 680–94. doi:10.1016/j.neuron.2011.01.020

Damasio, A. R. (1996). The somatic marker hypothesis and the possible functions of the prefrontal cortex. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *351*(1346), 1413–20. doi:10.1098/rstb.1996.0125

Davis, D. S., & Carson, M. J. (2013). Neural-Immune Interactions in Brain Function and Alcohol Related Disorders, 3–29. doi:10.1007/978-1-4614-4729-0

Deitmer, J. W., Singaravelu, K., & Lohr, C. (2009). Calcium ion signaling in astrocytes. In V. Parpura & P. G. Haydon (Eds.), *Astrocytes in (Patho)Physiology of the Nervous System* (pp.201-224). New York, NY: Springer Science+Business Media, LLC.

Delatour, B., & Gisquet-verrier, P. (1996). Prelimbic cortex specific lesions disrupt delayed-variable response tasks in the rat. *Behavioral Neuroscience*, *110*(6), 1282–98. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8986332

Delatour, B., & Gisquet-verrier, P. (1999). Lesions of the Prelimbic-Infralimbic Cortices in Rats Do Not Disrupt Response Selection Processes but Induce Delay-Dependent Deficits: evidence for a Role in Working Memory? *Behavioral Neuroscience*, *113*(5), 941–55. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10571477

Delatour, B., & Gisquet-Verrier, P. (2000). Functional role of rat prelimbic-infralimbic cortices in spatial memory: evidence for their involvement in attention and behavioural flexibility. *Behavioural Brain Research*, *109*(1), 113–28. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10699663

Dere, E., De Souza-Silva, M. a., Frisch, C., Teubner, B., Sohl, G., Willecke, K., & Huston, J. P. (2003). Connexin30-deficient mice show increased emotionality and decreased rearing activity in the open-field along with neurochemical changes. *European Journal of Neuroscience*, *18*(3), 629–638. doi:10.1046/j.1460-9568.2003.02784.x

DesJardins, K. C., & Morell, P. (1983). Phosphate groups modifying myelin basic proteins are metabolically labile; methyl groups are stable. *The Journal of Cell Biology*, *97*(2), 438–46. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6193125

Di Ciano, P., Benham-Hermetz, J., Fogg, a P., & Osborne, G. E. C. (2007). Role of the prelimbic cortex in the acquisition, re-acquisition or persistence of responding for a

drug-paired conditioned reinforcer. *Neuroscience*, *150*(2), 291–8. doi:10.1016/j.neuroscience.2007.09.016

Di Pietro, N. C., Black, Y. D., & Kantak, K. M. (2006). Context-dependent prefrontal cortex regulation of cocaine self-administration and reinstatement behaviors in rats. *The European Journal of Neuroscience*, *24*(11), 3285–98. doi:10.1111/j.1460-9568.2006.05193.x

Dias, R., & Aggleton, J. P. (2000). Effects of selective excitotoxic prefrontal lesions on acquisition of nonmatching- and matching-to-place in the T-maze in the rat: differential involvement of the prelimbic-infralimbic and anterior cingulate cortices in providing behavioural flexibility. *The European Journal of Neuroscience*, *12*(12), 4457–66. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11122356

Dietrich, J. B. (2009). Alteration of blood-brain barrier function by methamphetamine and cocaine. *Cell and Tissue Research*, *336*(3), 385–92. doi:10.1007/s00441-009-0777-y

Duvauchelle, C. L., & Ettenberg, A. (1991). Haloperidol attenuates conditioned place preferences produced by electrical stimulation of the medial prefrontal cortex. *Pharmacology, Biochemistry, and Behavior*, *38*(3), 645–50. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2068202

Dworkin, S. I., Co, C., & Smith, J. E. (1995). Rat brain neurotransmitter turnover rates altered during withdrawal from chronic cocaine administration. *Brain Reseach*, *682*, 116–126. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7552301

Dworkin, S. I., Mirkis, S., & Smith, J. E. (1995). Response-dependent versus response-independent presentation of cocaine: differences in the lethal effects of the drug.

Psychopharmacology, 117(3), 262–6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7770601

Dyer, C. A. (1993). Novel oligodendrocyte transmembrane signaling systems. Investigations utilizing antibodies as ligands. *Molecular Neurobiology*, *7*(1), 1–22. doi:10.1007/BF02780606

Dyer, C. A., Philibotte, T. M., & Wolf, M. K. (1994). Myelin Basic Protein Mediates Extracellular Signals That Regulate Microtubule Stability in Oligodendrocyte Membrane Sheets. *Journal of Neuroscience Research*, *107*, 97–107. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7528819

Edgar, J. M., McLaughlin, M., Werner, H. B., McCulloch, M. C., Barrie, J. A., Brown, A., … Griffiths, I. R. (2009). Early ultrastructural defects of axons and axon-glia junctions in mice lacking expression of Cnp1. *Glia*, *57*(16), 1815–24. doi:10.1002/glia.20893

Ellenbroek, B. A., Budde, S., & Cools, A. R. (1996). Prepulse inhibition and latent inhibition: the role of dopamine in the medial prefrontal cortex. *Neuroscience*, *75*(2), 535–42. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8931016

Ennaceur, A., Neave, N., & Aggleton, J. P. (1997). Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Experimental Brain Research*, *113*(3), 509–19. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9108217

Ernst, C., Nagy, C., Kim, S., Yang, J. P., Deng, X., Hellstrom, I. C., … Turecki, G. (2011). Dysfunction of astrocyte connexins 30 and 43 in dorsal lateral prefrontal cortex of suicide completers. *Biological Psychiatry*, *70*(4), 312–9. doi:10.1016/j.biopsych.2011.03.038

Ernst, M., Bolla, K., Mouratidis, M., Contoreggi, C., Matochik, J. A., Kurian, V., … London, E. D. (2002). Decision-making in a risk-taking task: a PET study. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *26*(5), 682–91. doi:10.1016/S0893-133X(01)00414-6

Ettenberg, A., Raven, M. A., Danluck, D. A., & Necessary, B. D. (1999). Evidence for Opponent-Process Actions of Intravenous Cocaine. *Pharmacology, Biochemistry, and Behavior*, *64*(3), 507–12. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10548263

Everitt, B. J., & Robbins, T. W. (2005). Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nature Neuroscience*, *8*(11), 1481–9. doi:10.1038/nn1579

Fattore, L., Puddu, M. C., Picciau, S., Cappai, A., Fratta, W., Serra, G. P., & Spiga, S. (2002). Astroglial in vivo response to cocaine in mouse dentate gyrus: a quantitative and qualitative analysis by confocal microscopy. *Neuroscience*, *110*(1), 1–6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11882367

Feja, M., & Koch, M. (2014). Ventral medial prefrontal cortex inactivation impairs impulse control but does not affect delay-discounting in rats. *Behavioural Brain Research*, *264*, 230–9. doi:10.1016/j.bbr.2014.02.013

Fellows, L. K., & Farah, M. J. (2005). Different underlying impairments in decision-making following ventromedial and dorsolateral frontal lobe damage in humans. *Cerebral Cortex (New York, N.Y. : 1991)*, *15*(1), 58–63. doi:10.1093/cercor/bhh108

Ferrer, J. M., Cobo, M., & Mora, F. (1987). The basolateral limbic circuit and self-stimulation of the medial prefrontal cortex in the rat. *Physiology & Behavior*, *40*(3), 291–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3659145

Ferry, A. T., Ongür, D., An, X., & Price, J. L. (2000). Prefrontal cortical projections to the striatum in macaque monkeys: evidence for an organization related to prefrontal networks. *The Journal of Comparative Neurology*, *425*(3), 447–70. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10972944

