The Role of Dopamine in MK-801-Induced Appetitive Extinction Responding and Associated Changes in Striatal Phosphorylated ERK

by

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Abstract

MK-801 influences learning and reward systems but triggers a dopamine (DA) dependant appetitive extinction deficit and changes phosphorylated ERK (pERK) in the striatum. The goal of this study was to elucidate the role of DA in extinction by comparing the MK-801-induced extinction deficit to the DA agonist cocaine along with D1/D2 antagonist pre-treatment, examining how DA agonists GBR 12909 and apomorphine affect extinction, and assess locomotion and striatal pERK expression. It was hypothesized that DA agonist drugs would potentiate extinction responding and increase pERK in the striatum. The results suggested that DA agonist drugs are capable of increasing responding during extinction but the results do not conclusively suggest that the MK-801 extinction profile results solely from altering the reward system. The magnitude of the extinction profile after MK-801 was not matched using any of the DA agonist drugs used in this study, implicating a synergism between reward potentiation and a learning deficit.

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Introduction

In all living organisms, biological homeostasis must be maintained by satisfying various energy demands. At a basic level, this is achieved through intake of resources like food and water. Satisfying basic needs and returning the body to homeostatic balance produces the subjective feeling of reward and this rewarding experience triggers leaning and the formation of memories that serve to reinforce future attainment of the reward (Berridge et al., 2010). Conversely, through the experience of pain or discomfort, experiential learning shapes behaviour so that stimuli that pose a threat are avoided (Jeffrey and Schulkin, 1998). Failure to satiate internal systems, for example by regulating food and water intake, results in an intense drive or craving (Hull, 1943; Hull, 1952; for review see Berridge et al., 2010). From an adaptive standpoint, craving can be thought of as a subjective, motivational drive that is meant to encourage behaviours associated with acquiring the specific resource that is in need. In order to reinforce the need for basic necessities crucial to survival, stimuli that are predictive of the reinforcer also come to be associated with the subjective experience of the primary reward (Berridge et al., 2010).

The formation of goal-directed and reward-seeking behaviours serves an adaptive purpose by promoting health, reproduction and survival. Organisms possess an innate drive to obtain nourishment, procure resources and approach pleasurable stimuli. These behaviours are crucial to normal functioning, and this is clearly demonstrated by the wide range of reward-based pathologies that result from dysfunction in this system. Taken together, motivation and reward learning involves interplay from multiple environmental

factors and biological changes that combine to produce complex and often long lasting behavioural alterations.

Uncovering the dynamics and interplay between learning and reward seeking has been a main focus of learning and addictions research. Maladaptive reward seeking is characteristic of numerous biological and behavioural problems like obesity and substance abuse (Everitt et al., 2008). When maladaptive reward seeking behaviors are formed, they are often persistent and difficult to resolve. These kinds of uncontrollable behaviours are the product of maladaptive learning that stems from the development of persistent biological changes and plasticity in the brains reward system (Everitt et al., 2008). Although there have been significant advances in understanding of how these systems interact, there are still many unanswered questions. We require a better understanding of how learning and reward-seeking behaviours manifest biologically and how these influences produce persistent behavioural changes. Uncovering the mechanisms in the extinction of reward-based learned behaviour would contribute to the development of pharmaceutical and therapeutic treatments that target disorders that that are characterized by learning and motivational deficits.

Adaptive learning and reward seeking

In nature, there are elements from an animal's environment that are constantly changing. In particular the accessibility of resources like food and water that are crucial to day-to-day survival is often intermittent and must be obtained quickly and efficiently. In response to the erratic nature of these environmental elements, animals have developed the ability to predict when certain resources will become available. This triggers a

motivational response that facilitates the procurement of the resource. This anticipatory behaviour, referred to as a predictive memory or a conditioned response, is an adaptive characteristic that involves interplay between peripheral signals and neurobiological changes in response to environmental cues (for review see Baxter and Byrne, 2006). For example, feeding behaviour arises from homeostatic need, but there is also a strong hedonic component. In general, homeostatic feeding results from hormonal signaling between the brain and the gut, while the hedonic component of feeding involves activation of reward pathways in the brain. Furthermore hedonic feeding can be triggered in response to various environmental cues, such as the sight or odor of a rewarding food (Malik et al., 2008). But these systems are not mutually exclusive. Some hormones that stimulate homeostatic feeding behaviour also influence hedonic reward pathways, can be triggered by environmental cues, and play a major role in food-based reward seeking (Malik et al., 2008).

Classical or Pavlovian conditioning

Classical conditioning refers to the assignment of meaning to an environmental cue or conditioned stimulus (CS) whose presentation will elicit an anticipatory biological response (Pavlov, 1927; Skinner, 1935; for review see Baxter et al., 2006). When an environmental cue consistently predicts the presentation of a relevant event or reward (unconditioned stimulus, US) the organism will make an association between the two stimuli. Eventually, the CS will signal that presentation of the US will follow and a conditioned reflexive response will ensue. Under normal circumstances the CS will be related to the US in some logical way (ex. the smell of food will trigger salivation) but a

logical connection between these stimuli is not required in order to form the necessary association (Baxter et al., 2006).

The classic example of this behavioural phenomenon was the famous experiment by Pavolv (1927) where an arbitrary sound (a tone) was presented to dogs before they were given food. Initially the dogs did not respond to the tone, but eventually the dogs formed an association. Once an association between the CS and US is established, the animal will exhibit biological responses and initiate anticipatory behaviours (conditioned response, CR) following presentation of the CS. In the case of Pavolv's dogs, once the association was formed, the dogs exhibited a conditioned response (salivation) upon presentation of the tone. Classical conditioning illustrates that reflexive biological responses can be elicited in response to environmental cues. The ability to predict and quickly act upon favorable opportunities in the environment maximizes the chances of obtaining a reward, further illustrating the adaptive nature of classical conditioning (Baxter et al., 2006).

Operant conditioning

Operant conditioning or instrumental learning refers to the process whereby an animal will learn to perform a goal-directed behaviour in order to control the presentation of a stimulus. In operant conditioning the animal makes an association between their behaviour and an event. In the case of appetitive operant conditioning, the animal will learn to press a lever to receive a food pellet. In most cases, the animal will quickly learn to modify their behaviour in such a way as to either maximize reward or minimize

punishment, depending on the environmental conditions (Thorndike, 1911; Skinner, 1938; for review see de Wit and Dickinson, 2009).

In operant reward-based conditioning, once the association between the behaviour and the presentation of reward has been formed, goal directed behaviours aimed at procurement of the reward will increase in frequency. Once operant conditioning has been established, consistent presentation of reward must occur following the conditioned behaviour in order to maintain the association. Failure to do so will result in a weakening of the association, a process referred to as extinction (McLaughlin and Floresco, 2007; for review see Killeen and Sanabria, 2009).

Operant conditioning is a useful tool to assess both learning and goal directed behaviour. In rodent studies, subjects are placed inside an operant box, where they can activate a lever that alters the presentation or removal of a stimulus. There are a number of methods that can be employed to assess motivation and reward learning using operant conditioning, making it an excellent model in learning and addictions research (Killeen et al., 2009). Food-based operant responding has been used extensively to assess the rewarding properties of palatable food and how motivation for food is altered by manipulating biology, pharmacology or behaviour. Self-administration studies constitute another class of operant-based behavioral tasks where activation of a lever triggers an intravenous infusion of a particular drug (Baxter et al., 1974; for review see Panlilio and Goldberg, 2007). They are considered to be comprehensive animal models of drug abuse and addiction, with more addictive drugs producing increased operant responding (Richardson and Roberts, 1996; Chiodo, 2008). By applying these methods, operant conditioning experiments allow researchers to focus on multiple characteristics of reward

seeking and learning, beyond merely the acquisition and maintenance of learning. For example, extinction, spontaneous recovery or reinstatement of reward seeking can be assessed (Knackstedt, 2010; Rescorla, 2007), which can provide a more accurate profile of complex behaviours like learning and motivation.

Extinction learning

The extinction of operant behaviour occurs when a previously conditioned instrumental response no longer triggers the presentation of a rewarding or aversive stimulus (McLaughlin and Floresco, 2007; for review see Killeen and Sanabria, 2009). In the case of appetitive extinction, rats that are trained to lever press for palatable food pellets will exhibit a decrease in responding during an extinction trial, where activation of the lever does not deliver a food reward (Hironaka et al., 2004; Woods and Bouton, 2008; Holahan, Clarke and Hines, 2010). In fear conditioning, an extinction session will produce a decline in fear responses when an aversive stimulus, for example a shock, is no longer presented in conjunction with previously conditioned environmental cue (Woods et al., 2008).

There has been a surge of interest in the mechanisms underlying extinction learning. The goal of extinction-based clinical therapies is to reduce the expression of compulsive and cue induced reward seeking and fear responses via exposure and response prevention. For example exposure therapy has been used clinically to treat eating disorders (McIntosh et al., 2010; (Steinglass and Parker, 2011), gambling (Jimenez-Murcia et al., 2011), and drug addiction (Havermans et al., 2006), as well a variety of fear and anxiety disorders (Bolton and Perrin, 2008; Hoyer et al., 2009).

Clinical studies show that extinction-based exposure therapy is generally quite effective for fear-based extinction (Feske and Chambless, 1995; Foa et al., 1999; Öst et al., 2001; Quirk et al., 2010) but has only achieved limited success in the treatment of compulsive behaviours that are prone to relapse (Maxwell, 2000; Price et al., 2010; for review see Conklin and Tiffany, 2002). A better understanding of the factors that influence extinction learning could lead to improvement in extinction based therapeutic techniques or the identification of potential complementary pharmacological treatments.

Extinction is believed to occur in three phases, acquisition, consolidation and retrieval (for review see Quirk and Mueller, 2008). The acquisition of extinction refers to the first phase of learning that corresponds to a decrease in operant responding during an extinction trial. Consolidation of the extinction memory must then occur and the extinction memory enters long-term storage. Retrieval is said to occur when the conditioned stimulus is presented and the previously extinguished behaviour returns (Keiflin et al., 2008; Quirk et al., 2008).

A number of distinct brain regions have been highlighted for their prominent role in extinction learning. In general, the prefrontal cortex (PFC), hippocampus and amygdala play central role in extinction learning. In appetitive extinction learning, activation of the reward system, such as the striatum and substantia nigra, has been identified as critical (Quirk et al., 2008; Noonan et al., 2010). In fear extinction activation of regions that involve fear responses, for example the periaqueductal grey seems to be an essential feature (Quirk et al., 2008). It is clear that extinction learning is, at least in part, a memory dependent mechanism, but in the case of reward-based extinction, pharmacological activation of the reward system can also alter extinction behaviour

(Holahan et al., 2010). This is an indication that reward-based extinction is likely the result of interactions between learning and reward mechanisms, rather than being exclusively learning dependent.

Extinction of operant learning is generally believed to occur from the formation of a new memory that suppresses or inhibits the old association between the instrumental response with the reward (Cammarota et al., 2004). Older theories attributed extinction to 'unlearning' the initial association, but the endurance of the previously reinforced association cannot be disputed; spontaneous recovery of a previously extinguished operant behaviour occurs readily (Rescorla, 2007) and reinstatement of a previously extinguished behaviour will occur rapidly when the presentation of the reward or previously paired cues are reestablished (Keiflin et al., 2008).

Spontaneous Recovery

Spontaneous recovery is the partial resurgence of a previously extinguished behaviour (for review see Rescorla, 2004). Spontaneous recovery occurs when a second extinction test is administered, normally on the day following the initial extinction trial (Rescorla, 2007). During a second extinction trial, if there is an increase in operant responding compared to the previous, initial extinction session, spontaneous recovery has occurred (Rescorla, 2004; Rescorla, 2007). The recurrence of a previously extinguished behaviour indicates that the learning during acquisition and memories of the operant association have persisted, despite inhibition by extinction learning. Also, spontaneous recovery responding declines with multiple extinction sessions, suggesting that repeated

extinction learning sessions increase inhibition of the initial operant association (Rescorla, 2004).

Dopamine

Reward-seeking behaviour is complex and involves input from multiple neurotransmitters and neural pathways in the brain. Motivation to obtain reward arises from a combination of interactions between various neural systems, but the vast majority of research points to dopamine (DA) as the key reward neurotransmitter. DA has been identified as playing a fundamental role in both reward seeking (Salamone, 1994; Chocyk et al., 2008) and the initiation and regulation of goal-directed movement (Thomsen and Caine, 2011; Goto and Grace, 2005). Stimuli, such as food or water, as well as environmental conditions paired with these reinforcing stimuli, elicit the synthesis and release of DA into various terminal regions within the reward system (Kalivas and O'Brien, 2007; Carlezon and Wise, 2009; Berridge et al., 2010). This signals to the organism that the stimulus is beneficial to survival and to initiate the necessary behaviours needed to procure it (Kalivas, 2007).

DA is a catecholamine neurotransmitter that is synthesized from L-Tyrosine. The enzyme Tyrosine hydroxylase catalyses the conversion of L-Tyrosine to L-Dihydroxyphenylalanine (L-DOPA) and is the rate limiting step in this synthesis chain (Fernstrom and Fernstrom, 2007). DOPA decarboxylase converts L-DOPA to DA. Once DA is synthesized it acts within many regions of the brain on various receptor subtypes (described below) before being recycled by the cell and reused, or degraded via methylation by catecho-O-methyltransferases (COMT) or deamination by monoamine

oxidase (MAO). It is important to remember that the catecholamine synthesis cycle does not terminate once DA has been formed, rather Dopamine β-hydroxlase may convert DA to Norepinephrine (NE), which can then be converted to Epinephrine via Phenylethanolamine *N*-methyltransferase (Fernstrom et al., 2007). This step in catecholamine synthesis is important to consider when investigating the role of DA in behaviour. Experimentally manipulating the DA system will also result in changes in NE, which has also been implicated in a wide range of behaviours from learning mechanisms to reward-based behaviours including sensitization and reinstatement of drug seeking (Sofuoglu and Sewell, 2009).

Five subtypes of DA receptors have been identified and grouped into two main classes according to intracellular changes brought about by ligand binding. DA receptors grouped within the D1-like class include D1 and D5 receptors (Heidbreder et al., 2005). When DA binds to a D1-like receptor, the Gs protein is activated. Activation of the Gs protein, in turn, triggers activation of the enzyme adenylate cyclase. Adenylate cyclase catalyses the conversion of adenosine triphosphate to cyclic adenosine monophosphate or cAMP, a second messenger associated with signal transduction, the activation of various protein kinases and the regulation of calcium through ion channels. Conversely, when DA binds to receptors within the D2 class, including D2, D3 and D4 subtypes, this activates the Gi protein, which in turn inhibits adenylyl cyclase and reduces the synthesis of cAMP (Fernstrom et al., 2007; Tirotta et al., 2010; Shiflett et al., 2011).

DA receptors can be grouped according to class, but significant differences in the behavioural correlates induced by receptor subtype activation have been identified. Pharmacologically altering receptor binding and modeling behaviour in DA receptor

mutant animals has demonstrated that each receptor subtype is associated with differential effects on locomotion and reward based behaviours.

D1 Receptor

The D1 receptor has been heavily implicated in locomotor behaviour and reward-based learning. In a study conducted by Xu et al. (1994) D1-deficient mice exhibited hypolocomotion, suggesting this receptor may be an important regulator of the motor system. D1-deficiency is also characterized by decreased locomotor responses to cocaine, indicating that the D1 receptor facilitates acute locomotor potentiation by psychostimulant drugs (Xu et al., 1994) and sensitization to the locomotor effects of repeated psychostimulant administration (Xu et al., 2000).

The D1 receptor is also linked to the acquisition of operant behaviours. In a study conducted by Baldwin, Sadeghian and Kelley (2002), infusion of the D1 receptor antagonist SCH 23390 into the prefrontal cortex (PFC) of food-deprived rats resulted in a dose-dependent decrease in acquisition responding. This finding suggests that appetitive instrumental learning, or the motor responses necessary to complete the task, is mediated by activation of the D1 receptor. D1 receptor plays a central role in psychostimulant-induced locomotion, but inactivation of this receptor does not seem to decrease the rewarding properties of psychostimulant drugs. For example, in a study by Miner et al. (1995) D1-deficient mice exhibited reduced locomotor responses to cocaine, but still developed conditioned place preference, suggesting that the rewarding properties of the drug were not disrupted.