Fields, R. D. (2008). Oligodendrocytes changing the rules: action potentials in glia and oligodendrocytes controlling action potentials. *The Neuroscientist : A Review Journal Bringing Neurobiology, Neurology and Psychiatry*, *14*(6), 540–3. doi:10.1177/1073858408320294

Fillmore, M. T., & Rush, C. R. (2002). Impaired inhibitory control of behavior in chronic cocaine users. *Drug and Alcohol Dependence*, *66*, 265–73. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12062461

Fischer-Smith, K. D., Houston, A. C. W., & Rebec, G. V. (2012). Differential effects of cocaine access and withdrawal on glutamate type 1 transporter expression in rat nucleus accumbens core and shell. *Neuroscience*, *210*, 333–9. doi:10.1016/j.neuroscience.2012.02.049

Fontanez-Nuin, D. E., Santini, E., Quirk, G. J., & Porter, J. T. (2011). Memory for fear extinction requires mGluR5-mediated activation of infralimbic neurons. *Cerebral Cortex (New York, N.Y. : 1991)*, *21*(3), 727–35. doi:10.1093/cercor/bhq147

Frantz, K. J., O'Dell, L. E., & Parsons, L. H. (2007). Behavioral and neurochemical responses to cocaine in periadolescent and adult rats. *Neuropsychopharmacology :*

Official Publication of the American College of Neuropsychopharmacology, *32*(3), 625–37. doi:10.1038/sj.npp.1301130

Fuchs, R. A., Branham, R. K., & See, R. E. (2006). Different neural substrates mediate cocaine seeking after abstinence versus extinction training: a critical role for the dorsolateral caudate-putamen. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *26*(13), 3584–8. doi:10.1523/JNEUROSCI.5146-05.2006

Fuchs, R. A., Eaddy, J. L., Su, Z.-I., & Bell, G. H. (2007). Interactions of the basolateral amygdala with the dorsal hippocampus and dorsomedial prefrontal cortex regulate drug context-induced reinstatement of cocaine-seeking in rats. *The European Journal of Neuroscience*, *26*(2), 487–98. doi:10.1111/j.1460-9568.2007.05674.x

Fuchs, R. A., Evans, K. A., Ledford, C. C., Parker, M. P., Case, J. M., Mehta, R. H., & See, R. E. (2005). The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *30*(2), 296–309. doi:10.1038/sj.npp.1300579

Fumagalli, F., Moro, F., Caffino, L., Orrù, A., Cassina, C., Giannotti, G., … Cervo, L. (2013). Region-specific effects on BDNF expression after contingent or non-contingent cocaine i.v. self-administration in rats. *The International Journal of Neuropsychopharmacology / Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, *16*(4), 913–8. doi:10.1017/S146114571200096X

Gass, J. T., & Chandler, L. J. (2013). The Plasticity of Extinction: Contribution of the Prefrontal Cortex in Treating Addiction through Inhibitory Learning. *Frontiers in Psychiatry*, *4*(May), 46. doi:10.3389/fpsyt.2013.00046

George, O., Mandyam, C. D., Wee, S., & Koob, G. F. (2008). Extended access to cocaine self-administration produces long-lasting prefrontal cortex-dependent working memory impairments. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *33*(10), 2474–82. doi:10.1038/sj.npp.1301626

Ghasemzadeh, M. B., Vasudevan, P., Mueller, C., Seubert, C., & Mantsch, J. R. (2009). Region specific alterations in glutamate receptor expression and subcellular distribution following extinction of cocaine self-administration. *Brain Research*, *1267*, 89–102. doi:10.1016/j.brainres.2009.01.047

Giedd, J. N., Blumenthal, J., Jeffries, N. O., Castellanos, F. X., Liu, H., Zijdenbos, A., … Rapoport, J. L. (1999). Brain development during childhood and adolescence: a longitudinal MRI study. *Nature Neuroscience*, *2*(10), 861–3. doi:10.1038/13158

Giguere, M., & Goldman-Rakic, P. S. (1988). Mediodorsal nucleus: areal, laminar, and tangential distribution of afferents and efferents in the frontal lobe of rhesus monkeys. *The Journal of Comparative Neurology*, *277*(2), 195–213. doi:10.1002/cne.902770204

Goeders, N. E., & Smith, J. E. (1986). Reinforcing properties of cocaine in the medical prefrontal cortex: primary action on presynaptic dopaminergic terminals. *Pharmacology, Biochemistry, and Behavior*, *25*(1), 191–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3018792

Gorelova, N., & Yang, C. R. (1997). The course of neural projection from the prefrontal cortex to the nucleus accumbens in the rat. *Neuroscience*, *76*(3), 689–706. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9135043

Graeber, M. B. (2010). Changing face of microglia. *Science (New York, N.Y.)*, *330*(6005), 783–8. doi:10.1126/science.1190929

Grant, S., London, E. D., Newlin, D. B., Villemagne, V. L., Liu, X., Contoreggi, C., … Margolint, A. (1996). Activation of memory circuits during cue-elicited cocaine craving. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(21), 12040–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8876259

Grimm, J. W., Hope, B. T., Wise, R. A., & Shaham, Y. (2001). Neuroadaptation. Incubation of cocaine craving after withdrawal. *Nature*, *412*(6843), 141–2. doi:10.1038/35084134

Guzman, D., Moscarello, J. M., & Ettenberg, A. (2009). The effects of medial prefrontal cortex infusions of cocaine in a runway model of drug self-administration: evidence of reinforcing but not anxiogenic actions. *European Journal of Pharmacology*, *605*(1-3), 117–22. doi:10.1016/j.ejphar.2009.01.003

Haber, S. N., Kunishio, K., & Mizobuchi, M. (1995). The Orbital and Medial Prefrontal Basal Ganglia Circuit Through the Primate. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *15*(7 Pt 1), 4851–67. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7623116

Hare, T. A., O'Doherty, J., Camerer, C. F., Schultz, W., & Rangel, A. (2008). Dissociating the role of the orbitofrontal cortex and the striatum in the computation of goal values

and prediction errors. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *28*(22), 5623–30. doi:10.1523/JNEUROSCI.1309-08.2008

Harvey, R. C., Dembro, K. A., Rajagopalan, K., Mutebi, M. M., & Kantak, K. M. (2009). Effects of self-administered cocaine in adolescent and adult male rats on orbitofrontal cortex-related neurocognitive functioning. *Psychopharmacology*, *206*(1), 61–71. doi:10.1007/s00213-009-1579-3

Heidbreder, C. a, & Groenewegen, H. J. (2003). The medial prefrontal cortex in the rat: evidence for a dorso-ventral distinction based upon functional and anatomical characteristics. *Neuroscience & Biobehavioral Reviews*, *27*(6), 555–79. doi:10.1016/j.neubiorev.2003.09.003

Heil, S. H., Johnson, M. W., Higgins, S. T., & Bickel, W. K. (2006). Delay discounting in currently using and currently abstinent cocaine-dependent outpatients and non-drug-using matched controls. *Addictive Behaviors*, *31*(7), 1290–4. doi:10.1016/j.addbeh.2005.09.005

Hellemans, J., Mortier, G., De Paepe, A., Speleman, F., & Vandesompele, J. (2007). qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biology*, *8*(2), R19. doi:10.1186/gb-2007-8-2-r19

Hemby, S. E., Co, C., Koves, T. R., Smith, J. E., & Dworkin, S. I. (1997). Differences in extracellular dopamine concentrations in the nucleus accumbens during response-dependent and response-independent cocaine administration in the rat. *Psychopharmacology*, *133*(1), 7–16. doi:10.1007/s002130050365

Hill, C. M. D., & Harauz, G. (2005). Charge effects modulate actin assembly by classic myelin basic protein isoforms. *Biochemical and Biophysical Research Communications*, *329*(1), 362–9. doi:10.1016/j.bbrc.2005.01.151

Hill, C. M. D., Libich, D. S., & Harauz, G. (2005). Assembly of tubulin by classic myelin basic protein isoforms and regulation by post-translational modification. *Biochemistry*, *44*(50), 16672–83. doi:10.1021/bi050646+

Hoover, W. B., & Vertes, R. P. (2007). Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. *Brain Structure & Function*, *212*(2), 149–79. doi:10.1007/s00429-007-0150-4

Hu, Y., Doudevski, I., Wood, D., Moscarello, M., Husted, C., Genain, C., … Israelachvili, J. (2004). Synergistic interactions of lipids and myelin basic protein. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(37), 13466-71. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15353595

Ikemoto, S., & Donahue, K. M. (2005). A five-minute, but not a fifteen-minute, conditioning trial duration induces conditioned place preference for cocaine administration into the olfactory tubercle. *Synapse (New York, N.Y.)*, *56*(1), 57–9. doi:10.1002/syn.20124

Inagawa, K., Watanabe, S., & Tsukada, Y. (1988). The role of myelination in learning performance observed in two strains of myelin-deficient mutant mice (shiverer and mld). *Behavioral and Neural Biology*, *50*(2), 184–92.

Inoue, Y., Nakamura, R., Mikoshiba, K., & Tsukada, Y. (1981). Fine structure of the central myelin sheath in the myelin deficient mutant Shiverer mouse, with special reference to the pattern of myelin formation by oligodendroglia. *Brain Research*, *219*(1), 85–94. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7260630

Inoue, Y., Nakamura, R., Mikoshiba, K., & Tsukada, Y. (1982). The formation patterns of central myelin sheaths in the myelin deficient mutant Shiverer mouse. *Okajimas Folia Anatomica Japonica*, *58*(4-6), 613–26. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7122012

Isaac, W. L., Nonneman, A. J., Neisewander, J., Landers, T., & Bardo, M. T. (1989). Prefrontal cortex lesions differentially disrupt cocaine-reinforced conditioned place preference but not conditioned taste aversion. *Behavioral Neuroscience*, *103*(2), 345–55. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2706078

Jacobs, E. H., Smit, A. B., de Vries, T. J., & Schoffelmeer, A. N. M. (2003). Neuroadaptive effects of active versus passive drug administration in addiction research. *Trends in Pharmacological Sciences*, *24*(11), 566–73. doi:10.1016/j.tips.2003.09.006

Jang, E. Y., Ryu, Y.-H., Lee, B. H., Chang, S.-C., Yeo, M. J., Kim, S. H., … Kim, H. Y. (2014). Involvement of reactive oxygen species in cocaine-taking behaviors in rats. *Addiction Biology*. Advance online publication. doi:10.1111/adb.12159

Jentsch, J. D., & Taylor, J. R. (1999). Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. *Psychopharmacology*, *146*(4), 373–90. doi:10.1007/PL00005483

Ji, G., & Neugebauer, V. (2012). Modulation of medial prefrontal cortical activity using in vivo recordings and optogenetics. *Molecular Brain*, *5*, 36. doi:10.1186/1756-6606-5-36

Jinks, A. L., & Mcgregor, I. S. (2006). Modulation of anxiety-related behaviours following lesions of the prelimbic or infralimbic cortex in the rat. *Brain Research, 772* (1-2), 181–90. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9406971

Kalivas, P. W., & Volkow, N. D. (2005). Reviews and Overviews The Neural Basis of Addiction: A Pathology of Motivation and Choice. *The American Journal of Psychiatry, 162* (8), 1403–13. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16055761

Kalivas, P. W., Volkow, N., & Seamans, J. (2005). Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission. *Neuron*, *45*(5), 647–50. doi:10.1016/j.neuron.2005.02.005

Kantak, K. M., Goodrich, C. M., & Uribe, V. (2007). Influence of sex, estrous cycle, and drug-onset age on cocaine self-administration in rats (Rattus norvegicus). *Experimental and Clinical Psychopharmacology*, *15*(1), 37–47. doi:10.1037/1064-1297.15.1.37

Káradóttir, R., & Attwell, D. (2007). Neurotransmitter receptors in the life and death of oligodendrocytes. *Neuroscience*, *145*(4), 1426–38. doi:10.1016/j.neuroscience.2006.08.070

Kasperski, S. J., Vincent, K. B., Caldeira, K. M., Garnier-Dykstra, L. M., O'Grady, K. E., & Arria, A. M. (2011). College students' use of cocaine: results from a longitudinal study. *Addictive Behaviors*, *36*(4), 408–11. doi:10.1016/j.addbeh.2010.12.002

Kau, K. S., Madayag, A., Mantsch, J. R., Grier, M. D., Abdulhameed, O., & Baker, D. A. (2008). Blunted cystine-glutamate antiporter function in the nucleus accumbens promotes cocaine-induced drug seeking. *Neuroscience*, *155*(2), 530–7. doi:10.1016/j.neuroscience.2008.06.010

Kaufman, J. N., Ross, T. J., Stein, E. A., & Garavan, H. (2003). Cingulate hypoactivity in cocaine users during a GO-NOGO task as revealed by event-related functional magnetic resonance imaging. *The Journal of Neuroscience : The Official Journal of the Society*

for Neuroscience, *23*(21), 7839–43. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12944513

Kelland, E. E., & Toms, N. J. (2001). Group I metabotropic glutamate receptors limit AMPA receptor-mediated oligodendrocyte progenitor cell death. *European Journal of Pharmacology, 424*(3), R3-4. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11672568

Kerstetter, K. A., & Kantak, K. M. (2007). Differential effects of self-administered cocaine in adolescent and adult rats on stimulus-reward learning. *Psychopharmacology*, *194*(3), 403–11. doi:10.1007/s00213-007-0852-6

Kidd, G., & Trapp, B. D. (2010). Molecular organization of the oligodendrocyte and myelin. In P. J. Armati & E. K. Mathey (Eds.), *The Biology of Oligodendrocytes* (pp.64-102). New York, NY: Cambridge University Press.

Kirby, K. N., & Petry, N. M. (2004). Heroin and cocaine abusers have higher discount rates for delayed rewards than alcoholics or non-drug-using controls. *Addiction, 99*, 461–71. doi:10.1111/j.1360-0443.2004.00669.x

Knackstedt, L. A, & Kalivas, P. W. (2009). Glutamate and reinstatement. *Current Opinion in Pharmacology*, *9*(1), 59–64. doi:10.1016/j.coph.2008.12.003

Knackstedt, L. A, Melendez, R. I., & Kalivas, P. W. (2010). Ceftriaxone restores glutamate homeostasis and prevents relapse to cocaine seeking. *Biological Psychiatry*, *67*(1), 81–4. doi:10.1016/j.biopsych.2009.07.018

Kodama, Y., Kikusui, T., Takeuchi, Y., & Mori, Y. (2007). Effects of early weaning on anxiety and prefrontal cortical and hippocampal myelination in male and female wistar rats. *Developmental Psychobiology, 50*(4), 332-42. doi:10.1002/dev.20289

Kolb, B., Buhrmann, K., McDonald, R., & Sutherland, R. J. (1994). Dissociation of the medial prefrontal, posterior parietal, and posterior temporal cortex for spatial navigation and recognition memory in the rat. *Cerebral Cortex, 4*(6), 664-80. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7703691

Kovalevich, J., Corley, G., Yen, W., Rawls, S. M., & Langford, D. (2012). Cocaine-induced loss of white matter proteins in the adult mouse nucleus accumbens is attenuated by administration of a β-lactam antibiotic during cocaine withdrawal. *The American Journal of Pathology*, *181*(6), 1921–7. doi:10.1016/j.ajpath.2012.08.013