D5 Receptor

Knockout studies indicate that the D5 receptor also plays a role in locomotor behaviour, but to a lesser degree than D1 receptors (for review see Tirotta et al., 2010). In a study conducted by Elliot, Sibley and Katz (2003), D5-deficient mice exhibited an attenuated locomotor response to cocaine, comparable to locomotion observed in SCH 23390-treated animals. This suggests that the D5 receptor mediates psychostimulant-induced locomotion in a manner similar to the D1 receptor. And, similar to D1-deficient mice, D5 knockouts can discriminate between cocaine and saline treatment (Elliot et al., 2003) suggesting that the psychoactive effects of the drug are not disrupted by D5 inactivation.

D2 Receptor

Studies indicate that D2-deficient mice exhibit significant motor impairments, linking this receptor subtype to locomotion, posture and coordination (for review see Tirotta et al., 2010). It has also been identified as central mediator of reward-based behaviours. For example, D2 receptors were linked to appetitive reward seeking in an early study by Spyraki, Fibiger and Phillips (1982) where pretreatment with the D2 antagonist haloperidol prevented the development of conditioned place preference for food. This finding suggests that the D2 receptor mediates food seeking and reward. In a more recent study conducted by Johnson and Kenny (2010) a downregulation of D2 receptors was observed in obese rats, which may be indicative of a compensatory mechanism attempting to counteract the regular intake of highly palatable foods that activate reward pathways and increase activation of the D2 receptor.

With regards to drug seeking behaviour, the D2 receptor has been linked to the reinforcing properties of drugs of abuse. For example, knocking out the D2 receptor disrupts drug self-administration and blunts conditioned place preference (Tirotta et al., 2010). Knockout studies show that D2-deficient mice also display reduced locomotion in response to cocaine (Tirotta et al., 2010). Furthermore, D2 receptor downregulation is observed in human cases of psychostimulant addiction (Volkow et al., 1993; Volkow et al., 2001; Adinoff, 2004), suggesting that compensatory mechanisms may be occurring to offset the unnaturally high levels of D2 occupancy elicited by regular drug use.

D3 Receptor

Knockout studies indicate that the D3 receptor plays a role in locomotion with D3-deficiency producing potentiated locomotor activity (Tirotta et al., 2010). The D3 receptor has also been implicated in reward seeking and compulsive behaviours. Thanos et al. (2008) assessed operant responding for palatable food and found that the D3 receptor antagonist SB-277011A significantly decreased operant responding, suggesting that the D3 receptor plays a role in food reward. SB-277011A decreased overall food intake in both groups, suggesting that it may also play a role in mediating satiety. These findings are indicative of the D3 receptors fundamental role in the reinforcing and motivational properties of food.

The D3 receptor has also been linked to instrumental reward learning. Hitchcott, Bonardi and Phillips (1997) examined the role of the D3 receptor in cue induced reward approach in rats and found that infusing the D3 receptor agonist 7-OH-DPAT into the amygdala altered conditioned approach behaviour. It was observed that 7-OH-DPAT

dose dependently facilitated conditioned approach toward a stimulus that had been previously paired with sucrose water, implicating this receptor in motivational responses to reward based cues. The D3 receptor has also been implicated in the reinforcing properties of drugs. In a study conducted by Peng and colleagues (2009) pretreatment with the D3 antagonist S33138 significantly attenuated cocaine self-administration, suggesting that the D3 receptor mediates the rewarding properties of psychostimulants. Furthermore, pretreatment with low doses of the D3 antagonist SB-277011 reduces operant responding for cocaine and blocks cocaine reinstatement (Heidbreder et al., 2005), further implicating this receptor subtype in the hedonic value of reinforcers.

D4 Receptor

The D4 receptor has been linked to locomotion; with knockouts exhibiting altered locomotor behaviour and increased sensitivity to the locomotor stimulant effects of drugs, but the finding are somewhat inconsistent and the role of the D4 receptor in locomotion has not been clearly defined (Tirotta et al., 2010). Also, unlike the other DA receptor subtypes of this class, the D4 receptor does not appear to mediate reward based behaviour. In a study conducted by Thanos et al. (2010) D4 knockouts did not differ significantly in food or cocaine operant responding, suggesting that the D4 receptor plays a negligible role in reward seeking.

Dopamine systems and reward

The DA system is comprised of three main pathways: mesocorticolimbic, nigrostriatal, and tuberinfundibular. These pathways are implicated in an eclectic range of behaviours, including reward seeking and learning.

The Mesocorticolimbic Dopamine System

This pathway originates in the ventral tegmental area (VTA) and projects to regions in the limbic system including the nucleus accumbens (Nac), amygdala, hippocampus and medial prefrontal cortex (mPFC) (Carlezon & Thomas, 2009). These are brain regions that have been linked to goal-directed behaviours in multiple ways, such as reward-based learning, the initiation of goal-directed actions, planning and coordinating behaviours and emotional contribution to reward (Carlezon & Thomas, 2009). The mesocorticolimbic DA system is of particular interest in the neurobiology of motivation, reward and addiction (Wise, 2009) and this pathway is heavily implicated in reinforcement-based learning, the hedonic or aversive value of stimuli and addictive behaviour (Goto et al., 2005; Heidbreder, 2005; Kalivas, 2007).

The Nigrostriatal Dopamie System

The nigrostriatal DA system is also implicated in the neurobiology of goal seeking and reward (Wise, 2009). This pathway originates in the substantia nigra and projects to the striatum (Leyton et al, 2005). The striatum is a large sub cortical structure that can be broken down into dorsal and ventral subdivisions. The ventral subdivision of the striatum is also known as the Nac. Although the majority of DA input to the Nac originates in the VTA (Carlezon et al., 2009), the nigrostriatal DA system is also believed to project to this region (Deutch and Cameron, 1991). The ventral subdivision plays a fundamental role in motivation and reward learning, while the dorsal subdivision is more often associated with locomotor activity and goal-directed movement, but there is a degree of functional overlap between the subdivisions (Robbins and Everitt, 1992).

The Tuberoinfundibualar Dopamine System

The cell bodies for this DA pathway are located in the hypothalamus and project to the pituitary gland (for review see Fitzgerald and Dinan, 2008). Antagonizing DA receptors along this pathway triggers an increase in prolactin secretion. Prolactin is a luteotropic hormone that plays a role in various biological systems including lactation, the immune system and various cell cycle related functions (Fitzgerald et al., 2008).

Pharmacological Agonists of the Dopamine System

There are multiple advantages to using pharmacological agents to assess changes in learning and reward-seeking behaviour. For example, pharmacological treatments can be employed during the acquisition of an operant response (Ranaldi et al., 2011) or prior to the extinction session (Aparicio, 2010; Holahan et al., 2010; Plaza-Zabala et al., 2010; Vurbic et al., 2011) to investigate how altering brain chemistry influences the different phases of reward–based operant tasks. In appetitive extinction, pharmacological manipulation also allows researchers to assess how altering neurotransmitter release peripherally or centrally affects learning and extinction behaviour, and which systems in the brain are implicated in extinction enhancement or resistance (Holahan et al., 2010; Ranaldi et al., 2011).

Biological and Behavioural Influences by Direct and Indirect Dopamine Agonists

MK-801

(+)-5-methyl-10,11- dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK-801) is a non-competitive antagonist of the N-methyl-d-aspartate (NMDA)

receptor. The majority of research has focused primarily on the specific glutamateric actions of MK-801 to experimentally assess deficits in learning and memory (Butelman et al., 1989; Kant et al., 1991; Brown et al. 2008). A secondary effect of MK-801 is the activation of the dopamine (DA) system via inhibition of the medial prefrontal cortex (mPFC) (Jackson, Frost and Maghaddam, 2001).

Normally the PFC exerts inhibitory control over the DA system. Karreman and Moghaddam (1996) elucidated the regulatory role of the PFC in DA excitability by inhibiting cells in the PFC and recording the resulting changes in DA transmission in the striatum. They demonstrated that disinhibiting the PFC with the GABA antagonist bicuculine resulted in an increase in DA in the striatum, suggesting that the PFC plays an inhibitory role in DA transmission in this region. Furthermore, antagonizing NMDA receptors in the VTA blocked this bicuculine induced increase in striatal DA. This observation is indicative of excitatory glutamatergic input from the PFC to the VTA.

Cells in the PFC terminate on GABA neurons that project to the Nac (Sesack and Pickel, 1992; Carlezon et al., 1996; Carr and Sesack, 2000). Blocking excitatory Glu transmission in the PFC inhibits activation of cells that normally stimulate GABAergic neurons that, in turn, inhibit the Nac. When this GABA release is inhibited by administration of NMDA receptor antagonists like MK-801, it triggers an increase in DA along the mesolimbic pathway (Jackson et al., 2001). Also, reducing glutamatergic activity in the PFC will reduce its excitatory influence on the VTA. In concordance with these observation, injections of MK-801 have been reported to increase DA output in the striatum (Ali et al., 1994) likely by increasing firing rates of mesolimbic dopaminergic neurons (Murase et al., 1993; Zhang et al., 1992).

The outcome of this MK-801-induced elevation in DA output is seen in the effect of MK-801 on reward-like behaviors. Animals can be trained to self-administer MK-801 (Carlezon et al., 1996), suggesting that the drug is reinforcing. Studies investigating the effect of pretreatment with MK-801 on cocaine reinforcment have yielded mixed findings. MK-801 has been reported to decrease cocaine self administration in monkeys (Platt, Rowlett and Spealman, 2008) but rat studies report an increase in cocaine responding that is indicative of a potentiation in the rewarding effects of the drug (Pierce, Meil and Kalivas, 1997; Hyytia, Backstrom and Liljequiest, 1999; Allen et al., 2005). MK-801 also produces a conditioned place preference at doses ranging from 0.03 mg/kg to 0.75 mg/kg in rats (Layer et al., 1993; Panos et al., 1999; Papp et al., 1996; Steinpreis et al., 1995), further suggesting that MK-801 has rewarding properties.

MK-801 has been reported to potentiate reward-seeking behaviour in a variety of experimental models of reward. In a study conducted by Cabenza de Vaca and Carr (1998), the effect of MK-801 pretreatment on intracranial self-stimulation (ICSS) was investigated. Systemic MK-801 produced an increase in ICSS responding and this effect was potentiated by food restriction. This finding suggests that MK-801 treatment will increase hedonic based stimulation responding and that food deprivation can further potentiate this effect. MK-801 has also been reported to increase the rewarding properties of food. For example, rat studies indicate that MK-801 increases palatable food intake in food-restricted animals (Burns and Ritter, 1997; Covasa et al., 2004). Taken together, the research suggests that MK-801 treatment enhances the rewarding properties of both conventional and pharmacologic reinforcers and it is likely that it is through this mechanism that MK-801 induces an appetitive extinction deficit.

Cocaine

Cocaine is an indirect DA agonist with extremely high abuse potential. According to Kalivas (2007) the abnormal levels of DA that result from long term cocaine administration trigger neuroplastic changes that facilitate learning, and therefore the acquisition of reward seeking behaviours. Cocaine inhibits the reuptake of dopamine (DA) by blocking its transporter protein. Transporter proteins facilitate reuptake by pumping neurotransmitters back in to the presynaptic cell, which signals to the cell to terminate further chemical release (Zahniser & Sorkin, 2009).

By inhibiting the reuptake of DA, cocaine administration indirectly increases the transmission of DA, resulting in increase in DA levels along the nigrostriatal and mesocorticolimbic DA pathways. DA increase in the Nac following cocaine administration has been repeatedly demonstrated and is believed to play a central role in the development of cocaine addiction (Weiss et al., 1992; Aragona et al., 2008). Furthermore, central infusion of DA agonists into the Nac have been shown to elicit reinstatement of cocaine self-administration following an extinction session, while DA antagonist infusion in the same region blocks reinstatement of drug seeking (Bachtell, Whisler, Karanian and Self, 2005).

To a lesser extent, cocaine also binds to the transporters for serotonin (5-HT) and NE (Serafine & Riley, 2009). NE originates in the Locus coeruleus (LC) and is involved in alertness and arousal states (Macey et al., 2003). By blocking the NE transporter (NET), cocaine also indirectly increases NE., This would initiate sympathetic nervous system activation contributing to anxiety following cocaine use and withdrawal (Macey

et al., 2003). By binding to the serotonin transporter (SERT), cocaine acts as an indirect 5-HT agonist, likely contributing to the psychostimulant and mood altering effects of the drug (for review see Kirby, Zeeb and Winstanley, 2011).

Animals will readily learn to self-administer cocaine and will develop a conditioned place preference for environments associated with cocaine administration (Ettenberg et al., 1982; Hyytia et al., 1999; Allen et al, 2005; Botreau et al., 2006; Chiodo et al., 2008; Keiflin et al., 2008; Knackstedt et al., 2010), clearly demonstrating the reinforcing properties of the drug. Administration of DA antagonists results in a decrease in cocaine self-administration in rats, suggesting that the subjective rewarding and reinforcing characteristics of cocaine are DA dependent (Ettenberg et al., 1982). This finding is substantiated by clinical studies suggesting that DA neurotransmission is directly related to motivational states associated with cocaine reward (Leyton et al., 2005).

GBR-12909

Similar to cocaine, GBR-12909 binds with a high affinity to the DAT, causing an increase in extracellular DA (Villemagne et al., 1999; Singh, 2000). Unlike cocaine, GBR-12909 has a much longer half-life and produces a gradual increase in DA, rather than the massive and instantaneous rise in concentration observed following cocaine treatment (Singh, 2000; for review see Rothman et al., 2008). Because of these qualities, GBR-12909 has been used extensively in pharmacological and preclinical addiction research and some research suggests that it has potential for the treatment of cocaine addiction (Rothman et al., 1991; Baumann et al., 1994; Rothman et al., 2008).

Researchers have suggested that, like methadone treatment for heroin addiction, GBR-12909 could be useful as a cocaine replacement therapy (Rothman et al., 1991; Baumann et al., 1994). While both GBR-12909 and cocaine work via similar mechanisms and both are associated with activation of reward pathways, it is believed that the significantly lengthened half-life of the GBR-12909 produces lessened subjective feelings of reward in comparison to cocaine.

Despite the observation that GBR-12909 has such a high affinity for the DA transporter, studies show that pretreatment attenuates the increase in DA elicited by cocaine (Baumann et al., 1994). Also, systemic pretreatment with high doses of GBR-12909 reduce self-administration of cocaine in animal studies (Villemagne et al., 1999; Singh, 2000; Schenk, 2002), likely by preventing cocaine binding to the DAT transporter.

Although GBR-12909 has been suggested as a replacement therapy for cocaine addiction, studies show that GBR-12909 still has addictive potential. Animals will self-administer GBR-12909 and treatment triggers reinstatement of extinguished drug-seeking behavior (Wojnicki and Glowa, 1996; Stafford et al., 2001). Animals that have been trained to self-administer cocaine will maintain responding when cocaine is replaced with GBR-12909 (Roberts, 1993) and GBR-12909 treatment has been reported to produce locomotor sensitization in a manner similar to cocaine (Kelley and Lang, 2002). Also, animals that receive repeated administrations of cocaine will exhibit mild cross-tolerance to GBR-12909 (Elmer et al., 1996), and systemic administration of GBR-12909 dose-dependently reinstates cocaine and methamphetamine responding (Gardner, 2000; Schenk, 2002; Carati and Schenk, 2011), suggesting the GBR-12909 acts via similar

mechanisms and can serve as a cue to reinstate previously extinguished drug seeking behaviours.

Apomorphine (APO)

APO is a direct, nonselective DA agonist that increases the transmission of DA by enhancing the affinity of DA at the receptor (Braga et al., 2009). Multiple electrophysiological and pharmacological studies indicate that APO is a potent agonist of the DA system at high doses, but that low doses in the 0.05mg/kg range act presynaptically to reduce DA (Van Ree and Wolterink, 1981; Cary et al., 2004; Braga, Dias, Carey and Carrera, 2009). Low doses of APO reduce DA transmission by acting upon presynaptic autoreceptors (Aghajanian and Bunney, 1977; Cary et al., 2004), while high doses of APO mimic the action of DA on various postsynaptic receptors to increase the firing of DAergic cells (Argiolas and Hedlund, 2001).