Koya, E., Uejima, J. L., Wihbey, K. A., Bossert, J. M., Hope, B. T., & Shaham, Y. (2009). Neuropharmacology Role of ventral medial prefrontal cortex in incubation of cocaine craving. *Neuropharmacology*, *56*, 177–185. doi:10.1016/j.neuropharm.2008.04.022

Kristiansen, L. V, Bannon, M. J., & Meador-Woodruff, J. H. (2009). Expression of transcripts for myelin related genes in postmortem brain from cocaine abusers. *Neurochemical Research*, *34*(1), 46–54. doi:10.1007/s11064-008-9655-3

Kurtuncu, M., Arslan, A. D., Akhisaroglu, M., Manev, H., & Uz, T. (2004). Involvement of the pineal gland in diurnal cocaine reward in mice. *European Journal of Pharmacology*, *489*(3), 203–5. doi:10.1016/j.ejphar.2004.03.010

Lacroix, L., Broersen, L. M., Weiner, I., & Feldon, J. (1998). The effects of excitotoxic lesion of the medial prefrontal cortex on latent inhibition, prepulse inhibition, food hoarding, elevated plus maze, active avoidance and locomotor activity in the rat. Neuroscience, *82*(2), 431–42. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9539214

Lacroix, L., Spinelli, S., White, W., & Feldon, J. (2000). The effects of ibotenic acid lesions of the medial and lateral prefrontal cortex on latent inhibition, prepulse inhibition and amphetamine-induced hyperlocomotion. *Neuroscience*, *97*(3), 459–68. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10828529

LaLumiere, R. T., Niehoff, K. E., & Kalivas, P. W. (2010). The infralimbic cortex regulates the consolidation of extinction after cocaine self-administration. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *17*(4), 168–75. doi:10.1101/lm.1576810

LaLumiere, R. T., Smith, K. C., & Kalivas, P. W. (2012). Neural circuit competition in cocaine-seeking: roles of the infralimbic cortex and nucleus accumbens shell. *The European Journal of Neuroscience*, *35*(4), 614–22. doi:10.1111/j.1460-9568.2012.07991.x

Lehrmann, E., Oyler, J., Vawter, M. P., Hyde, T. M., Kolachana, B., Kleinman, J. E., … Freed, W. J. (2003). Transcriptional profiling in the human prefrontal cortex: evidence for two activational states associated with cocaine abuse. *The Pharmacogenomics Journal*, *3*(1), 27–40. doi:10.1038/sj.tpj.6500146

Leslie, F. M., Loughlin, S. E., Wang, R., Perez, L., Lotfipour, S., & Belluzzia, J. D. (2004). Adolescent development of forebrain stimulant responsiveness: insights from animal studies. *Annals of the New York Academy of Sciences*, *1021*, 148–59. doi:10.1196/annals.1308.018

Li, C., & Frantz, K. J. (2009). Attenuated incubation of cocaine seeking in male rats trained to self-administer cocaine during periadolescence. *Psychopharmacology*, *204*(4), 725–33. doi:10.1007/s00213-009-1502-y

Li, L., & Shao, J. (1998). Restricted lesions to ventral prefrontal subareas block reversal learning but not visual discrimination learning in rats. *Physiology & Behavior*, *65*(2), 371–379. doi:10.1016/S0031-9384(98)00216-9

Li, P., Wu, P., Xin, X., Fan, Y.-L., Wang, G.-B., Wang, F., … Lu, L. (2014). Incubation of alcohol craving during abstinence in patients with alcohol dependence. *Addiction Biology*. Advance online publicaiton. doi:10.1111/adb.12140

Lim, K. O., Choi, S. J., Pomara, N., Wolkin, A., & Rotrosen, J. P. (2002). Reduced frontal white matter integrity in cocaine dependence: a controlled diffusion tensor imaging study. *Biological Psychiatry, 51*(11), 890-5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12022962

Lim, K. O., Wozniak, J. R., Mueller, B. a, Franc, D. T., Specker, S. M., Rodriguez, C. P., … Rotrosen, J. P. (2008). Brain macrostructural and microstructural abnormalities in cocaine dependence. *Drug and Alcohol Dependence*, *92*(1-3), 164–72. doi:10.1016/j.drugalcdep.2007.07.019

Little, K. Y., Ramssen, E., Welchko, R., Volberg, V., Roland, C. J., & Cassin, B. (2009). Decreased brain dopamine cell numbers in human cocaine users. *Psychiatry Research*, *168*(3), 173–80. doi:10.1016/j.psychres.2008.10.034

Liu, X., Matochik, J. A., Ph, D., Cadet, J., & London, E. D. (1998). Smaller volume of prefrontal lobe in polysubstance abusers: a magnetic resonance imaging study. *Neuropsychopharmacology: Official Publication of the American College on Neuropsychopharmacology*, *18*(4), 243-52. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9509492

Loftis, J. M., Choi, D., Hoffman, W., & Huckans, M. S. (2011). Methamphetamine causes persistent immune dysregulation: a cross-species, translational report. *Neurotoxicity Research*, *20*(1), 59–68. doi:10.1007/s12640-010-9223-x

London, E. D., Ernst, M., & Grant, S. (2000). Orbitofrontal cortex and human drug abuse: functional imaging. *Cerebral Cortex, 10*(3), 334–42. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10731228

Loos, M., Pattij, T., Janssen, M. C. W., Counotte, D. S., Schoffelmeer, A. N., Smit, A. B., … van Gaalen, M. M. (2010). Dopamine receptor D1/D5 gene expression in the medial prefrontal cortex predicts impulsive choice in rats. Advance online publication. doi:10.1093/cercor/bhp167

Luna, B., Padmanabhan, A., & O'Hearn, K. (2010). What has fMRI told us about the development of cognitive control through adolescence? *Brain and Cognition*, *72*(1), 101–13. doi:10.1016/j.bandc.2009.08.005

Luo, X., Zhang, S., Hu, S., Bednarski, S. R., Erdman, E., Farr, O. M., … Li, C.-S. R. (2013). Error processing and gender-shared and -specific neural predictors of relapse in cocaine dependence. *Brain : A Journal of Neurology*, *136*(Pt 4), 1231–44. doi:10.1093/brain/awt040

Luyt, K., Varadi, A., & Molnar, E. (2003). Functional metabotropic glutamate receptors are expressed in oligodendrocyte progenitor cells. *Journal of Neurochemistry*, *84*(6), 1452–64. doi:10.1046/j.1471-4159.2003.01661.x

Ma, L., Hasan, K. M., Steinberg, J. L., Narayana, P. a, Lane, S. D., Zuniga, E. a, … Moeller, F. G. (2009). Diffusion tensor imaging in cocaine dependence: regional effects of

cocaine on corpus callosum and effect of cocaine administration route. *Drug and Alcohol Dependence*, *104*(3), 262–7. doi:10.1016/j.drugalcdep.2009.05.020

Ma, Y.-Y., Lee, B. R., Wang, X., Guo, C., Liu, L., Cui, R., … Dong, Y. (2014). Bidirectional modulation of incubation of cocaine craving by silent synapse-based remodeling of prefrontal cortex to accumbens projections. *Neuron*, 1453–67. doi:10.1016/j.neuron.2014.08.023

Madayag, A., Lobner, D., Kau, K. S., Mantsch, J. R., Abdulhameed, O., Hearing, M., … Baker, D. A. (2007). Repeated N-acetylcysteine administration alters plasticity-dependent effects of cocaine. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *27*(51), 13968–76. doi:10.1523/JNEUROSCI.2808-07.2007

Malarkey, E. B. & Parpura, V. (2009). Mechanisms of transmitter release from astrocytes. In V. Parpura & P. G. Haydon (Eds.), *Astrocytes in (Patho)Physiology of the Nervous System* (pp.301-350). New York, NY: Springer Science+Business Media, LLC.

Mathey, E. K., Arthur, A., & Armati, P.J. (2010). CNS oligarths: the rise of the oligodendrocyte in a neuron-centric culture. In P. J. Armati & E. K. Mathey (Eds.), *The Biology of Oligodendrocytes* (pp.64-102). New York, NY: Cambridge University Press.

Mark, G. P., Hajnal, A., Kinney, A. E., & Keys, A. S. (1999). Self-administration of cocaine increases the release of acetylcholine to a greater extent than response-independent cocaine in the nucleus accumbens of rats. *Psychopharmacology*, *143*(1), 47–53. doi:10.1007/s002130050918

Martinez, Z. A., Ellison, G. D., Geyer, M. A., & Swerdlow, N. R. (1999). Effects of sustained cocaine exposure on sensorimotor gating of startle in rats. *Psychopharmacology*, *142*(3), 253–260. doi:10.1007/s002130050887

Matthieu, J. M., & Amiguet, P. (1990). Myelin/oligodendrocyte glycoprotein expression during development in normal and myelin-deficient mice. *Developmental Neuroscience*, *12*(4-5), 293–302. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2289413

McBride, W. J., Murphy, J. M., & Ikemoto, S. (1999). Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. *Behavioural Brain Research*, *101*(2), 129–52. doi:10.1016/S0166-4328(99)00022-4

McFarland, K., & Kalivas, P. W. (2001). The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *21*(21), 8655–63. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11606653

McFarland, K., Lapish, C. C., & Kalivas, P. W. (2003). Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *23*(8), 3531–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1276962

McGregor, I. S., Atrens, D. M., & Jackson, D. M. (1992). Cocaine facilitation of prefrontal cortex self-stimulation: a microstructural and pharmacological analysis. *Psychopharmacology*, *106*(2), 239–47. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1312730

McLaughlin, J., & See, R. E. (2003). Selective inactivation of the dorsomedial prefrontal cortex and the basolateral amygdala attenuates conditioned-cued reinstatement of extinguished cocaine-seeking behavior in rats. *Psychopharmacology*, *168*(1-2), 57–65. doi:10.1007/s00213-002-1196-x

Miguel-Hidalgo, J. J. (2009). The role of glial cells in drug abuse. *Current Drug Abuse Reviews*, *2*(1), 72–82. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19630738

Miller, E. K. (2000). The prefrontal cortex and cognitive control. *Nature Reviews. Neuroscience*, *1*(1), 59–65. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/112527969

Min, Y., Kristiansen, K., Boggs, J. M., Husted, C., Zasadzinski, J. A., & Israelachvili, J. (2009). Interaction forces and adhesion of supported myelin lipid bilayers modulated by myelin basic protein. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(9), 3154–9. doi:10.1073/pnas.0813110106

Mitchell, L. S., Gillespie, S. C., Mcallister, F., Fanarraga, M. L., Kirkham, D., Kelly, B., … Kennedy, P. G. E. (1992). Developmental expression of major myelin protein genes in the CNS of X-linked hypomyelinating mutant rumpshaker. *Journal of Neuroscience Research, 33*(2), 205-17. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1280692

Moeller, F. G., Hasan, K. M., Steinberg, J. L., Kramer, L. A, Dougherty, D. M., Santos, R. M., … Narayana, P. A. (2005). Reduced anterior corpus callosum white matter integrity is related to increased impulsivity and reduced discriminability in cocaine-dependent subjects: diffusion tensor imaging. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *30*(3), 610–7. doi:10.1038/sj.npp.1300617

Moeller, F. G., Hasan, K. M., Steinberg, J. L., Kramer, L. A, Valdes, I., Lai, L. Y., … Narayana, P. A. (2007). Diffusion tensor imaging eigenvalues: preliminary evidence for altered myelin in cocaine dependence. *Psychiatry Research*, *154*(3), 253–8. doi:10.1016/j.pscychresns.2006.11.004

Montis, M. G. De, Co, C., Dworkin, S. I., & Smith, J. E. (1998). Modifications of dopamine D1 receptor complex in rats self-administering cocaine..*European Journal of Pharmacology, 362* (1), 9-15. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9865524

Mora, F., & Cobo, M. (1990). The neurobiological basis of prefrontal cortex self-stimulation: a review and an integrative hypothesis. *Progress in Brain Research*, *85*, 419–31. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2094908

Mora, F., & Ferrer, J. M. (1986). Neurotransmitters, pathways and circuits as the neural substrates of self-stimulation of the prefrontal cortex: facts and speculations. *Behavioural Brain Research*, *22*(2), 127–40. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2878671

Morán, O., & Mateu, L. (1983). Loosening of paranodal myelin by repetitive propagation of action potentials. *Nature*, *304*(5924), 344–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6877354

Morris, G. P., Clark, I. A, Zinn, R., & Vissel, B. (2013). Microglia: a new frontier for synaptic plasticity, learning and memory, and neurodegenerative disease research. *Neurobiology of Learning and Memory*, *105*, 40–53. doi:10.1016/j.nlm.2013.07.002

Murphy, E. R., Dalley, J. W., & Robbins, T. W. (2005). Local glutamate receptor antagonism in the rat prefrontal cortex disrupts response inhibition in a visuospatial attentional task. *Psychopharmacology*, *179*(1), 99–107. doi:10.1007/s00213-004-2068-3

Murray, N., & Steck, A. J. (1984). Impulse conduction regulates myelin basic protein phosphorylation in rat optic nerve. *Journal of Neurochemistry, 43*(1), 243-8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6202844

Mutschler, N. H., Miczek, K. A., & Hammer, R. P. (2000). Reduction of zif268 messenger RNA expression during prolonged withdrawal following "binge" cocaine self-administration in rats. *Neuroscience*, *100*(3), 531–8. doi:10.1016/S0306-4522(00)00298-0

Narayana, P. A., Ahobila-Vajjula, P., Ramu, J., Herrera, J., Steinberg, J. L., & Moeller, F. G. (2009). Diffusion tensor imaging of cocaine-treated rodents. *Psychiatry Research*, *171*(3), 242–51. doi:10.1016/j.pscychresns.2008.04.004

Nie, D., Ma, Q., Law, J. W., Chia, C. P., Dhingra, N. K., Shimoda, Y., … Xiao, Z. C. (2006). Oligodendrocytes regulate formation of nodes of Ranvier via the recognition molecule OMgp, *2*(3), 151–64. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17364021

Nielsen, D. A., Huang, W., Hamon, S. C., Maili, L., Witkin, B. M., Fox, R. G., … Moeller, F. G. (2012). Forced Abstinence from Cocaine Self-Administration is Associated with DNA Methylation Changes in Myelin Genes in the Corpus Callosum: a Preliminary Study. *Frontiers in Psychiatry*, *3*(June), 60. doi:10.3389/fpsyt.2012.00060

O'Leary, M. E., & Hancox, J. C. (2010). Role of voltage-gated sodium, potassium and calcium channels in the development of cocaine-associated cardiac arrhythmias. *British Journal of Clinical Pharmacology*, *69*(5), 427–42. doi:10.1111/j.1365-2125.2010.03629.x

Obara, I., Bell, R. L., Goulding, S. P., Reyes, C. M., Larson, L. A., Ary, A. W., … Szumlinski, K. K. (2009). Differential effects of chronic ethanol consumption and withdrawal on homer/glutamate receptor expression in subregions of the accumbens and amygdala of P rats. *Alcoholism, Clinical and Experimental Research*, *33*(11), 1924–34. doi:10.1111/j.1530-0277.2009.01030.x

Oberheim, N. A., Wang, X., Goldman, S., & Nedergaard, M. (2006). Astrocytic complexity distinguishes the human brain. *Trends in Neurosciences*, *29*(10), 547–53. doi:10.1016/j.tins.2006.08.004