APO has been used experimentally to assess the rewarding and stimulating effects of DA agonism. In a study by Braga et al. (2009) a dose-dependent increase in locomotor behaviour was observed, with high doses of APO (0.5 and 2.0mg/kg) producing hyperlocomotion and behavioural sensitization and low doses inducing hypolocomotion. Rats will self-administer APO at doses ranging from 0.125-1 mg/kg, and DA antagonist pretreatment blocks this effect (Baxter et al., 1974; Woolverton, Goldberg and Ginos, 1984; Norman et al., 2011). Taken together, these results suggest that APO treatment does activate the DA system and produces behaviours that are similar to those observed with other psychostimulant drugs like cocaine.

Learning Mechanisms and Reward Learning

Glutamate

It is clear that DA plays a fundamental role in goal-directed behaviour. The reward system, however, is much more complex and activation of a single neurotransmitter system cannot explain the entirety of its behavioral outcomes. Glutamate- (Glu) dependent learning and synaptic plasticity occurs in conjunction with and in response to activation of the DA reward system (for review see Kalivas et al., 2007). These complex neurotransmitter systems interact within various brain regions to produce reward-based learning and motivational responses to environmental cues.

Glu is an excitatory neurotransmitter that plays a critical role in learning and memory formation (Meldrum, 2000; Kemp and McKernan, 2002). There are two ways in which the strength of neural synapses can be altered: long term potentiation (LTP) and long term depression (LTD) (Thomas, Kalivas, & Shaham, 2008). Both LTP and LTD of neural synapses will result in biological changes that include the strengthening or weakening of synapses and modifications in the branching of dendrites (Meldrum, 2000; Kemp and McKernan, 2002; Thomas et al., 2008). There are multiple forms of LTP and LTD but all known mechanisms involve activation of the Glu system in some manner, demonstrating the importance of this neurotransmitter in learning and synaptic plasticity. Glutamate receptors can be divided into two broad categories according to the mechanism that triggers cellular activation, ionotropic and metabotropic. The ionotropic Glu receptors include subtypes N-Methyl-D-aspartate (NMDA), Kainate and 2-amino-3-(5-methyl-3-oxo-1,2- oxazol-4-yl)propanoic acid (AMPA), while only one class of

metabotropic Glu receptor (mGluR) has been identified (Meldrum, 2000; Mameli and Luscher, 2011).

While a neuron is at rest, NMDA receptor ion channel pores are blocked by magnesium ions (Mg^{2+}) that disrupt the flow of Ca^{2+} ions through the channel (Meldrum, 2000). When sufficient depolarization of the cell occurs, the Mg^{2+} block is relieved and Glu binding can trigger entry of Ca^{2+} (Meldrum, 2000; for review see Luscher and Frerking, 2001). This mechanism then proceeds to trigger a cascade of intracellular events that lead to the formation of AMPA receptors. The upregulation of AMPA receptors strengthens the excitatory connections on the postsynaptic cell, thus making the cell more excitable (Meldrum, 2000; Mameli and Luscher, 2011).

Conversely, in LTD AMPA receptors are removed from the cellular membrane. This process occurs when weak signals on the postsynaptic cell slightly activate NMDA receptors, allowing a small amount of Ca^{2+} to enter (Selig et al., 1995; Morishita et al., 2005). Insufficient intracellular Ca^{2+} leads to the activation of phosphatases that catalyze the removal of phosphate groups (dephosphorylation) from AMPA receptors, which in turn leads to AMPA receptor downregulation (Selig et al., 1995; Morishita et al., 2005).

Glutamate systems and reward

Glu activation is a vital component of the reward system and triggers multiple learning and signaling mechanisms in the brain. For instance, Kelley, Smith-Roe and Holahan (1997) demonstrated that Glu transmission in the core portion of the Nac is required for the acquisition of an instrumental task. It was observed that infusing the core of the Nac with the NMDA receptor antagonist 2-amino-5-phosphonopentanoic acid

(AP-5) interfered with the acquisition of food based operant learning. A similar but blunted effect was observed after infusion of AP-5 into the Nac shell region. Furthermore, AP-5 administration did not interfere with operant responding once the task had been learned, suggesting that the acquisition of operant instrumental learning occurs via a glutamatergic dependant mechanism in the Nac.

In another study by Zellner and colleagues (2009) the role of Glu activation in the VTA on acquisition of food seeking operant behaviour was assessed. Microinjections of the NMDA receptor antagonist AP-5 into the VTA prior to conditioning produced a deficit in reward-based learning. In a similar study by Ranaldi et al. (2011) AP-5 infusion in the VTA also impaired the acquisition of conditioned approach and this deficit occurred in conjunction with significantly reduced cellular activation in the Nac. MK-801 has been reported to block behavioural sensitization to cocaine (Kalivas and Alesdatter, 1993) and conditioned place preference (Kim et al., 1996), indicating that the formation of reward based behaviours may be dependent upon Glu signaling. Additionally, research indicates that intracellular signaling mechanisms are activated by Glu and contribute significantly to learning and the development of reward seeking behaviours.

There is a complex interplay between Glu-dependant learning, LTP and DA activation in the formation of addictive behaviours but it is unclear how these factors interact synergistically to produce persistent reward-seeking behaviours (for review see Mameli et al., 2011). Various cortical and limbic structures innervate the striatum via excitatory glutamatergic afferents (Shiflett & Balleine, 2011). Projections from the ventral prefrontal cortex and limbic structures terminate in the Nac, while the dorsal

striatum receives glutamatergic projections from the sensory motor cortex (Shiflett & Balleine, 2011).

The majority of neurons in the striatum are γ-Aminobutyric acid (GABA) medium spiny output neurons (MSN) projecting to the globus pallidus, a region within the basal ganglia that exerts inhibitory control over the substantia nigra, thus preventing involuntary motor movements while at rest. Under resting conditions these striatal MSN's are hyperpolarized to ensure that disinhibition of the globus pallidus' inhibitory control over the midbrain motor system does not occur (Shiflett et al., 2011). MSN's express both DA and Glu receptors and it has been proposed that DA signaling during the hyperpolarized MSN down state may serve to regulate MSN Glu induced excitability, while DA transmission when MSN's are in an active state is thought to facilitate Glu mediated cellular activation (Shiflett et al., 2011).

The coordination of DA and Glu signaling within the striatum serves the complex function of modulating the excitability of striatal MSN's, thereby regulating synaptic plasticity, synaptogenesis, and cellular excitability (Mameli et al., 2011; Shiflett et al., 2011). The interplay of these neurotransmitter systems is key to regulating the function of the reward pathways and the degree to which synaptic plasticity will take place following activation of the DA system. It is through this mechanism that the induction of reward-based learning occurs and motivated behaviours are formed (Mameli et al., 2011). Furthermore, at a cellular level, DA and Glu transmission alter multiple intracellular signaling pathways that can influence the characteristics of cell function, such as gene transcription and other factors that affect resting state cellular excitability (Gould and Husseini, 2005; Shiflett et al., 2011).

Protein Kinase Signaling Pathways

As mentioned previously, neurotransmitters are released from presynaptic cells and bind to receptors on postsynaptic cells. These cellular mechanisms not only alter the activity of the cell in the short term, but also activate multiple downstream signaling molecules that initiate widespread changes in cellular mechanisms and gene expression (Gould and Husseini, 2005; Davis and Laroche, 2006; Shiflett et al., 2011). The MAPK/ERK kinase cascade is made up of Mitogen-activated protein (MAP) kinases that become activated through phosphorylation. This triggers a chain of downstream cellular events that mediate various characteristics of cell activity and communication (Davis et al., 2006; Shiflett et al., 2011).

Intracellular signaling plays a vital role in processes involving cell function, gene transcription and cell survival, and disruptions in these systems have been experimentally linked to numerous biological and behavioural problems such as learning and reward-based deficits (Shiflett et al., 2011). The importance of these complex mechanisms in an eclectic range of molecular actions makes these signaling cascades particularly important in the understanding of how altering neural systems shapes behaviour.

With regard to reward and motivation, one of the central signaling pathways is driven by the cAMP dependent enzyme protein kinase A (PKA). PKA catalyzes the transfer of phosphate groups from the energy store adenosine triphosphate (ATP) to various cellular targets that influence function and activity (Shiflett et al., 2011). Activation of PKA initiates phosphorylation to multiple targets in the MAPK/ERK cascade. When DA binds to the D1 receptor it triggers activation of cAMP. The increase in cAMP activity triggers production of protein kinase A (PKA). Conversely, D2 receptor

activation exerts the opposite effect, and produces a reduction in cAMP and a corresponding decrease in PKA (Fernstrom et al., 2007; Shiflett et al., 2011).

The MAPK/ERK signaling pathway plays a fundamental role in transcriptional regulation in corticostriatal circuits and has been implicated in the acquisition of goal directed behaviours, acquisition of reward learning, and extinction (Baldwin et al., 2002; Holahan et al., 2010; Shiflett et a., 2011). For example, in a study by Baldwin et al. (2002) altering PKA expression interfered with acquisition of an appetitive-learning task. In this study PKA was inhibited and stimulated, and both conditions produced impairment in learning. This finding suggests that PKA expression facilitates reward learning. Furthermore, dysregulation of PKA through under or over expression can detrimentally affect reward learning, so it is likely that expression of PKA must be maintained at an ideal level in order for this system to function normally.

Glu also plays a fundamental role in regulating ERK signaling (Gould and Husseini, 2005; Davis and Laroche, 2006; Shiflett et al., 2011). Activation of NMDA, AMPA and metabotropic glutamate receptor R1 (mGluR1) on MSN's in the striatum facilitate influx of Ca^{2+}, which in turn triggers a cascade of downstream effects that can result in inhibition of PKA and therefore a reduction of pERK (Shiflett et al., 2011). On the other hand, Glu transmission also initiates alternate downstream mechanisms that result in an increase in pERK, indicating that, alongside DA, Glu plays a central role in mediating activation of this pathway (Gould and Husseini, 2005; Shiflett et al., 2011).

Dopamine- and cAMP-regulated neuronal phosphoprotein (DARPP-32) is the key mediator in DA and Glu regulated ERK activation (Gould and Husseini, 2005; Shiflett et

al., 2011). DARPP-32 is located on DA expressing MSN's in the striatum and is differentially regulated by DA and Glu, depending upon the location of threonine (Thr) phosphorylation. In a study by Tropea, Kosofsky and Rajadhyaksha (2008) the degree of DARPP-32 and ERK phosphorylation in response to cocaine induced conditioned place preference was investigated. The researchers found a significant increase in phosphorylation in both cases, indicating that activation of this pathway may play a role in the formation of reward seeking behaviours. D1 receptor activation dependent PKA release promotes the phosphorylation of DARPP-32 at the Thr-34 residue, which in turn activates ERK by allowing DARPP-32 to inhibit protein phosphates-1 (PP-1), a potent inhibitor of ERK phosphorylation. Conversely, Glu receptor activation dephosphorylates DARPP-32 at the Thr-75 residue, allowing DARPP-32 to inhibit PKA, thereby inhibiting ERK (Shiflett et al., 2011). It is through this bidirectional mechanism that DARPP-32 can act as a molecular switch that integrates DA and Glu signaling to regulate ERK phosphorylation in response to the activity of the cell (Gould and Manji, 2005; Shiflett et al., 2011).

Another important MAPK/ERK regulated signaling cascade to consider involves the activation of calmodulin-dependant kinase II (CamKII) via Glu elicited Ca^{2+} release. This is the second mechanism whereby Glu transmission mediates pERK. CamKII activates ERK by triggering the ras-ref signaling cascade (Gould and Manji, 2005; Davis et al., 2006; Shiflett et al., 2011). Thus, Glu can regulate the phosphorylation of ERK in two ways, via DARPP-32 and CamKII. It is clear that DA and Glu systems interact, and it is the integration of these two systems that determines the net degree of pERK mediated plasticity and learning (Shiflett et al., 2011). It appears that when Glu and DA

release are synchronized, DA will facilitate Glu driven cortical plasticity, with the optimal conditions for the phosphorylation of ERK being during coordinated activation of both DA and Glu systems.

Phosphorylated ERK has been identified as a pivotal regulator of the transcription factor cAMP response element-binding (CREB), which binds to cAMP response elements (CRE) and controls the expression of various downstream genes (Gould and Manji, 2005; Shiflett et al., 2011). CREB is believed to play a role in learning and the formation of goal directed behaviours and an increase in the phosphorylation of CREB in the striatum has been experimentally linked to the formation of conditioned place preference to cocaine (Tropea et al., 2008). An increase in CREB phosphorylation has also been linked to response learning. For example, when Brightwell and colleagues (2008) used a mutant mouse model to block CREB function in the striatum they observed a significant deficit in response learning. Taken together, studies show that CREB likely facilitates learning by acting upon regions within limbic and corticostriatal circuits and that the activity of this transcription factor is likely mediated, at least in part, by pERK.

Rationale and study design

Studies indicate that administration of dopamine (DA) antagonists facilitates extinction for food and drug seeking (Burns and Ritter, 1997; Cabenza de Vaca and Carr, 1998; Covasa et al., 2004), suggesting that there is a reward and motivational component coming into play when an animal exhibits a resistance to reward-based extinction. Furthermore, pretreatment with MK-801 induces an extinction deficit (Holahan et al., 2010), likely due to DA activation. It is clear that both DA and Glu transmission

influence the degree of ERK phosphorylation in corticostriatal circuits and that this has widespread effects on motivation, reward based learning and synaptic plasticity (Gould et al., 2005; Tropea et al., 2008; Holahan et a., 2010; Shiflett et al., 2011). It is still unclear, however, how these systems interact to produce an operant extinction deficit and the role that pERK in striatal regions plays in this system.

The goal of the present study was to elucidate the role of Glu and DA systems in extinction resistance, and how pharmacologically manipulating these systems affected pERK expression in the striatum. Studies conducted in this laboratory have demonstrated that MK-801 interferes with appetitive extinction and occurs with a marked reduction in pERK in the piriform and infralimbic cortices and an increase in pERK in the Nac (Holahan, Clarke and Hines, 2010). It was also found that the extinction deficit was abolished with co-administration of DA antagonists, suggesting DA drives the extinction deficit. This observation is in agreement with previous reports that DA efflux increases during food-based extinction (Ahn and Phillips, 2007) and DA transporter knockout mice, that show elevated levels of DA in the synaptic cleft, also show resistance to extinction for food-based learning (Hironaka et al., 2004).

MK-801 influences both learning and reward systems but it remains unclear how these mechanisms interact to interfere with extinction or the role that pERK plays in this behavioural deficit. The importance of DA in reward seeking has been repeatedly demonstrated (Yokel and Wise, 1976; Carr et al., 2000) and the MK-801 induced extinction deficit seems to be contingent upon DA activation, but the interruption in learning mechanisms via NMDA receptor antagonism and changes in downstream pERK signaling may also play a pivotal role. In a study by Botreau, Paolone and Steward (2006)

administration of D-cycloserine, an NMDA receptor agonist, facilitated extinction of cocaine conditioned place preference, which is indicative of an enhancement in the learning mechanisms of extinction. Considering the positive effects of agonizing NMDA receptors on extinction learning, the role of NMDA receptor antagonism in the MK-801 induced extinction deficit cannot be ruled out.

Given what we know about the need for coordinated DA and Glu transmission in the initiation of signaling pathways, phosphorylation of ERK and consequently the induction of reward-based learning and synaptic plasticity, it seems fitting that the MK-801-induced extinction deficit may be mediated by NMDA receptor antagonism and the reduction in pERK resulting in learning deficits. Since MK-801 treatment decreases binding to the NMDA receptor, it is likely that this affect reduces the degree to which Glu binding initiates the MAPK/ERK signaling cascade. As we know, this cascade is required to phosphorylate ERK and trigger the synaptic plasticity and increased cellular excitability needed to extinguish a reward based behaviour. Since MK-801 is also an indirect DA agonist that triggers an increase in pERK in the Nac (Holahan et al., 2010) the absence of sufficient glutamatergic mediation of these downstream pathways likely plays a significant role in throwing off the balance of this system.

Although the above statements are logical and fit the theoretical model of the pERK cascade, the role of DA in MK-801 extinction resistance remains elusive. If the MK-801 extinction resistance is due to a reduction in Glu transmission alone, then the administration of DA antagonist treatment should not, in theory, have an effect on the MK-801 extinction deficit. On the other hand, since DA antagonism has such a profound effect on facilitating extinction, and is powerful enough to block the extinction deficit

produced by MK-801, then DA agonism alone should theoretically produce a similar interference in extinction, without contribution from NMDA receptor antagonism.