Olds, J., & Milner, P. (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *Journal of Comparative and Physiological Psychology*, *47*(6), 419–27. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/13233369

Padhi, B. K., & Pelletier, G. (2012). Perturbation of myelin basic protein (Mbp) splice variant expression in developing rat cerebellum following perinatal exposure to methylmercury. *Toxicology Letters*, *213*(3), 374–80. doi:10.1016/j.toxlet.2012.07.011

Perea, G., & Araque, A. (2002). Communication between astrocytes and neurons: a complex language. *Journal of Physiology, Paris*, *96*(3-4), 199–207. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12445897

Perry, J. L., Anderson, M. M., Nelson, S. E., & Carroll, M. E. (2007). Acquisition of i.v. cocaine self-administration in adolescent and adult male rats selectively bred for high and low saccharin intake. *Physiology & Behavior*, *91*(1), 126–33. doi:10.1016/j.physbeh.2007.02.001

Perry, J. L., Larson, E. B., German, J. P., Madden, G. J., & Carroll, M. E. (2005). Impulsivity (delay discounting) as a predictor of acquisition of IV cocaine self-administration in female rats. *Psychopharmacology*, *178*(2-3), 193–201. doi:10.1007/s00213-004-1994-4

Peters, J., LaLumiere, R. T., & Kalivas, P. W. (2008). Infralimbic prefrontal cortex is responsible for inhibiting cocaine seeking in extinguished rats. *The Journal of*

Neuroscience : The Official Journal of the Society for Neuroscience, *28*(23), 6046–53. doi:10.1523/JNEUROSCI.1045-08.2008

Peters, J., Vallone, J., Laurendi, K., & Kalivas, P. W. (2008). Opposing roles for the ventral prefrontal cortex and the basolateral amygdala on the spontaneous recovery of cocaine-seeking in rats. *Psychopharmacology*, *197*(2), 319–26. doi:10.1007/s00213-007-1034-2

Phillips, A. G., & Fibiger, H. C. (1978). The role of dopamine in maintaining intracranial self-stimulation in the ventral tegmentum, nucleus accumbens, and medial prefrontal cortex. *Canadian Journal of Psychology*, *32*(2), 58–66. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/737577

Piechota, M., Korostynski, M., Solecki, W., Gieryk, A., Slezak, M., Bilecki, W., … Przewlocki, R. (2010). The dissection of transcriptional modules regulated by various drugs of abuse in the mouse striatum. *Genome Biology*, *11*(5), R48. doi:10.1186/gb-2010-11-5-r48

Popko, B., Puckett, C., Lai, E., Shine, H. D., Readhead, C., Takahashi, N., … Hood, L. (1987). Myelin deficient mice: expression of myelin basic protein and generation of mice with varying levels of myelin. *Cell*, *48*(4), 713–21. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2434243

Porter, J. T., & McCarthy, K. D. (1997). Astrocytic neurotransmitter receptors in situ and in vivo. *Progress in Neurobiology, 51*(4), 439-55. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9106901

Pum, M. E., Carey, R. J., Huston, J. P., & Müller, C. P. (2008). Role of medial prefrontal, entorhinal, and occipital 5-HT in cocaine-induced place preference and

hyperlocomotion: evidence for multiple dissociations. *Psychopharmacology*, *201*(3), 391–403. doi:10.1007/s00213-008-1296-3

Reichel, C. M., Chan, C. H., Ghee, S. M., & See, R. E. (2012). Sex differences in escalation of methamphetamine self-administration: cognitive and motivational consequences in rats. *Psychopharmacology*, *223*(4), 371–80. doi:10.1007/s00213-012-2727-8

Reid, J. M., Jacklin, D. L., & Winters, B. D. (2014). Delineating prefrontal cortex region contributions to crossmodal object recognition in rats. *Cerebral Cortex (New York, N.Y. : 1991)*, *24*(8), 2108–19. doi:10.1093/cercor/bht061

Reissner, K. J., Brown, R. M., Spencer, S., Tran, P. K., Thomas, C. A., & Kalivas, P. W. (2014). Chronic administration of the methylxanthine propentofylline impairs reinstatement to cocaine by a GLT-1-dependent mechanism. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *39*(2), 499–506. doi:10.1038/npp.2013.223

Rogers, R. D., Everitt, B. J., Baldacchino, A., Blackshaw, A. J., Swainson, R., Wynne, K., … Robbins, T. W. (1999). Dissociable deficits in the decision-making cognition of chronic amphetamine abusers, opiate abusers, patients with focal damage to prefrontal cortex, and tryptophan-depleted normal volunteers: evidence for monoaminergic mechanisms. *Neuropsychopharmacology: Official publication of the American College on Neuropsychopharmacology, 20*(4), 322-39. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10088133

Rose, J. E., & Woolsey, C. N. (1948). The orbitofrontal cortex and its connections with the mediodorsal nucleus in rabbit, sheep and cat. *Research Publications - Association for*

Research in Nervous and Mental Disease, *27*(1), 210–32. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18106857

Sadahiro, S., Yoshikawa, H., Yagi, N., Yamamoto, Y., Yanagihara, T., Kimura, M., & Sakoda, S. (2000). Morphometric analysis of the myelin-associated oligodendrocytic basic protein-deficient mouse reveals a possible role for myelin-associated oligodendrocytic basic protein in regulating axonal diameter. *Neuroscience*, *98*(2), 361–7. doi:10.1016/S0306-4522(00)00111-1

Sanfey, A. G. (2007). Social decision-making: insights from game theory and neuroscience. *Science (New York, N.Y.)*, *318*(5850), 598–602. doi:10.1126/science.1142996

Sari, Y., Smith, K. D., Ali, P. K., & Rebec, G. V. (2009). Upregulation of GLT1 attenuates cue-induced reinstatement of cocaine-seeking behavior in rats. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *29*(29), 9239–43. doi:10.1523/JNEUROSCI.1746-09.2009

Scemes, E. & Spray, D. C. (2009). Connexin expression (gap junctions and hemichannels) in astrocytes. In V. Parpura & P. G. Haydon (Eds.), *Astrocytes in (Patho)Physiology of the Nervous System* (pp.101-150). New York, NY: Springer Science+Business Media, LLC.

Schenk, S., Horger, B. A., Peltier, R., & Shelton, K. (1991). Supersensitivity to the reinforcing effects of cocaine following 6-hydroxydopamine lesions to the medial prefrontal cortex in rats. *Brain Research*, *543*(2), 227–235. doi:10.1016/0006-8993(91)90032-Q

Schilman, E. A., Uylings, H. B. M., Galis-de Graaf, Y., Joel, D., & Groenewegen, H. J. (2008). The orbital cortex in rats topographically projects to central parts of the caudate-

putamen complex. *Neuroscience Letters*, *432*(1), 40–5. doi:10.1016/j.neulet.2007.12.024

Schmidt, H. D., & Pierce, R. C. (2010). Cocaine-induced neuroadaptations in glutamate transmission: potential therapeutic targets for craving and addiction. *Annals of the New York Academy of Sciences*, *1187*, 35–75. doi:10.1111/j.1749-6632.2009.05144.x

Schramm-Sapyta, N. L., Cauley, M. C., Stangl, D. K., Glowacz, S., Stepp, K. A., Levin, E. D., & Kuhn, C. M. (2011). Role of individual and developmental differences in voluntary cocaine intake in rats. *Psychopharmacology*, *215*(3), 493–504. doi:10.1007/s00213-011-2216-5

Sesack, S. R., Deutch, A. Y., Roth, R. H., & Bunney, B. S. (1989). Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. *The Journal of Comparative Neurology*, *290*(2), 213–42. doi:10.1002/cne.902900205