It is important to remember that with DA agonist drugs, Glu activation will induce plasticity and trigger reward learning (Mameli et al., 2011; Shiflett et al., 2011) and it is likely that DA agonist drugs alone will promote the phosphorylation of ERK. Also, because DA agonists increase motivation to obtain reward, pretreatment with DA agonists should produce an extinction deficit. This statement also applies to the extinction deficit brought about by MK-801. If MK-801 is indirectly agonizing DA, then perhaps the extinction deficit is not related to NMDA receptor antagonism per se, rather the increase in DA may be sufficiently activating the reward system.

In order to expand upon previous research findings, DA agonism effects on appetitive extinction responding was examined by: (1) comparing the MK-801-induced extinction deficit to the DA agonist cocaine along with D1/D2 antagonists pre-treatment with SCH23390 (D1), Haloperidol (D2) and Flupenthixol (nonselective), (2) examining the effect of DA agonist drugs GBR 12909 and apomorphine (APO) on extinction responding, (3) assessing the effects of drug treatment on locomotor behaviour and (4) investigating striatal pERK expression across drug conditions. If the MK-801 extinction deficit is entirely due to DA agonism, DA agonists alone should produce the same potentiated extinction response. It was hypothesized that NMDA receptor antagonism with MK-801 would elevate extinction responding through a dopamine-dependent process and that the DA agonists used in this study would produce an elevation in extinction responding similar to that observed with MK-801. It was further hypothesized

that the induction of an extinction deficit by both MK-801 and DA agonists would occur in conjunction with measurable changes in pERK in the striatum.

Cocaine, being a reuptake inhibitor via DAT blockade, was a fitting choice for comparison with MK-801 due to its potentiating effect of the DA system and its well-founded connections to the motivational system and reward-based pathologies (Ettenberg et al., 1982; Hyytia et al., 1999; Allen et al, 2005; Botreau et al., 2006; Chiodo et al., 2008; Keiflin et al., 2008; Knackstedt et al., 2010). GBR-12909 provided somewhat of a positive control, due to its characteristic high specificity to the DAT system, in comparison to cocaine, which also blocks NET and SERT transporters to a lesser degree. Also, GBR-12909, because of its prolonged half-life, is not associated with the same degree of addictive potential as cocaine (Singh, 2000; Rothman et al., 2008). Comparing the extinction behaviour elicited by both cocaine and GBR-12909 may shed some light on the importance of these factors in extinction resistance.

Apomorphine, being a direct DA agonist, should theoretically provide an estimate of the effect of direct vs. indirect DA agonism on extinction learning. Also, by modeling a dose response, the effect of APO low dose DA antagonism on extinction may be compared to higher doses that should, theoretically, agonize DA (Van Ree and Wolterink, 1981; Cary et al., 2004; Braga, Carey and Carrera, 2009) and produce an extinction deficit. If low dose DA antagonism is achieved and can be modeled alongside high dose DA agonism, the resulting effects on pERK expression will provide an interesting comparison of the role of DA transmission in pERK mediated plasticity.

It was also of interest to assess how these drugs affect locomotor behavior. It is important to consider that the striatum mediates movement-based behaviours, particularly when an animal is initiating actions that are goal directed (Thomsen and Caine, 2011; Goto and Grace, 2005). If the drugs induce hyperlocomotion, it might be unclear if operant responding after drug exposure is due to an increase in motivation to obtain reward, or just an artifact of the locomotor effects. Therefore, locomotor behaviour was assessed alongside operant extinction responding. If hyperlocomotion is induced, and this effect results in increased lever responding, assessing both correct and incorrect lever responses may provide an indication of the degree that motivation to obtain reward is coming into play. A locomotor elicited increase in operant responding should produce an indiscriminate pattern of lever pressing, rather than a reward-seeking pattern that is characterized by the majority of lever pressing being upon the active lever.

DA antagonism has been previously linked to hypolocomotion (Shen, Crabbe and Phillips, 1995; Chausmer and Katz, 2001). Because of this potential confound, the inclusion of a locomotor measure is necessary to ensure that the mediation of MK-801 extinction resistance by DA is not due to the effect of reduced DA transmission on blunting locomotion, but instead is due to the effect of blocking DA reward pathways. In order to control for this potential extraneous factor, a comparison of the locomotor effects of combined DA antagonism and MK-801 with a saline control is needed. If the combined treatment produces a significant impairment in locomotion compared to saline treated animals, then an adjustment of DA antagonist dosing will be required.

Materials and Methods

Subjects

A total of 98 male Long Evans rats (190-250 grams) from Charles River, Quebec were used in this study. Animals were housed individually in clear plastic cages (26 × 20 × 45 cm), given water ad *libitum* and subjected to a standard 12-h light/dark cycle (lights on at 8:00 a.m.). Food was restricted until animals reached 85% of their free-feeding baseline, which was maintained throughout the duration of the experiment. During the food restriction period, animals were given 5 chocolate pellets (45 mg) in their home cage. Animals were handled for 5 minutes daily and weights were recorded. On the two days prior to the start of behavioral training, animals were given 0.3 ml of saline subcutaneously (s.c.) or intraperitoneally (i.p.), depending on the treatment group. Care of rats and all procedures was conducted in accordance with the guidelines of the Canadian Council on Animal Care and protocols approved by the Carleton University Animal Care Committee as well as the Guide for the Use and Care of Laboratory Animals.

Drugs

All drugs used in this study were mixed on the day of experimentation. The noncompetitive NMDA receptor antagonist, MK-801 (Sigma-Aldrich), was stored frozen as a stock solution of 1.0 mg/ml in 0.9% sterile saline. It was thawed and diluted to 0.05 mg/kg; pH 7.4 with 0.9% sterile saline. Haloperidol (Sigma-Aldrich) was mixed into 50% ethanol at a 1-mg/ml stock solution. Hydrochloric acid (5 M) was added until the solute went into solution (3–5 µl per 50 ml of total solution). It was stored at −20°C and

was diluted with sterile 0.9% saline to 0.1 mg/kg (pH 5.5). SCH 23390 (Sigma-Aldrich) was dissolved in 0.9% sterile saline and stored frozen as a 1-mg/ml stock solution (pH 7.4). It was thawed and diluted to 0.01 mg/ml. The doses of MK-801, Haloperidol and SCH 23390 were based on previous work conducted in this laboratory (Holahan, Clarke and Hines, 2010). The DA agonist drug apomorphine (APO) (Sigma-Aldrich) was stored frozen as a stock solution of 5.0mg/ml in 0.9% sterile saline and 0.1% ascorbic acid. It was thawed and diluted to 0.05, 0.10 and 2.0mg/ml with 0.9% sterile saline. APO doses were based on previous reports (Cary et al., 2004; Braga, Carey and Carrera, 2009). The DA agonist GBR-12909 (GBR) (Sigma-Aldrich) was dissolved in 0.9% sterile saline solution and DMSO was added until the solute went into solution (100 µl per 2.5 ml of total solution) according to doses based on previous work by Rothman et al., 2008. Cocaine (Sigma-Aldrich) was dissolved in 0.9% sterile saline and diluted to the working concentration of 10mg/ml. Dosing was based on previous reports (Tilley et al., 2007). The nonselective DA antagonist Flupenthixol (Sigma-Aldrich) was dissolved in 0.9% sterile saline and diluted to the working concentration of 300µg/kg. Dosing was based on previous reports (Veeneman et al., 2010).

Operant conditioning procedure

Acquisition

Twelve operant conditioning chambers (Coulbourn Instruments; 30.5 cm W×25.5 cm D×30.5 cm H) housed in insulated casings were used. Upon pressing the left lever two times (FR2), the house light extinguishes, the panel lights above the lever change from red to green and the pellet dispenser releases one 45-mg chocolate pellet

(BioServe, New Jersey) into the hopper. Presses on the left lever were considered correct and presses on the right lever were considered incorrect. During the 5-day acquisition phase, rats were placed into the chambers for 30 min each day. Cumulative lever presses are recorded.

Extinction

The extinction session was 30 min and took place on the third day after the last reinforced acquisition day. No food reward was delivered in response to correct lever pressing but the house light extinguished and the panel lights changed from red to green. Cumulative lever presses were recorded.

Rats (n=30) were trained for 5 days then assigned to one of 5 treatment groups (n=6): saline, MK-801, SCH 23390/Haloperidol, SCH 23390/Haloperidol + MK-801 and cocaine. Both SCH 23390 and Haloperidol were administered (i.p.) 20 min prior to the extinction session. MK-801 was administered (s.c.) 15 min prior to the extinction session. MK-801, Haloperidol and SCH 23390 incubation periods were based on previous work by Holahan et al. (2010). Cocaine was administered (i.p.) 5 minutes prior to the extinction session. The incubation period for cocaine was based on previous reports that drug effect peaks within 20 minutes post i.p. injection (Tilley et al., 2007).

Rats (n=24) were trained for 5 days then assigned to one of 3 treatment groups (n=8): saline, 5mg/ml and 10mg/ml GBR. All GBR doses were administered 30 min before the extinction session (See Rothman et al., 2008).

Rats (n=24) were trained for 5 days then assigned to one of 4 treatment groups (n=6): saline, 0.05mg/ml APO, 0.5mg/ml APO and 2.0mg/ml APO. All APO doses were

administered s.c.15 min prior to the 30 min extinction session. The incubation period for APO was based on previous work by Braga et al. (2009).

Assessment of locomotor behavior

The same rats were assigned to the same drug conditions as those used for extinction. Animals were given drug treatments and placed in an elevated plus maze (100 cm L x 6cm W x 8 cm H). The floor of the maze was black and the walls were constructed of transparent Plexiglas. Motor activity was recorded using the HVS tracking system (HVS Image TM). Rearing behavior was manually recorded and operationally defined as the animal lifting both front paws off of the surface of the maze. Total test time was 30 minutes.

Tissue Preparation

The same rats were assigned to the same drug conditions as those used for extinction and locomotor testing. Animals were given drug treatment and decapitated. Left hemispheres were immersion fixed in a 4% paraformaldehyde/0.1 M phosphate buffer solution (pH 7.4) and used for imunohistochemistry. The hemisphere was sectioned on a cryostat at 60 μm and collected in 0.1% sodium azide/0.1 M phosphate buffer.

Immunohistochemistry

Incubation in the primary antibodies (1:5000 rabbit anti-pERK1/2 from Chemicon/Millipore or 1:5000 mouse anti-pERK1 from Abcam) occurred overnight at room temperature. Incubation in the secondary antibodies (1:500 goat antirabbit 594 or

1:500 goat antimouse 488; Molecular Probes) occurred for 2 hours at room temperature. Sections were mounted on glass slides and cover slipped using Fluormount (Sigma). Fluorescent images of the striatum were captured at x20 magnification. Regions of interest were traced with reference to Paxinos and Watson (1998).

Statistics

In order to assess differences in acquisition responding between groups, two-way repeated measures ANOVAs on the mean number of correct (rewarded) presses were employed. For this analysis, the group was considered the between subjects factor and day was considered the within (repeated) subject factor. One-way ANOVAs were used to assess total extinction pressing on the correct and incorrect levers, nose pokes, horizontal locomotion and rearing behaviour for all experiments, while statistical differences between groups at each level of time for extinction and locomotor tests were analyzed by employing two-way ANOVAs (group by time bin).

Total pERK cell counts and differences in core and shell labeling were compared across groups by employing one-way ANOVAs and to examine differences in core and shell labeling within groups paired samples t-tests were employed. The Fisher's least significant difference (LSD) post hoc test was employed throughout the analysis and was deemed the appropriate statistic due to the small n's and unequal sample sizes in this study (Wilson, 2005).

Results

Experiment 1: MK-801, Cocaine and D1/D2 receptor antagonists SCH23390 and Haloperidol on behavioural extinction, locomotion and pERK expression

Operant Training and Extinction Responding

Acquisition

Daily cumulative acquisition data are shown in Appendix 1A, Figure 1. There was a main effect of day ($F(4,25)=125.191$, $p<0.001$) but no main effect of group ($F(4,25)=0.235$) and no interaction ($F(4,25)=0.235$), indicating that all groups exhibited similar rates of operant responding during the acquisition phase, prior to drug treatment.

Extinction

The extinction data for Experiment 1 are shown in Appendix 1A, Figures 2-5. Results revealed a significant effect of group ($F(4,25)=9.047$, $p<0.001$). Post hoc tests indicated that the MK-801 group pressed significantly more than saline ($p<0.002$), cocaine ($p<0.019$), the D1/D2 antagonist group ($p<0.001$) and the MK-801 group pretreated with the D1/D2 antagonists ($p<0.001$). The cocaine group pressed significantly more than group D1/D2 ($p<0.033$) and group D1/D2/ MK-801 ($p<0.011$).

Analysis of the mean number of correct (rewarded) presses revealed a main effect of treatment ($F(4,25)=9.776$, $p<0.001$). MK-801 exhibited significantly higher extinction pressing compared to saline from ten minutes onward to the end of the session ($p<0.02$ at 10 min and $p < 0.002$ at 30 min). The MK-801 group pressed significantly higher than the other groups within the first five minutes of testing. For the remainder of the

extinction session (twenty, twenty five and thirty minute time bins), MK-801 continued to press at a significantly higher rate than group D1/D2 ($p<0.001$) and group D1/D2 and MK-801 ($p<0.001$).

The cocaine-treated group pressed significantly lower than saline controls during the first five minutes of the extinction session ($p<0.029$). They exhibited rates of pressing similar to that of saline controls during the ten, fifteen and twenty five minute time bins ($p>0.05$). The cocaine groups correct responding was significantly higher than saline controls during the twenty ($p<0.017$), and thirty minute time bins ($p<0.011$). During the second half of the extinction session (twenty, twenty-five and thirty minutes) both MK-801 and cocaine groups exhibited statistically similar rates of pressing ($p>0.05$). There were no significant differences in extinction pressing identified between the D1/D2 treated animals and saline controls during any of the time bins during the extinction session ($p>0.05$), but the D1/D2 group pressed slightly lower than the saline controls during the first five minutes of testing. Group D1/D2 and MK-801 pressed significantly lower than saline controls during the first five minutes of testing ($p<0.003$) but pressing for this group was comparable to saline controls for the remainder of the testing session.

There were no differences in total incorrect presses during the extinction session ($F(4,25)=1.222$, $p>0.05$), but time bin analysis (Figure 4) revealed that the MK-801 group pressed significantly higher than the D1/D2 group during the five ($p=0.017$) and fifteen minute ($p=0.019$) time bins. MK-801 also exhibited significantly higher incorrect presses during the thirty-minute time bin than saline ($p=0.043$), cocaine ($p=0.047$) and D1/D2 and MK-801 ($p=0.032$) groups. Group D1/D2 was comparable to saline during the first five minutes of testing ($p=0.124$), but pressed at a rate significantly lower than

saline during the ten minute time bin ($p<0.043$). Extinction pressing for this group was comparable to saline for remainder of testing (fifteen, twenty, twenty-five and thirty minute time bins) ($p>0.05$). There were no other differences in incorrect pressing identified between any other treatment groups.

There were no differences in total nose poke data indentified during extinction ($F(2,25)=2.229$, $p>0.05$) but time bin by treatment analysis (Figure 5) revealed that at various time bins, MK-801 and cocaine exhibited significantly higher rates of nose poke behaviour than other groups. The MK-801 group exhibited significantly higher nose poke activity than saline, D1/D2 and D1/D2 and MK-801 from fifteen minutes onward. Cocaine exhibited significantly higher nose poke activity than saline, D1/D2 and D1/D2 and MK-801 at the end of the session ($p<0.006$; $p<0.003$; $p<0.001$). There were no other group differences detected.

Locomotion

The locomotor data for Experiment 1 are shown in Appendix 1B, Figures 1-4. Group differences in total locomotion and rearing during a thirty minute testing session were assessed (Figure 1). The results indicated that there was a significant main effect of treatment ($F(4,25)=15.445$, $p<0.001$). Post hoc analysis indicated that the D1/D2 group moved significantly fewer meters than saline controls ($p<0.041$), while MK-801-treated group moved significantly more than saline ($p<0.001$). There were no other differences between any of the other treatment groups and saline controls. Cocaine treated animals moved significantly more than group D1/D2 ($p<0.009$) but moved significantly less than group MK-801 ($p<0.001$).

In order to tease apart the profile of locomotor activation, locomotor data were broken down into horizontal and vertical (rearing) components and subsequently analyzed in five minute time bins. For group differences in horizontal locomotion across the testing period (Figure 2) the results indicated that MK-801 moved significantly more than saline controls across all six time bins of testing. The D1/D2 group moved significantly less than saline controls during the five and ten minute time bins ($p<0.019$; $p<0.024$). There were no other significant differences from saline detected in any of the other groups. MK-801 moved significantly more than group D1/D2 and MK-801 group. There were no differences in movement detected between MK-801 and cocaine treated animals by the end of the first five minutes of testing ($p>0.05$), but MK-801 moved significantly more for all other time bins ($p<0.001$). There were no other groups differences detected.

With regard to total rearing (Figure 3), there was a significant main effect of treatment ($F(4,25)=19.536$, $p<0.001$). Both Cocaine and MK-801 groups reared significantly more than saline ($p<0.026$; $p<0.001$). MK-801 also reared significantly more than cocaine ($p<0.001$), whereas the D1/D2 group reared significantly less than saline ($p<0.001$). There were no other group differences detected.

For group differences in vertical (rearing) locomotion across the testing period (Figure 4) the results indicated that group D1/D2 reared significantly less than saline across all time bins and similar findings were observed with group D1/D2 and MK-801. MK-801 reared significantly more than saline during the ten ($p<0.043$), twenty ($p<0.033$), twenty-five ($p<0.013$) and thirty ($p<0.009$) time bins. MK-801 also reared significantly more than groups D1/D2 and D1/D2 and MK-801 ($p<0.001$). Similarly, the

cocaine group reared significantly more than saline starting at the fifteen-minute time bin ($p<0.001$). Cocaine treated animals reared significantly less than MK-801 treated animals by then end of the ten minute time bin and this effect persisted throughout the duration of testing ($p<0.001$).

pERK expression

The pERK data for Experiment 1 are shown in Appendix 1C, Figures 1-2. For group differences in the total number of pERK labeled cells in the Nac (Figure 1) the results indicated that there was a significant effect of treatment ($F(4,25)=7.364$, $p<0.002$). The LSD indicated that the cocaine treated animals exhibited significantly more pERK labeling than saline ($p<0.025$), D1/D2 ($p<0.001$) and D1/D2 and MK-801 treated animals ($p<0.001$). MK-801 had more labeling than D1/D2 ($p<0.002$) and D1/D2 and MK-801 treated animals ($p<0.003$). There were no other significant differences detected between any other groups. The results of the ANOVA indicated that MK-801 exhibited significantly more pERK labeling than D1/D2 ($p<0.019$) and D1/D2 and MK-801 ($p<0.015$). There were no other group differences detected.

With regard to differences between pERK labeling in the core and shell of the nucleus accumbens (Figure 2) there was a significant effect of treatments ($F(4,25)=8.601$, $p<0.001$). The results of the LSD post hoc indicated that there was significantly more pERK labeling in the core of the Nac of the cocaine treated animals compared to saline ($p<0.003$), D1/D2 ($p<0.001$), and D1/D2 and MK-801 ($p<0.001$). There was significantly more pERK labeling in the core of the MK-801 group, compared to D1/D2 ($p<0.004$) and D1/D2 and MK-801 ($p<0.001$). There were no other group differences detected. For

differences in core and shell labeling within groups (Figure 2) the results indicated that there was significantly more pERK labeling in the core of the Nac, compared to the shell in the cocaine treated animals ($t(5)=5.604$, $p<0.004$ (two-tailed)). There were no other differences detected in any of the other groups.

Experiment 2: Cocaine and Flupenthixol on behavioural extinction, locomotion and pERK expression

Operant Training and Extinction Responding

Acquisition

Daily cumulative acquisition data are shown in Appendix 2A, Figure 1 and analysis revealed main effect of day ($F(1,16)=119.783$, $p<0.001$) but no main effect of groups ($F(3,16)=0.370$, $p>0.05$) and no interaction ($F(3,16)=0.228$, $p>0.05$).

Extinction

The extinction data for Experiment 2 are shown in Appendix 2A, Figures 2-5. The correct lever extinction data are shown in Figure 2. For differences in total extinction pressing the results did not identify an effect of treatment ($F(3,15)=2.307$, $p>0.05$). The post hoc analysis identified a significant difference between the cocaine group and the flupenthixol and cocaine group ($p<0.031$). For the mean number of correct (rewarded) presses (Figure 3) analysis did not identify significant main effect of treatment for correct lever presses during extinction ($F(3,15)=2.307$, $p>0.05$) but time bin post hoc analysis identified significant differences between treatment groups at various time bins. By the end of the first five minutes of testing, the flupenthixol and cocaine treated animals were pressing significantly lower than saline animals ($p<0.046$), with pressing in these groups

subsequently increasing to a comparable level to saline for the remainder of testing ($p>0.05$). There were no other significant differences identified between any of the treatment groups within the ten, fifteen and twenty minute time bins ($p>0.05$), however, during the last ten minutes of testing (time bins twenty-five and thirty) the cocaine treated animals pressed significantly more than saline ($p<0.009$; $p<0.017$). Additionally, the flupenthixol treated animals pressed significantly lower than cocaine animals during the twenty-five and thirty minute time bins ($p<0.007$; $p<0.002$).

The incorrect lever extinction data are shown in Figure 4. Analysis did not reveal a significant main effect of treatment ($F(3,15)=1.181$, $p>0.05$). Time bin post hoc analysis indicated that the flupenthixol treated animals pressed the incorrect lever significantly less than saline controls during the twenty- five minute time bin ($p<0.044$) but no other significant differences were identified.

The nose poke extinction data are shown in Figure 5. Analysis identified a significant main effect of treatment ($F(3,15)=4.155$, $p<0.026$. Time bin post hoc analysis indicated that all of the treatment groups (cocaine, flupenthixol, flupenthixol and cocaine) exhibited significantly lower nose poke activity than saline controls during the first five minute time bin ($p<0.001$; $p<0.024$; $p<0.001$). There were no significant differences in nose poke activity during the ten, fifteen or twenty minute time bins between any of the treatment groups ($p>0.05$) but differences reemerged toward the end of testing with the flupenthixol and flupenthixol and cocaine groups displaying lower nose poke activity than the cocaine group ($p<0.0191$; $p<0.038$). For the flupenthixol and cocaine group this difference was also present during the thirty-minute time bin ($p<0.013$). There were no other group differences detected in this analysis.

Locomotion

The locomotor data for Experiment 2 are shown in Appendix 2B, Figures 1-4. For group differences in total horizontal locomotion during a thirty minute testing session (Figure 1) the results indicated that there was a significant main effect of treatment ($F(3,15)=4.202$, $p<0.025$). Post hoc analysis indicated that the flupenthixol and flupenthixol and cocaine groups moved significantly less than cocaine ($p<0.007$; $p<0.012$). The results indicated that the flupenthixol group moved significantly less than the saline group ($p<0.046$). Analysis for group differences in horizontal locomotion across the testing period (Figure 2) reveled that, after the first five minutes of testing, the saline group moved less than all other groups ($p < 0.05$). With exception of the first time bin, cocaine moved significantly more than the flupenthixol group for the duration of testing. The cocaine group continued to move significantly more than saline for the remainder of testing. There were no other significant differences detected.

Assessment of group differences in total vertical (rearing) locomotion during a thirty minute testing session (Figure 3) indicated that there was no significant main effect of treatment ($F(3,15)=1.525$). For group differences in vertical (rearing) locomotion across the testing period (Figure 4) the results indicated that the flupenthixol group reared significantly less than the cocaine group by the end of the ten and thirty minute time bins ($p<0.033$; $p<0.0291$). There were no other group differences detected.

pERK expression

The pERK data for Experiment 2 are shown in Appendix 2C, Figures 1-2. Assessment of group differences total number of pERK labeled cells in the Nac (Figure

1) indicated that there was no significant effect of treatment ($F(3,15)=3.180$, $p<0.0551$). The post hoc analysis revealed that there was significantly more pERK labeling in the cocaine group, compared to flupenthixol and flupenthixol and cocaine groups ($p<0.016$; $p<0.024$). The cocaine group did not exhibit more pERK labeling than the saline group ($p<0.059$). There were no other group differences detected.

Analysis of differences between pERK labeling in the core and shell of the Nac (Figure 2) indicated that there was no significant difference in core ($F(3,15)=2.244$, $p>0.05$) or shell ($F(3,15)=2.689$, $p>0.05$) pERK labeling across treatments. Post hoc analysis revealed that there was significantly more pERK labeling in the core of Nac following cocaine treatment compared to flupenthixol ($p<0.024$). There was also significantly more pERK labeling in the cocaine shell compared to saline ($p<0.038$) and flupenthixol and cocaine animals ($p<0.025$). There were no other group differences detected. Assessment of differences in core and shell labeling within groups (Figure 2) indicated that there was significantly more pERK labeling in the core of the Nac, compared to the shell in the saline treated animals ($t(3)=-10.011$, $p<0.003$ (two-tailed)). There were no other differences detected in any of the other groups ($p>0.05$).

Experiment 3: GBR-12909 dose-response curve on behavioural extinction, locomotion and pERK expression

Operant Training and Extinction Responding

Acquisition: Daily cumulative acquisition data are shown in Appendix 3A, Figure 1. Analysis revealed a main effect of day ($F(4,104)=79.119$, $p<0.001$) but no interaction ($F(12,104)=0.753$) and no effect of treatment ($F(3,26)=0.953$).

Extinction

The extinction data for Experiment 3 are shown in Figures 2-5. Assessment of differences in total extinction pressing (Figure 2) did not identify an effect of treatment ($F(3,26)=1.475$, $p>0.05$). Post hoc analysis confirmed that there were no significant differences in total presses across treatments. Analysis of the mean number of correct (rewarded) presses and did not find a significant main effect of group ($F(3,26)=1.475$, $p>0.05$) (Figure 3). Post hoc analysis was employed to assess groups differences across time and revealed that the 20mg/kg GBR-12909 group pressed significantly more than saline by the end of the twenty minute time bin ($p<0.032$). There were also significant differences identified by the end of the thirty minute time bin where all GBR-12909 doses (5, 10, 20mg/kg) pressed the correct lever significantly more than saline controls ($p<0.027$; $p<0.035$; $p<0.021$).

Analysis of incorrect (unrewarded) presses revealed a significant main effect of group ($F(3,26)=6.207$, $p<0.004$) (Figure 4). Post hoc analysis indicated that the 10mg/kg GBR-12909 group pressed significantly more than the 5mg/kg group ($p<0.043$) during the twenty minute time bin of testing and the 20mg/kg group pressed significantly less than saline during the twenty minute time bin.

Analysis of nose poke activity (Figure 5) revealed a significant main effect of treatment ($F(3,26)=3.341$, $p<0.036$). Post hoc analysis indicated that the 20mg/kg dosed animals exhibited a significant increase in nose poke activity compared to saline, 5mg/kg and 10mg/kg treated animals ($p<0.005$; $p<0.014$; $p<0.012$) by the end of the first five minutes of testing. These differences did not persist throughout testing and all groups

nose poke activity was statistically similar by the end of the ten minute time bin ($p>0.05$). By the end of the fifteen minute time bin all doses of GBR12909 exhibited similar nose poke activity, however, the 20mg/kg group was significantly increased compared to saline ($p<0.015$). There were also differences present at the end of testing, with the 10 and 20mg/kg groups exhibiting higher nose poke activity than saline ($p<0.044$; $p<0.023$). There were no other differences detected.

Locomotion

The locomotor data for Experiment 3 are shown in Appendix 3B, Figures 1-4. Assessment of group differences in total horizontal locomotion (Figure 1) indicated that there was no main effect of treatment ($F(3,26)=0.617$). Assessment of group differences in horizontal locomotion across the testing period (Figure 2) indicated that the 20mg/kg group moved significantly more than saline during the first five minutes of testing ($p<0.019$). There were no other group differences detected.

For group differences in total vertical (rearing) locomotion during a thirty minute testing session (Figure 3) the results indicated that there was no significant main effect of treatment ($F(3,26)=2.028$, $p>0.05$). Assessment of group differences in vertical (rearing) locomotion across the testing period (Figure 4) indicated that the 5mg/kg group reared significantly more than saline during the five ($p<0.048$), fifteen ($p<0.010$) and twenty-five ($p<0.038$) minute time bins. There were no other group differences detected.

pERK expression

The pERK data for Experiment 3 are shown in Appendix 3C, Figures 1-2. For group differences total number of pERK labeled cells in the Nac (Figure 1) the results indicated

that there was a significant effect of treatment ($F(3,26)=7.191$, $p<0.002$). Post hoc tests confirmed that the 20mg/kg treated animals had significantly more pERK labeled cells than all other groups (saline ($p<0.010$), 5mg/kg ($p<0.01$), 10mg/kg ($p<0.001$)). There were no other group differences detected.

Assessment of differences between pERK labeling in the core and shell of the nucleus accumbens (Figure 2) indicated that the increase in pERK labeled cells in the 20mg/kg was confined to the core of the Nac, where this group had significantly more labeled cells than saline ($p<0.008$), 5mg/kg ($p<0.018$) and 10mg/kg ($p<0.001$). There were no significant differences in the shell between any of the groups ($p>0.05$). For differences in core and shell labeling within groups (Figure 2) the results indicated that the 5mg/kg group exhibited significantly more pERK labeled cells in the core of the Nac ($t(7)=2.954$, $p<0.022$). Similarly, the 20mg/kg group exhibited significantly more pERK staining in the core of the Nac ($t(5)=11.983$, $p<0.001$).

Experiment 4: Apomorphine dose-response curve on behavioural extinction, locomotion and pERK expression

Operant Training and Extinction Responding

Acquisition

Daily cumulative acquisition data are shown in Appendix 4A, Figure 1. Analysis of the mean number of correct (rewarded) presses revealed a main effect of day ($F(4,100)=88.382$, $p<0.001$) but no main effect of groups ($F(4,25)=0.042$, $p>0.05$) and no interaction ($F(16,100)=0.159$, $p>0.05$).

Extinction

The extinction data for Experiment 4 are shown in Appendix 4A, Figures 2-5 (Figure 2). Assessment of the mean number of correct (rewarded) presses for each group (Figure 3) revealed a significant main effect of treatment ($F(4,25)=10.335$, $p<0.001$). Post hoc analysis determined that the 0.05mg/kg APO, 2.0APO and 5.0 APO groups pressed significantly lower than saline controls during the first five ($p<0.001$), ten ($p<0.002$), and fifteen ($p<0.003$; $p<0.002$; $p<0.001$) and twenty minute time bins ($p<0.006$; $p<0.016$; $p<0.001$; $p<0.001$). While during the twenty-five minute time bin both 0.05 and 0.5mg/kg doses increased correct pressing to a comparable level to saline ($p>0.05$), both the 2.0 and 5.0mg/kg groups continued to press significantly lower than controls ($p<0.016$; $p<0.014$), but both groups were comparable to saline at the end of the testing period ($p>0.05$). Additionally, during the last five minutes of testing (30 minute time bin) the 0.5 mg/kg group pressed significantly higher than saline ($p<0.002$).

For the majority of testing, the 0.05mg/kg APO group pressed consistently higher than all other doses (0.5, 2.0, 5.0mg/kg) with significant differences occurring during the five ($p<0.001$) and ten ($p<0.027$; $p<0.025$; $p<0.024$) minute time bins. While the 0.5mg/kg APO group increased pressing and was comparable to the 0.05mg/kg group for the remainder of the session ($p>0.05$), and surpassed the pressing of the 0.05mg/kg group during the last five minutes ($p<0.006$). The 2.0 and 5.0mg/kg treated animals continued to press at a significantly lower rate than the 0.05mg/kg group during the fifteen ($p<0.017$; $p<0.01$), twenty-five ($p<0.016$; $p<0.014$) and thirty ($p<0.001$) minute time bins. This group also pressed significantly lower than the 0.5mg/kg group during the twenty-five ($p<0.004$) and thirty ($p<0.001$) minute time bins.