Shen, H., Gipson, C. D., Huits, M., & Kalivas, P. W. (2014). Prelimbic cortex and ventral tegmental area modulate synaptic plasticity differentially in nucleus accumbens during cocaine-reinstated drug seeking. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *39*(5), 1169–77. doi:10.1038/npp.2013.318

Smith, H. R., Beveridge, T. J. R., Nader, M. A., & Porrino, L. J. (2014). Regionally-specific alterations in myelin proteins in nonhuman primate white matter following prolonged cocaine self-administration. *Drug and Alcohol Dependence*, *137*, 143–7. doi:10.1016/j.drugalcdep.2014.01.015

Smith, J. E., Koves, T. R., & Co, C. (2003). Brain neurotransmitter turnover rates during rat intravenous cocaine self-administration. *Neuroscience*, *117*(2), 461–475. doi:10.1016/S0306-4522(02)00819-9

Sorg, B. A., Smith, M. M., & Campagnoni, A. T. (1987). Developmental expression of the myelin proteolipid protein and basic protein mRNAs in normal and dysmyelinating mutant mice. *Journal of Neurochemistry*, *49*(4), 1146–54. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2442307

Stapleton, J. M., Morgan, M. J., Phillips, R. L., Wong, D. F., Yung, B. C., Shaya, E. K., … London, E. D. (1995). Cerebral glucose utilization in polysubstance abuse. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *13*(1), 21–31. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8526968

Stefański, R., Ziółkowska, B., Kuśmider, M., Mierzejewski, P., Wyszogrodzka, E., Kołomańska, P., … Kostowski, W. (2007). Active versus passive cocaine administration: differences in the neuroadaptive changes in the brain dopaminergic system. *Brain Research*, *1157*, 1–10. doi:10.1016/j.brainres.2007.04.074

Stout, C. E., Costantin, J. L., Naus, C. C. G., & Charles, A. C. (2002). Intercellular calcium signaling in astrocytes via ATP release through connexin hemichannels. *The Journal of Biological Chemistry*, *277*(12), 10482–8. doi:10.1074/jbc.M109902200

Substance Abuse and Mental Health Services Administration, *Drug Abuse Warning Network, 2011: National Estimates of Drug-Related Emergency Department Visits*. HHS Publication No. (SMA) 13-4760, DAWN Series D-39. Rockville, MD: Substance Abuse and Mental Health Services Administration, 2013.

Substance Abuse and Mental Health Services Administration. (2013). *Results from the 2012 National Survey on Drug Use and Health : Summary of National Findings*. *NSDUH Series H-46, HHS*. Retrieved from http://store.samhsa.gov/home

Sullivan, R. M., & Gratton, A. (2002). Behavioral effects of excitotoxic lesions of ventral medial prefrontal cortex in the rat are hemisphere-dependent. *Brain Research*, *927*(1), 69–79. doi:10.1016/S0006-8993(01)03328-5

Sun, H., Cocker, P. J., Zeeb, F. D., & Winstanley, C. A. (2012). Chronic atomoxetine treatment during adolescence decreases impulsive choice, but not impulsive action, in adult rats and alters markers of synaptic plasticity in the orbitofrontal cortex. *Psychopharmacology*, *219*(2), 285–301. doi:10.1007/s00213-011-2419-9

Szumlinski, K. K., Lominac, K. D., Kleschen, M. J., Oleson, E. B., Dehoff, M. H., Schwarz, M. K., … Kalivas, P. W. (2005). Behavioral and neurochemical phenotyping of Homer1 mutant mice: possible relevance to schizophrenia. *Genes, Brain, and Behavior*, *4*(5), 273–88. doi:10.1111/j.1601-183X.2005.00120.x

Takagishi, M., & Chiba, T. (1991). Efferent projections of the infralimbic (area 25) region of the medial prefrontal cortex in the rat: an anterograde tracer PHA-L study. *Brain Research*, *566*(1-2), 26–39. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1726062

Tanaka, H., Ikenaka, K., & Isa, T. (2006). Electrophysiological abnormalities precede apparent histological demyelination in the central nervous system of mice overexpressing proteolipid protein. *Journal of Neuroscience Research*, *84*(6), 1206–16. doi:10.1002/jnr.21018

Tanaka, H., Ma, J., Tanaka, K. F., Takao, K., Komada, M., Tanda, K., … Ikenaka, K. (2009). Mice with altered myelin proteolipid protein gene expression display cognitive deficits accompanied by abnormal neuron-glia interactions and decreased conduction velocities. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *29*(26), 8363–71. doi:10.1523/JNEUROSCI.3216-08.2009

Thomas, K. L., Arroyo, M., & Everitt, B. J. (2003). Induction of the learning and plasticity-associated gene Zif268 following exposure to a discrete cocaine-associated stimulus. *European Journal of Neuroscience*, *17*(9), 1964–1972. doi:10.1046/j.1460-9568.2003.02617.x

Tran-Nguyen, L. T., Fuchs, R. A., Coffey, G. P., Baker, D. A., O'Dell, L. E., & Neisewander, J. L. (1998). Time-dependent changes in cocaine-seeking behavior and extracellular dopamine levels in the amygdala during cocaine withdrawal. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *19*(1), 48–59. doi:10.1016/S0893-133X(97)00205-4

Trantham, H., Szumlinski, K. K., Mcfarland, K., Kalivas, P. W., & Lavin, A. Ã. (2002). Repeated cocaine administration alters the electrophysiological properties of prefrontal cortical neurons. *Neuroscience, 113*(4), 749–53. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12182882

Trotter, J., Karram, K., & Nishiyama, A. (2010). NG2 cells: properties, progeny and origin. . *Brain Research Reviews*, *63*(1-2), 72–82. doi:10.1016/j.brainresrev.2009.12.006

Tzschentke, T. M. (2000). The medial prefrontal cortex as a part of the brain reward system. *Amino Acids,19*(1), 211–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11026491

Tzschentke, T. M. (2007). Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. *Addiciton Biology, 12*(3-4), 227–462. doi:10.1111/j.1369-1600.2007.00070.x

Tzschentke, T. M., & Schmidt, W. J. (1998). Discrete quinolinic acid lesions of the rat prelimbic medial prefrontal cortex affect cocaine- and MK-801- , but not morphine- and amphetamine-induced reward and psychomotor activation as measured with the place preference conditioning paradigm. *Behavioural Brain Research*, *97*(102), 115–27. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9867237

Ullian, E. M., Sapperstein, S. K., Christopherson, K. S., & Barres, B. A. (2001). Control of synapse number by glia. *Science (New York, N.Y.)*, *291*(5504), 657–61. doi:10.1126/science.291.5504.657

Ulmer, J. B. (1988). The phosphorylation of myelin proteins. *Progress in Neurobiology*, *31*(3), 241–59. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2458617

Uschkureit, T., Sporkel, O., Stracke, J., Bussow, H., & Stoffel, W. (2000). Early onset of axonal degeneration in double (plp-/-mag-/-) and hypomyelinosis in triple (plp-/-mbp-/-mag-/-) mutant mice. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *20*(14), 5225–33. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10884306

Uylings, H. B., & van Eden, C. G. (1990). Qualitative and quantitative comparison of the prefrontal cortex in rat and in primates, including humans. *Progress in Brain Research*, *85*, 31–62. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2094901

Uys, J. D., Knackstedt, L., Hurt, P., Tew, K. D., Manevich, Y., Hutchens, S., … Kalivas, P. W. (2011). Cocaine-Induced Adaptations in Cellular Redox Balance Contributes to

Enduring Behavioral Plasticity. *Neuropsychopharmacology*, *36*(12), 2551–2560. doi:10.1038/npp.2011.143

Vartanian, T., Dawson, G., Soliven, B., Nelson, D. J., & Szuchet, S. (1989). Phosphorylation of myelin basic protein in intact oligodendrocytes: inhibition by galactosylsphingosine and cyclic AMP. *Glia*, *2*(5), 370–9. doi:10.1002/glia.440020509

Verkhratsky, A. (2009). Neurotransmitter receptors in astrocytes. In V. Parpura & P. G. Haydon (Eds.), *Astrocytes in (Patho)Physiology of the Nervous System* (pp.49-68). New York, NY: Springer Science+Business Media, LLC.

Vives, F., Morales, A., & Mora, F. (1986). Lesions of connections of the medial prefrontal cortex in rats: differential effects on self-stimulation and spontaneous motor activity. *Physiology & Behavior*, *36*(1), 47–52. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3952183

Volkow, N. D., & Fowler, J. S. (2000). Addiction, a disease of compulsion and drive: involvement of the orbitofrontal cortex. *Cerebral Cortex, 10*(3), 318–25. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10731226

Volkow, N. D., Valentine, A., & Kulkarni, M. (1988). Radiological and neurological changes in the drug abuse patient: a study with MRI. *Journal of Neuroradiology. Journal de Neuroradiologie*, *15*(3), 288–93. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3266757

Volkow, N. D., Wang, G., Fowler, J. S., Ph, D., Hitzemann, R., Angrist, B., … Shea, C. (1999). Association of methylphenidate-induced craving with changes in right striato-orbitofrontal metabolism in cocaine abusers: implications in addiction, *The American*

Journal of Psychiatry, 156(1), 19–26. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9892293

Volterra, A., & Meldolesi, J. (2005). Astrocytes, from brain glue to communication elements: the revolution continues *Nature Review. Neuroscience*, *6*(8), 626–40. doi:10.1038/nrn1722

Wake, H., Lee, P. R., & Fields, R. D. (2011). Control of local protein synthesis and initial events in myelination by action potentials *Science, 333*(6049), 1647-51. doi:10.1126/science.1206998

Walton, M. E., Bannerman, D. M., & Rushworth, M. F. S. (2002). The role of rat medial frontal cortex in effort-based decision making. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *22*(24), 10996–1003. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12486195

Wang, E., Dimova, N., Sperle, K., Huang, Z., Lock, L., McCulloch, M. C., … Cambi, F. (2008). Deletion of a splicing enhancer disrupts PLP1/DM20 ratio and myelin stability. *Experimental Neurology*, *214*(2), 322–30. doi:10.1016/j.expneurol.2008.09.001

Wang, G. J., Volkow, N. D., Fowler, J. S., Cervany, P., Hitzemann, R. J., Pappas, N. R., … Felder, C. (1999). Regional brain metabolic activation during craving elicited by recall of previous drug experiences. *Life Sciences*, *64*(9), 775–84. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10075110

Wang, G., Shi, J., Chen, N., Xu, L., Li, J., Li, P., … Lu, L. (2013). Effects of length of abstinence on decision-making and craving in methamphetamine abusers. *PloS One*, *8*(7), e68791. doi:10.1371/journal.pone.0068791

Warburton, E. C., & Brown, M. W. (2010). Findings from animals concerning when interactions between perirhinal cortex, hippocampus and medial prefrontal cortex are necessary for recognition memory. *Neuropsychologia*, *48*(8), 2262–72. doi:10.1016/j.neuropsychologia.2009.12.022

Weissenborn, R., Robbins, T. W., & Everitt, B. J. (1997). Effects of medial prefrontal or anterior cingulate cortex lesions on responding for cocaine under fixed-ratio and second-order schedules of reinforcement in rats. *Psychopharmacology*, *134*(3), 242–257. doi:10.1007/s002130050447

Wiktorowicz, M., & Roach, A. (1991). Regulation of myelin basic protein gene transcription in normal and shiverer mutant mice. *Developmental Neuroscience*, *13*(3), 143–50. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1721568

Willoughby, T., Good, M., Adachi, P. J. C., Hamza, C., & Tavernier, R. (2013). Examining the link between adolescent brain development and risk taking from a social-developmental perspective. *Brain and Cognition*, *83*(3), 315–23. doi:10.1016/j.bandc.2013.09.008

Winstanley, C. A., Olausson, P., Taylor, J. R., Jentsch, J. D., Discounting, D., Reaction, F. S., … Angeles, L. (2010). Insight Into the Relationship Between Impulsivity and Substance Abuse From Studies Using Animal Models, *34*(8), 1306–1318. doi:10.1111/j.1530-0277.2010.01215.x

Wise, S. P. (2008). Forward frontal fields: phylogeny and fundamental function. *Trends in Neurosciences*, *31*(12), 599–608. doi:10.1016/j.tins.2008.08.008

Wong, W. C., Ford, K. A., Pagels, N. E., McCutcheon, J. E., & Marinelli, M. (2013). Adolescents are more vulnerable to cocaine addiction: behavioral and

electrophysiological evidence. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *33*(11), 4913–22. doi:10.1523/JNEUROSCI.1371-12.2013

Yamamoto, Y., Yoshikawa, H., Nagano, S., Kondoh, G., Sadahiro, S., & Gotow, T. (1999). Myelin-associated oligodendrocytic basic protein is essential for normal arrangement of the radial component in central nervous system myelin. *The European Journal of Neuroscience*, *11*(3), 847–55. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10103078

Yamazaki, Y., Hozumi, Y., Kaneko, K., Sugihara, T., Fujii, S., Goto, K., & Kato, H. (2007). Modulatory effects of oligodendrocytes on the conduction velocity of action potentials along axons in the alveus of the rat hippocampal CA1 region. *Neuron Glia Biology*, *3*(4), 325–34. doi:10.1017/S1740925X08000070

Yates, J. R., Perry, J. L., Meyer, A. C., Gipson, C. D., Charnigo, R., & Bardo, M. T. (2014). Role of medial prefrontal and orbitofrontal monoamine transporters and receptors in performance in an adjusting delay discounting procedure. *Brain Research*, *1574*, 26–36. doi:10.1016/j.brainres.2014.06.004

You, Z., Tzschentke, T. M., Brodin, E., & Wise, R. A. (1998). Electrical stimulation of the prefrontal cortex increases cholecystokinin, glutamate, and dopamine release in the nucleus accumbens: an in vivo microdialysis study in freely moving rats. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *18*(16), 6492–6500. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9698337

Zapata, A., Chefer, V. I., Ator, R., Shippenberg, T. S., & Rocha, B. A. (2003). Behavioural sensitization and enhanced dopamine response in the nucleus accumbens after

intravenous cocaine self-administration in mice. *European Journal of Neuroscience*, *17*(3), 590–596. doi:10.1046/j.1460-9568.2003.02491.x

Zavala, A. R., Weber, S. M., Rice, H. J., Alleweireldt, A. T., & Neisewander, J. L. (2003). Role of the prelimbic subregion of the medial prefrontal cortex in acquisition, extinction, and reinstatement of cocaine-conditioned place preference. *Brain Research*, *990*(1-2), 157–164. doi:10.1016/S0006-8993(03)03452-8

Zeeb, F. D., Floresco, S. B., & Winstanley, C. A. (2010). Contributions of the orbitofrontal cortex to impulsive choice: interactions with basal levels of impulsivity, dopamine signalling, and reward-related cues. *Psychopharmacology*, *211*(1), 87–98. doi:10.1007/s00213-010-1871-2

Zou, J., & Crews, F. (2010). Induction of innate immune gene expression cascades in brain slice cultures by ethanol: key role of NF-κB and proinflammatory cytokines. *Alcoholism, Clinical and Experimental Research*, *34*(5), 777–89. doi:10.1111/j.1530-0277.2010.01150.x