The incorrect lever extinction data for Experiment 4 are shown in Appendix 4A, Figure 4. Analysis revealed a significant effect of treatment ($F(4,25)=3.308$, $p<0.027$). Post hoc analysis indicated that the 0.5mg/kg group pressed significantly lower than saline during the five minute time bin ($p<0.034$) and, by the end of the ten minute time bin, the 0.5, 2.0 and 5.0mg/kg APO groups were all pressing significantly lower than saline ($p<0.007$; $p<0.030$; $p<0.018$). These group differences reemerged by the end of the twenty minute time bin ($p<0.019$; $p<0.011$; $p<0.015$) but during the last ten minutes of testing (twenty-five and thirty minute bins), only the 2.0 and 5.0mg.kg dosed animals were pressing significantly lower than saline ($p<0.016$; $p<0.021$). There were no other group differences in incorrect lever pressing identified.

The nose poke extinction data are shown in Appendix 4, Figure 5. Analysis revealed a significant effect of treatment ($F(4,25)=15.544$, $p<0.001$). Post hoc analysis revealed that the 0.5, 2.0 and 5.0mg/kg APO groups exhibited significantly less nose poke activity than saline after the first five minutes of testing ($p<0.001$). The 0.05APO group also exhibited higher nose poke activity then the 0.5, 2.0 and 5.0 APO dosed animals during this time bin ($p<0.001$), but by the end of the ten minute time bin all groups (0.05, 0.5, 2.0 and 5.0mg/kg) were lower than saline ($p<0.002$; $p<0.018$; $p<0.018$; $p<0.010$). For the 2.0 and 5.0 APO groups, nose poke activity remained lower than saline during the twenty minute time bin ($p<0.003$; $p<0.002$). While the 2.0 mg/kg group increased nose pokes after this point and was comparable to saline for the remainder of testing ($p>0.05$), the 5.0mg/kg group exhibited blunted nose poke activity compared to saline during the twenty-five ($p<0.024$) minute time bin.

Locomotion

The locomotor data for Experiment 4 are shown in Appendix 4B, Figures 1-4. Assessment of group differences in total horizontal locomotion during a thirty minute testing session (Figure 1) indicated that there was a significant main effect of group ($F(2,25)=5.339$, $p<0.004$). Post hoc test confirmed that 2.0 and 5.0mg/kg APO groups moved significantly less than saline ($p<0.003$; $p<0.004$) and 0.05 APO ($p<0.045$; $p<0.007$). The 5.0 APO group also moved significantly less overall than the 0.5mg/kg APO group

For group differences in horizontal locomotion across the testing period (Figure 2) the results indicated 0.05 APO group was statistically similar to saline throughout the testing session ($p>0.05$). The 0.5 APO group moved significantly less than saline during the five ($p<0.049$) and ten ($p<0.026$) minute bins of testing but with movement then increasing to a rate statistically similar to saline for the remainder of the session. The 2.0 and 5.0 APO groups moved significantly less than saline during the ten ($p<0.006$; $p<0.008$), fifteen ($p<0.011$; $p<0.006$), twenty ($p<0.01$; $p<0.006$), twenty-five ($p<0.018$; $p<0.02$) and thirty ($p<0.007$; $p<0.004$) minute time bins. They also moved significantly less than the 0,05 APO group after the ten ($p<0.007$; $p<0.0091$), fifteen ($p<0.011$; $p<0.006$), twenty ($p<0.002$), twenty-five ($p<0.044$; $p<0.0491$) and thirty ($p<0.007$; $p<0.0091$) minute time bins.

Assessment of group differences in total vertical (rearing) locomotion (Figure 3) indicated that there was a significant effect of treatment ($F(4,25)=15.280$, $p<0.001$). Post hoc analysis indicated that the 0.5, 2.0 and 5.0 APO groups reared significantly less than

saline overall ($p<0.002$; $p<0.001$; $p<0.001$). They were also lower than the 0.05APO group ($p<0.004$; $p<0.001$; $p<0.001$).

Assessment of group differences in vertical (rearing) locomotion across the testing period (Figure 4) indicated that the 0.5 APO group began by rearing significantly less than saline during the five ($p<0.001$), ten ($p<,0.001$), fifteen ($p<,0.001$) and twenty ($p< 0.036$) minute time bins. This groups rearing increased toward the end of the testing period with 0.5 APO rearing statistically similar to saline and 0.05APO ($p>0.05$). 0.5 APO also reared less than 0.05 APO during the five ($p<0.001$), ten ($p<0.001$), fifteen ($p<0.003$), and twenty ($p<0.018$) minutes time bins. Similar to observations of horizontal locomotion, the 2.0 and 5.0 APO groups pressed significantly lower than saline for the five ($p<0.001$), ten ($p<0.001$), fifteen ($p<0.001$), twenty ($p<0.003$; $p<0.002$), twenty-five ($p< 0.001$) and thirty ($p<0.001$) minute time bins. These groups were also significantly lower than the 0.05 APO groups across all time bins.

pERK expression

The pERK data for Experiment 4 are shown in Appendix 4C, Figures 1-2. Analysis of group differences in total number of pERK labeled cells in the Nac (Figure 1) indicated no significant effect of group ($F(4,25)=0.329$, $p>0.05$). Assessment of differences between pERK labeling in the core and shell of the nucleus accumbens (Figure 2) indicated that there were no significant differences between core and shell staining between any of the treatment groups ($p>0.05$).

For differences in core and shell labeling within groups (Figure 2) the results indicated that the saline group exhibited significantly more pERK staining in the core

compared to the shell ($t(5)=3.088$, $p<0.028$). Higher core than shell pERK labeling was identified with the 0.5 APO ($t(5)=4.292$, $p<0.009$), 2.0 APO ($t(5)=3.905$, $p<0.012$) and 5.0 APO ($t(5)=2.862$, $p<0.036$).

Discussion

Experiment 1: MK-801, Cocaine and D1/D2 receptor antagonists SCH23390 and Haloperidol on behavioural extinction, locomotion and pERK expression

Consistent with extinction, the saline control groups in all experiments exhibited high rates of bar pressing at the beginning of testing but without reinforcement, responding promptly diminished and remained low for the rest of the session. This observation across 4 separate experiments substantiates the experimental design and demonstrates the robust reliability of this behaviour. Conversely, the 0.05mg/kg dose of MK-801 induced an extinction deficit that persisted for the majority of the extinction session; a result consistent with several previous findings (Holahan et al., 2010, 2011).

It is important to note that during the extinction test, the MK-801 group exhibited rates of correct pressing similar to controls during the first 5 minutes of testing but, unlike controls, pressing remained consistently elevated for the duration of the test. Characteristic of an operant extinction deficit, MK-801 treatment did not increase operant responding *per se*, rather the MK-801-treated group maintained steady state of pressing. This behaviour could result from impairment in extinction learning mechanisms, activation of reward pathways, hyperlocomotion, or a combination of these factors.

Why do rats continue bar pressing after systemic MK-801 treatment in the absence of food reinforcement?

Previous studies have reliably demonstrated that MK-801 increases food intake and food seeking behaviour (Burns and Ritter, 1997; Covasa et al., 2004), and this effect was found to be particularly salient during food deprivation (Burns et al., 1997). These

observations are indicative of the notion that MK-801 treatment increases food craving and seeking, possibly by interfering with satiety signals or by increasing the rewarding properties of food. Regardless of the effect that MK-801 has on craving and satiety, it is unlikely that a difference in hunger states between MK-801 and saline groups is the underlying cause of the extinction deficit observed. Previous studies by Balleine and Dickinson (1995) did not observe differences in appetitive extinction responding between food-deprived and satiated rats, suggesting that food deprivation and hunger states has little effect on instrumental responding during extinction. Rather, previous studies have demonstrated that MK-801 increases the rewarding effects of food and triggers reward seeking behaviour. For example, in a study conducted by Yonghui and colleagues (2006) the influence of systemic MK-801 treatment on conditioned place preference (CPP) was assessed. It was observed that MK-801 treated animals display an enhanced CPP for food that occurs independently of hyperlocomotion and memory impairments. It was concluded that MK-801 treatment elicited activation within reward circuits that lead to the potentiation of food seeking. However, research has reliably demonstrated that MK-801 impairs learning (Butelman et al., 1989; Kant et al., 1991; Brown et al. 2008) such as that which would underlie extinction (Cammarota et al., 2004; for review see Quirk and Devin Mueller, 2008). Thus, it is likely that the deficit in extinction responding observed in this experiment was the result of a combination of food reward potentiation and/or a learning deficit, rather than gastric influences (Covasa, Hung, Ritter and Burns, 2004).

Could hyperlocomotion be the underlying cause of the increase in extinction pressing associated with MK-801 treatment?

When interpreting the effect of MK-801 on appetitive extinction, it is important to consider locomotor effects. Consistent with the literature, it was observed that MK-801 treatment significantly increased horizontal activity (Irifune, Shimizu, Nomoto and Fukuda, 1995; Yarkov, Der and Joyce, 2010) and rearing (Asadpour and Sadeghnia, 2011) compared to all other treatments (saline, cocaine, D1/D2, D1/D2+MK-801). Excessive rearing behaviour is viewed as a form of locomotor stereotypy (Creese and Iversen, 1974) that normally accompanies treatment with psychostimulant drugs like cocaine or amphetamine (Creese et al., 1974; Blanchard et al., 2000; Canales and Graybiel, 2000). Furthermore, the potentiated rearing behaviour that is characteristic of psychostimulant drug exposure is contingent upon an increase in extracellular DA (Tilley and Gu, 2008) and has been found to be striatal dependent (Canales et al., 2000), suggesting that this behaviour is a product of reward system activation.

Although the MK-801 group did press the incorrect lever slightly more than controls during one of the 5 minute time bins of testing, incorrect presses occurred drastically less than correct presses in all cases, regardless of treatment. Overall, extinction pressing was largely confined to the correct lever, rather than the indiscriminate pressing on both levers that would result from an increase in activity. In a study by Holahan et al. (2010) MK-801 treatment was characterized by increased lever pressing during extinction and hyperlocomotion but employing a second extinction test differentiated these behaviours. The MK-801 group pressed the correct lever at a lower rate than observed in the first session, suggesting that increased locomotion was not

solely responsible for elevated pressing behaviour. Taken together, despite the induction of hyperlocomotion, the profile of extinction responding does not support the notion that increased activity is the underlying cause of the deficit in extinction, but still cannot be discounted as a contributing factor.

Could MK-801 extinction pressing result from activation of the reward system?

Further substantiating the notion that MK-801 is acting upon reward mechanisms, combined treatment of DA antagonist drugs with MK-801 successfully reversed the extinction deficit. This group (D1/D2+ MK-801) exhibited lower rates of pressing compared to saline controls during the first 5 minutes of testing, but responded at a similar rate to controls for the remainder of the session. This observation could indicate that the combination of DA antagonism with MK-801 treatment induces a minor cognitive or locomotor impairment, or possibly a combination of both that induces a slight interference in correct lever responding early in the extinction session. DA antagonism has been previously linked to hypolocomotion (Shen, Crabbe and Phillips, 1995; Chausmer and Katz, 2001) but observations during locomotor testing did not support the notion that locomotion was impaired during the extinction session.

While the MK-801 group exhibited consistently elevated locomotor and rearing behaviour, the D1/D2+ MK-801 group exhibited a locomotor profile similar to saline controls throughout the entire activity session. Additionally, D1/D2+ MK-801 animals exhibited statistically similar incorrect lever responding and nose poke activity as saline controls. Considering that MK-801 exhibited generally higher rates of both correct

presses and nose poke behaviour, antagonizing the DA system appeared to normalize these behaviours, rather than impair them.

Although it was not as pronounced, the 10mg/kg dose of cocaine produced a potentiation in correct lever responding similar to the observations made with MK-801 treatment. Unlike MK-801, cocaine treated animals began the extinction session pressing significantly less than saline. This could be suggestive of decreased reward seeking during this time, or could also be the result of increased exploratory and/or locomotor behaviour upon entry into the operant chambers thereby "distracting" the rats from the levers. Interestingly, during locomotor assessment, the cocaine group did not exhibit hyperactivity compared to saline, despite consistent reports in the literature that acutely this dose of cocaine triggers hyperlocomotion (Tilley, et al., 2007; Tirotta et al., 2010). It is possible that other factors, such as the anxiogenic effects of cocaine in response to the novel testing environment, disrupted locomotor activity. If this was the case, because the animals were exposed to the operant boxes multiple times prior to extinction testing, habituation likely took place and the anxiogenic effects of the drug would not have been as pronounced in this familiar environment (Carey, DePalma and Damianopoulos, 2005).

Regardless of the differences in correct pressing during the first time bin of extinction, the cocaine group also exhibited incorrect lever responding rates similar to those of saline controls, and incorrect lever responding was also significantly lower than correct lever responding across all time bins of testing. Possibly indicative of increased reward seeking, nose poke activity was also increased in this group compared to saline. When considering the profile of behaviours in the cocaine group during extinction and the similarities to MK-801, the results of this study suggest that correct lever responding

was goal directed and resulted from activation of DA and reward pathways. Indicative of an appetitive potentiation, the cocaine group exhibited pressing that was similar to the operant responding profile of the MK-801 group, responding was fairly consistent across time, and was significantly higher than rates of pressing by saline controls at the end of testing.

The potentiation of reward seeking behaviour with cocaine treatment suggests that nonselective activation of the DA system may be capable of over-riding extinction learning mechanisms. Previous research suggests that acute cocaine treatment dose not impair learning mechanisms (Cestari and Castellano, 1996), so it is unlikely that a learning impairment underlies the cocaine-induced extinction profile deficit. Additionally, it has been reported that feeding behaviour is reduced following acute cocaine treatment in the non food deprived rat (Cooper and van der Hoek, 1993) suggesting that the 10mg/kg dose combined with food deprivation was sufficient to overcome this effect. The findings of this experiment suggest that by potentiating reward-seeking behaviour the 10mg/kg cocaine increases correct lever responding during extinction similar to that observed following MK-801. Since there is no basis to suggest that this behaviour has cognitive or locomotor underpinnings, it is logical to attribute the change in behaviour to reward system activation that triggers an increase in food seeking under these conditions.

How do the various drug treatments differentially alter the pattern of ERK activation in the nucleus accumbens?

In order to assess activation of the reward system by the different drugs, immunohistochemisty for ERK phosphorylation in the nucleus accumbens was employed. The phosphorylation of ERK results from an interplay between DA D1 receptors and glutamatergic input (for review see Shiflett and Balleine, 2011). To assess how the various drug treatments differentially altered the ERK cascade in the nucleus accumbens (Nac), tissue from each drug treatment was collected using the same time scale as was used to mark the onset of extinction and locomotor testing. Consistent with the notion that pERK can be induced by activation of D1 receptors, this study identified significantly more pERK labeling in the Nac of cocaine treated animals compared to controls overall. Differentiating labeled cells within the core and the shell of the Nac revealed that the majority of pERK labeled cells were located in the core, while there were no differences in shell labeling. Contrary to expectations, there were no significant differences in pERK labeling in the MK-801 group compared to controls. Pretreatment with D1/D2 receptor antagonists did significantly reduce pERK labeling with MK-801, confirming previous observations that pERK is contingent upon DA activation.

Previous studies in this laboratory have shown that MK-801 treatment is associated with differential pERK labeling following an operant extinction session (Holahan, Clarke and Hines, 2010; Holahan, Westby and Albert, 2012). Specifically, it was observed that MK-801 treatment (0.05 mg/ kg) triggered a reduction in infralimbic pERK staining and elevated Nac pERK staining compared to controls. These findings were observed in rats that were tested on appetitive extinction immediately prior to

euthanasia. It was concluded that a reduction in infralimbic cortex pERK and increase in Nac pERK lead to a dysfunction of mesolimbic reward systems that are normally regulated by prefrontal circuits, resulting in a potentiation of non-reinforced pressing. These findings were substantiated by observations that pERK labeling in the shell of the Nac, a region identified as a regulator of motivation and reward, was significantly higher in the MK-801 group than saline. The results of the present thesis are not consistent with these published findings. MK-801 treatment did not elicit an increase in pERK in the shell of the Nac, despite having previously modeled the MK-801 extinction deficit.

One possible explanation for this discrepancy is the absence of an extinction session triggering learning mechanisms immediately prior to tissue collection. Experiences that trigger learning, particularly reward based striatal learning, have been previously associated with the induction of pERK and it is possible that initiation of the profile of pERK activation observed by Holahan et al., (2010; 2012) is contingent upon extinction learning immediately prior to tissue collection. The pERK expression that was observed in the present study also may reflect differences in the time course of drug administration. It is possible that the 15-minute incubation period that followed MK-801 treatment was not sufficient to trigger a robust pERK activation at this dose. In a study by Ahn et al. (2006) a 1.0mg/kg dose of MK-801 was sufficient to produce a persistent increase in pERK compared to saline controls that could be measured from 15-90 minutes post injection, but the dose used in this study (0.05mg/kg) was much lower and was injected subcutaneously, rather than intraperitoneally.

Following cocaine treatment, an increase in pERK labeling was observed in the core of the Nac compared to controls. Both the core and the shell of the Nac are heavily

implicated in reward seeking, but activation in the core has also been linked to the presentation of contextual cues previously paired with reward (Fricks-Gleason and Marshall, 2011; Miller and Marshall, 2005). For example, increased pERK in the core region of the Nac is characteristic of the formation of conditioned place preference for cocaine (Miller and Marshall, 2005). Considering that psychostimulant administration is normally associated with an increase in pERK in both the core and shell of the Nac (Valjent et al., 2000), the observation that pERK labeling was confined to the core could be indicative of DA activation in response to the presentation of contextual cues associated with the injection procedure (ex. the restraint towel, transportation on the cart etc).

Experiment 2: Cocaine and Flupenthixol on behavioural extinction, locomotion and pERK expression

The purpose of experiment 2 was to replicate the cocaine-induced non-reinforced potentiation in extinction pressing observed in experiment 1 and to assess the influence of DA antagonist pretreatment on the elicitation of these behaviours. The results of experiment 2 were consistent with the findings of experiment 1 in that the saline groups' pressing was consistent with operant extinction and cocaine treatment interfered with normal extinction behaviour. Similar to behaviours observed with either an extinction deficit or a potentiation of the reward system, the cocaine group pressed significantly more than saline controls at the end of the session.

Does the increase in cocaine extinction pressing result from activation of the reward system?

Pretreatment with the non-selective DA antagonist flupenthixol (Flu) blocked the cocaine-induced behavioural profile during the extinction session. Successfully reversing the potentiation of non-reinforced pressing with flupenthixol reaffirmed the previous findings in experiment 1, and demonstrated that other DA antagonist drugs other than Haloperidol and SCH23390 are capable of producing similar behavioural effects. These findings further confirm the hypothesis that an appetitive extinction deficit will result from activation of the DA reward system in the absence of glutamate antagonism.

Similar to the findings in experiment 1, the cocaine-treated animals exhibited significantly higher nose poke activity than controls at the end of the testing session. This observation is indicative of increased reward-seeking behaviour and further confirms the notion that acute cocaine treatment increased correct lever responding through activation of the reward system. Although the behaviour of the cocaine group suggests that the maintenance of correct lever pressing is due to activation of the DA reward pathways, the locomotor effects of cocaine may also play a role.

Consistent with the literature but inconsistent with the findings of experiment 1, the cocaine group moved significantly more than controls for the majority of the locomotor testing session (Tilley, et al., 2007; Tirotta et al., 2010). This suggests that hyperlocomotion may have played a role in the groups behaviour during the previous extinction test. It is important to consider that, consistent with findings in experiment 1, there were no differences in incorrect lever pressing and therefore no indication that the

correct pressing was not goal directed and was the result of the stimulating properties of cocaine, rather than increased reward seeking. Regardless, hyperlocomotion cannot be ruled out as an influence on bar pressing and must be considered when interpreting the results of the current study.

How do cocaine and flupenthixol differentially alter the pattern of ERK activation in the nucleus accumbens?

Similar to the findings in experiment 1, the cocaine treated animals exhibited more pERK labeling overall than the Flu and Flu + cocaine groups. This finding supports the notion that pERK is mediated by D1 receptor activation and that the dose of Flu used in this experiment was sufficient to block the increase in pERK normally characteristic of cocaine exposure. Unlike experiment 1, pERK labeling was not significantly higher in the cocaine group than saline controls. Additionally, assessment of core and shell labeling did not uncover the same degree of core labeling in the cocaine group that was observed in experiment 1, although core labeling for the cocaine group was still significantly higher than core labeling in the Flu group.

There was also significantly more pERK labeling in the Nac shell of cocaine animals compared to saline and Flu+ cocaine animals. Activation in the shell of the Nac has been experimentally linked to drug reward (Rodd-Henricks, McKinzie, Li, Murphy and McBride, 2002), DA dependent impulsive behaviour (Murphy et al., 2008), behavioural responses to conditioned stimuli (Floresco, McLaughlin and Haluk, 2008) and deficits in appetitive extinction responding (Holahan et al., 2010; 2012). Furthermore, animals will instrumentally respond for infusions of cocaine into the Nac

shell, but not the core (Rodd-Henricks et al., 2002), demonstrating the importance of this region in psychostimulant reward. Thus the increase in pERK labeling in the shell of the cocaine group could be due to a direct activation of the reward system that produced a potentiation of reward mechanisms and cocaine seeking, rather than the contextual cue pattern of pERK activation confined to the core of the Nac observed in the cocaine treated animals within experiment 1.

Another possible explanation for the inconsistent pERK labeling in the cocaine treated animals across the first two studies is the small group sizes for experiment 2. It is possible that the small n for this group (n=5) did not provide enough power to draw a meaningful comparison to the findings of experiment 1. It is possible that increasing the group sizes would tease apart the differences and trends identified in pERK expression in experiment 2 to better reflect the findings of experiment 1 and should certainly be an objective in future studies.

Experiment 3: GBR-12909 dose-response curve on behavioural extinction, locomotion and pERK expression

The purpose of exploring the dose dependent impact of GBR-12909 on appetitive extinction was to see how another DA agonist, similar mechanistically to cocaine but with a much higher affinity for DAT, altered extinction responding. Although GBR-12909 is a DAT blocker, it is unique in that it is not associated with the same degree of reward as cocaine and is much less addictive (Singh, 2000; Rothman et al., 2008). Despite this, GBR-12909 triggers reinstatement of extinguished drug-seeking behaviour

in both rats and primates (Wojnicki and Glowa, 1996; Stafford et al., 2001) and animals will operantly respond to receive infusions of the drug (Roberts, 1993).

Does increased GBR-12909 extinction pressing result from activation of the reward system?

Like cocaine, GBR-12909 treatment was associated with increased pressing at the end of the extinction period compared to controls across all doses. Furthermore, locomotor assessment did not reveal significant differences in horizontal locomotion overall and there were very few differences in locomotion observed in the time bin analysis, with only the highest dose resulting in more movement than controls during the first time bin. The locomotor findings are particularly significant because of the concurrent, albeit attenuated, elicitation of potentiated lever pressing. Although the GBR-12909 extinction profile was not as pronounced as the cocaine or MK-801 behaviour, the lack of hyperlocomotion with GBR-12909 is a demonstration that DA agonism can elicit a potentiation of non-reinforced pressing without altering locomotor behaviour. It is important to note that rearing was significantly increased for all GBR-12909 doses, suggesting that DA dependent locomotion and stereotypy was still moderately affected by drug treatment (Tilley et al., 2008) and could play a minor role in the extinction profile.

Although this group did exhibit behaviours consistent with an extinction deficit, it was to a lesser degree than that observed in the cocaine-treated groups from experiments 1 and 2. Furthermore, the profile of GBR-12909 correct presses resembled an extinction pattern, with a higher rate of pressing at the beginning of the session that diminished

toward the end of testing, whereas cocaine and MK-801 maintained a relatively steady rate of pressing for the entire session. It is possible that this results from the reduced addictive properties of GBR-12909 or the lack of SERT (Zolkowska et al., 2009) and NET (Barker and Blakely, 2000) blockade that accompanies cocaine treatment (Serafine & Riley, 2009).

Regardless of the profile of extinction pressing, all doses of GBR-12909 were characterized by increased correct lever responding at the end of the extinction period compared to controls and the 10 and 20mg/kg doses exhibited significantly higher rates of nose pokes at the end of testing. This finding is consistent with previous reports that GBR-12909 increases operant responding for food using similar doses (Kelley and Lang, 1989). Kelley et al. (1989) also reported these doses increased responding for conditioned reinforcers, suggesting that these behaviours are reward-based. Furthermore, the finding that an extinction deficit was induced with non-selective DA agonism and limited locomotor potentiation further substantiates the notion that DA agonism alone is sufficient to produce an appetitive extinction deficit. The findings of this experiment contribute to expanding evidence that suggests that the extinction deficits observed with cocaine and MK-801 are due to the drugs influences on DA reward pathways, rather than a disruption in extinction learning mechanisms.

Does GBR-12909 does dependently alter the pattern of ERK activation in the nucleus accumbens?

Only the highest dose of GBR-12909 (20mg/kg) triggered a significant overall increase in pERK and it was later discovered that this increase was confined to the core

of the Nac, where this group had significantly more labeled cells than all other treatments (saline, 5mg/kg and 10mg/kg). This finding suggests that a higher dose of GBR-12909 is required to elicit pERK in the Nac than cocaine, although differences in time course of tissue collection may account for the discrepancies between GBR-12909 and cocaine pERK labeling. The time course for GBR-12909 was based on previous experiments by Rothman et al., 2008 that reported GBR-12909 elicited DA levels peaking in the brain 30 minutes after administration but, using similar doses, a study by Espana, Roberts and Jones (2009) reported a rapid increase in DA influx within the first 5 minutes of treatment. It is possible that the pERK activation took place within this time period for the lower does, and was no long detectable by the end of the 30-minute incubation period.

Similar to the findings with cocaine in experiment 1, the core of the Nac exhibited significantly higher labeling than the shell for both the 5 and the 20mg/kg doses. It is possible that these doses triggered an increase in extracellular DA in the core in response to the contextual cues described previously, but further investigation into how the mechanisms and time course of GBR-12909 and cocaine block DA reuptake to increase DA is needed to fully understand the profile of pERK observed with GBR-12909 this study.

Experiment 4: Apomorphine dose-response curve on behavioural extinction, locomotion and pERK expression

A dose response with Apomorphine (APO) was employed to assess the influence of direct DA agonism on appetitive operant extinction. Because of the unique properties

of APO, the literature reports low doses of APO reducing DA release and high doses increasing it (Van Ree and Wolterink, 1981; Cary et al., 2004; Braga, Dias, Carey and Carrera, 2009). If it is assumed that in the previous studies an increase in DA release was responsible for the extinction deficits, the results of this study do not support this notion. It was originally hypothesized that, similar to observations following DA antagonist treatment, the low doses of APO (0.05, 0.5mg/kg) would facilitate operant extinction. Conversely, it was predicted that the high doses (2.0, 5.0mg/kg) would trigger an extinction deficit in much the same way as cocaine or GBR-12909.

Contrary to our expectations, the opposite effect was observed. The two highest doses of APO (2.0, 5.0mg/kg) were associated with a marked impairment in both lever responding and locomotion. The operant and locomotor effects were also very consistent across groups, which is indicative of a gross impairment, either cognitive, locomotor or both, at these doses. This finding is not supported by the literature, where doses within this range produce locomotor sensitization (Braga et al., 2009) and increase operant responding and reward seeking in a DA-dependent manner (Baxter et al., 1974; Woolverton, Goldberg and Ginos, 1984; Norman et al., 2011). It is possible that the high doses of APO triggered the emetic effects of the drug, rendering the animals nauseated and thus disturbing their food seeking and locomotion (Carpenter, Briggs and Strominger, 1984)

The lowest dose of APO (0.05mg/kg) produced operant response rates comparable to saline for correct, incorrect and nose poke activity, suggesting that the drug had little to no effect on behaviour. Additionally, locomotor behaviour for this group was statistically similar to controls. Various factors could have contributed to these

observations. For instance, it is possible that this dose of APO was not sufficient to produce either agonism or antagonism, despite reports in the literature that doses within this range alter behaviour and antagonize DA (Van Ree and Wolterink, 1981; Cary et al., 2004; Braga, Dias, Carey and Carrera, 2009)

Interestingly, the 0.5 APO group consistently exhibited significantly lower correct presses and nose pokes than controls for the first 15 minutes of testing, which increased toward the end of the 30-minute session. One possible interpretation of this result could be that the 0.5mg/kg dose was working on autoreceptors to reduce DA output during this time, and the drug effect did not persist for the duration of the session. The upward shift in extinction responding (correct lever) and reward seeking (nose pokes) could be a reflection of the drug effect diminishing across time and normalizing by the end of testing.

Interestingly, the 0.5mg/kg group exhibited a similar response profile during locomotor testing, with low horizontal distance moved and rearing during the first half of the session, which increased to achieve parity with controls by the last 10 minutes of testing. These observations are indicative of DA antagonism reducing locomotion, although previous studies report this dose increasing locomotion (Brown, Bae and Kiyatkin, 2006). Furthermore, the higher doses were also associated with operant and locomotor impairment, but this drug effect persisted throughout the entire session. If the 0.5 APO group did in fact model the DA antagonistic properties of low dose APO, and the higher doses (2.0, 5.0) induced emetic effects, it is possible that a more moderate dose within the 0.75-1.5mg/kg range will produce the DA agonist effect we were attempting to model in this experiment. Further experiments should be employed to test this possibility.

Does apomorphine dose dependently alter the pattern of ERK activation in the nucleus accumbens?

Contrary to expectations, there were no differences in pERK labeling in the Nac across treatments both overall or in the core or shell. It is possible that the incubation period was not sufficient to induce the changes in DA output required to initiate the dose-dependent phosphorylation of ERK that was expected to occur. Aside from differences across treatments, it was also noted that the control, 0.5, 2.0 and 5.0mg/kg treatments exhibited significantly higher labeling in the core than the shell of the Nac. This was a surprising finding that is difficult to reconcile, particularly with regard to control animals. Since the time course of drug administration and tissue collection differed for each group, it is difficult to compare labeling across treatments and it is possible that this lack of internal consistency underlies these findings.

Although it is likely that time course plays a role, the consistent differences between core and shell labeling across groups warrants further explanation. Another factor that should be considered is the use of ascorbic acid as a vehicle for the APO. Ascorbic acid is rather benign and was used in a very low concentration (0.1%) but its use was consistent across APO doses, suggesting it may be a potential contributor to observed changes in pERK. Previous studies have reported alterations in pERK following exposure to ascorbic acid. In fact, the influence of ascorbic acid on the DA system has been long since documented. For example, in an older study conducted by Tolbert and colleagues (1979) ascorbic acid was shown to inhibit DA in the striatum. More recent studies have extended these observations to include alterations in pERK resulting from ascorbic acid treatment (Peus et al., 1999; Pomerance et al., 2000), suggesting that this is

a possible contributing factor to the aberrant findings in this experiment but warrants further investigation into dose responsive effects before causal relationships are made.

Conclusions

The results of this thesis suggest that DA agonist drugs are indeed capable of inducing a potentiation of pressing on the correct lever during a non-reinforced session similar to observations with MK-801. Although DA agonism alone can produce these behaviours, the results of this study do not conclusively suggest that the MK-801 extinction profile results solely from DA-dependent processes altering the reward system. Rather, the magnitude of the extinction profile after MK-801 was not matched using any of the DA agonist drugs used in this study, suggesting that a synergism between reward potentiation and learning deficits likely underlies the behaviour.

Future studies should attempt to reconcile these issues, possibly through co-administration of NMDA receptor agonist drugs with MK-801 and/or assessment of drug effects on specific brain regions during extinction. It would also be useful to assess the dose-dependent effects of cocaine during extinction. It is possible that a higher dose would have elicited an extinction profile of the same magnitude as MK-801. Additionally, use of another direct DA agonist drug other than APO may be needed to properly assess the influence of direct vs. indirect DA agonism on extinction responding.

Unfortunately the assessment of pERK in these experiments provided little clarification of the role of the ERK cascade in the induction and maintenance of an appetitive extinction deficit. Future studies should model pERK activation across time, before, during and after extinction learning occurs in order to uncover the differences in

drug phosphorylation of ERK under these conditions. Despite the experimental limitations of this study, the results suggest that DA does play a fundamental role in the induction of an appetitive extinction deficit and should be further explored to assess the potential role for DA antagonism and Glu agonism in the extinction of persistent and maladaptive reward seeking behaviours.

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Appendix 1A: MK-801, Cocaine and D1/D2 receptor antagonists SCH23390 and Haloperidol Operant Responding

Figure Caption 1: A two-way repeated measures ANOVA with group as the between subjects factor and day as the within (repeated) subject factor on the mean number of correct (rewarded) presses revealed that the groups significantly increased lever pressing across acquisition days. There were no effects of group or interactions detected. This demonstrates that all groups were exhibiting statistically similar rates of operant responding during the acquisition phase, prior to drug treatment.

Figure 1: Acquisition

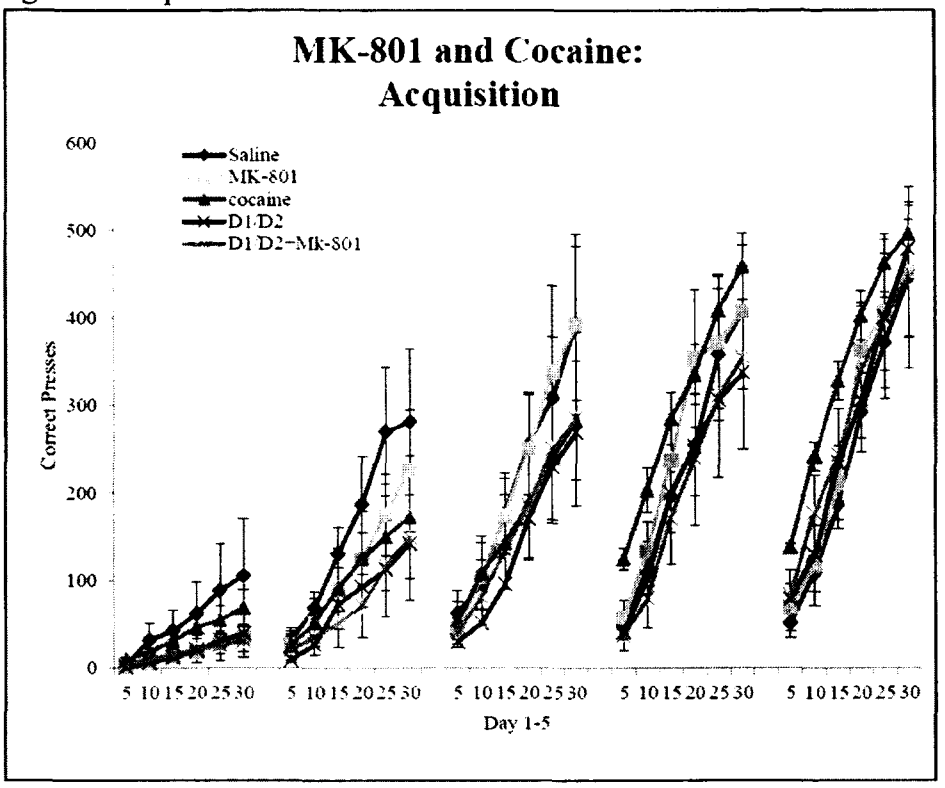


Figure Caption 2: Bar graph depicting the average total number of correct presses during the 30 minute extinction session. Analysis revealed that MK-801 (0.05mg/kg) pressed significantly more than all other treatments (saline, cocaine (10mg/kg), haloperidol (D2) (0.1mg/kg) and SCH23390 (D1) (0.01mg/kg) and D1/D2 and MK-801). Cocaine pressing overall was significantly higher than the D1/D2 and the D1/D2 + MK-801 treated animals. (*$p<0.05$, **$p<0.01$, ***$p<0.001$)

Figure Caption 3: Line graph depicting the average number of correct lever presses for each 5 minute time interval during the 30 minute extinction session. MK-801 (0.05mg/kg) and cocaine (10mg/kg) treatment was characterized by elevated correct lever pressing during extinction compared to all other treatments. Pre-treatment with DA antagonists haloperidol (D2) (0.1mg/kg) and SCH23390 (D1) (0.01mg/kg) blocked the MK-801- induced extinction deficit, suggesting that the increase in correct lever pressing is likely DA dependent (*$p<0.05$, **$p<0.01$).

Figure 2: Total Correct Lever

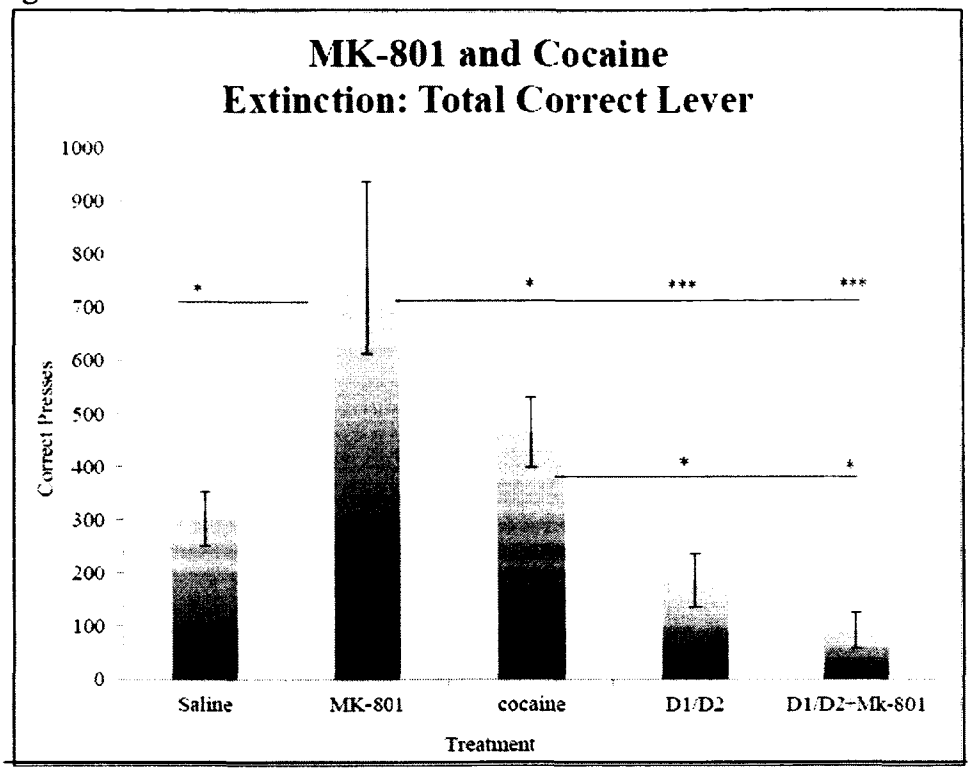


Figure 3: Time Bin Correct Lever

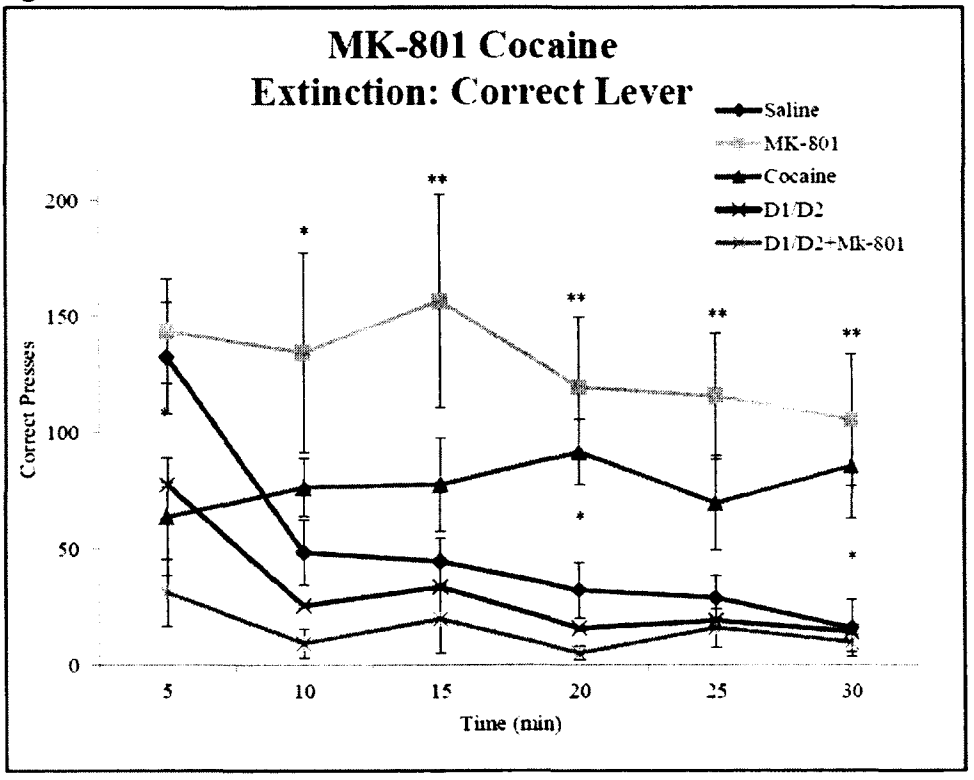


Figure Caption 4: Line graph depicting the average number of incorrect lever presses for each 5 minute time interval during the 30 minute extinction session. The MK-801 (0.05mg/kg) group pressed significantly higher than SCH23390 (D1) (0.01mg/kg) and haloperidol (D2) (0.1mg/kg) pretreated animals. MK-801 also exhibited significantly higher incorrect presses at the end of testing, within the thirty-minute time bin, compared to saline, cocaine (10mg/kg) and D1/D2 + MK-801 groups (*$p<0.05$, **$p<0.01$).

Figure Caption 5: Line graph depicting the average number of nose pokes for each 5 minute time interval during the 30 minute extinction session. MK-801 (0.05mg/kg) treated animals exhibited significantly higher nose poke activity than saline, SCH23390 (D1) (0.01mg/kg) and Haloperidol (D2) (0.1mg/kg) and D1/D2 + MK-801 during the second half of the extinction session. The cocaine (10mg/kg) group exhibited significantly higher nose pokes all groups other than MK-801 by the end of the thirty minute time bin. The increase in nose poke activity for both the MK-801 and cocaine groups and the reduction in nose pokes observed when MK-801 treatment is paired with DA antagonism suggest that MK-801 and cocaine trigger DA-dependent reward seeking. (*$p<0.05$, **$p<0.01$).

Figure 4: Time Bin Incorrect Presses

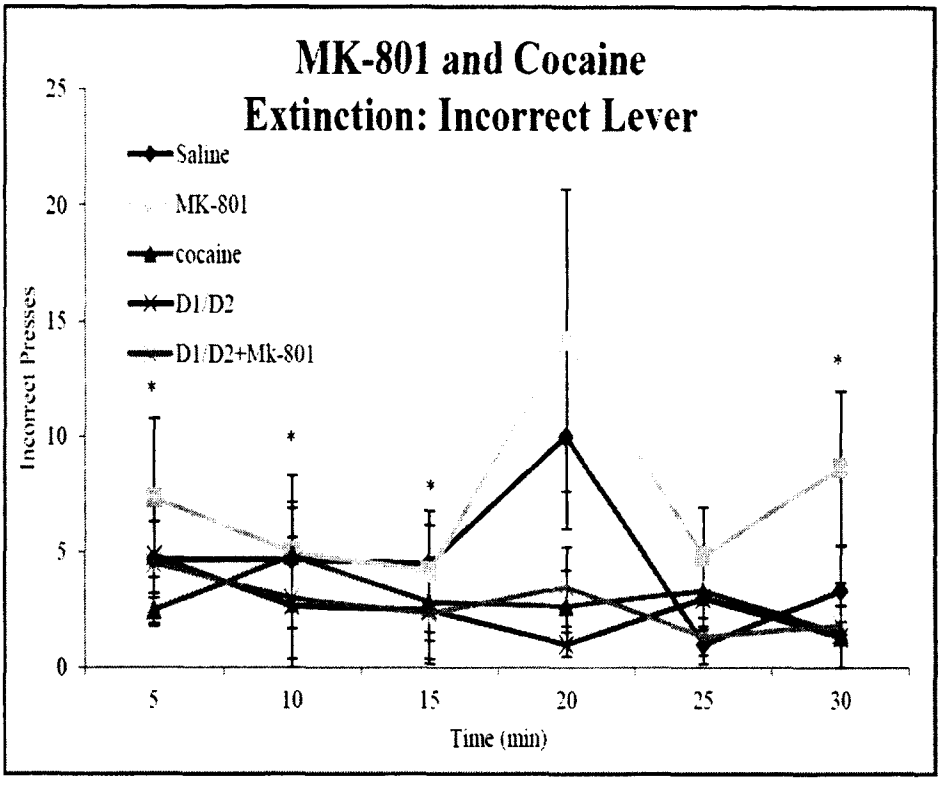
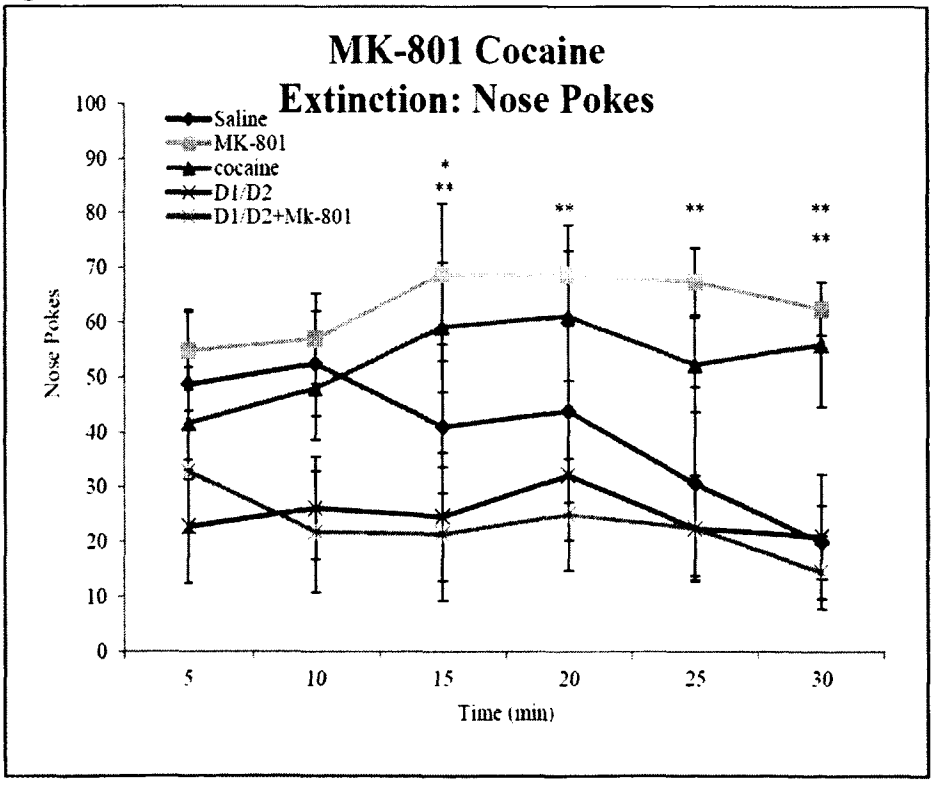


Figure 5: Time Bin Nose Pokes



Appendix 1B: MK-801, Cocaine and D1/D2 receptor antagonists SCH23390 and Haloperidol Locomotion

Figure Caption 1: Bar graph depicting average total distance moved during the 30 minute testing session. Overall, the MK-801 (0.05mg/kg) group exhibited hyperlocomotion throughout the session compared to all other treatment groups. Pre-treatment with DA antagonists haloperidol (D2) (0.1mg/kg) and SCH 23390 (D1) (0.01) blocked the MK- 801- induced hyperlocomotion. The D1/D2 group also moved significantly less than saline and cocaine (10mg/kg) groups. There were no other group differences detected. (*$p<0.05$, **$p<0.01$)

Figure Caption 2: Line graph depicting horizontal locomotion during the 30 minute testing session. MK-801 (0.05mg/kg) group exhibited hyperlocomotion throughout the session compared to all other treatment groups. Pre-treatment with DA antagonists haloperidol (D2) (0.1mg/kg) and SCH 23390 (D1) (0.01mg/kg) blocked the MK-801-induced hyperlocomotion. The D1/D2 group moved less than saline at the beginning of testing, but was comparable to saline during the second half of testing. D1/D2 also moved significantly less than cocaine treated animals for the duration of testing (*$p<0.05$, **$p<0.01$)

Figure 1: Total Locomotion

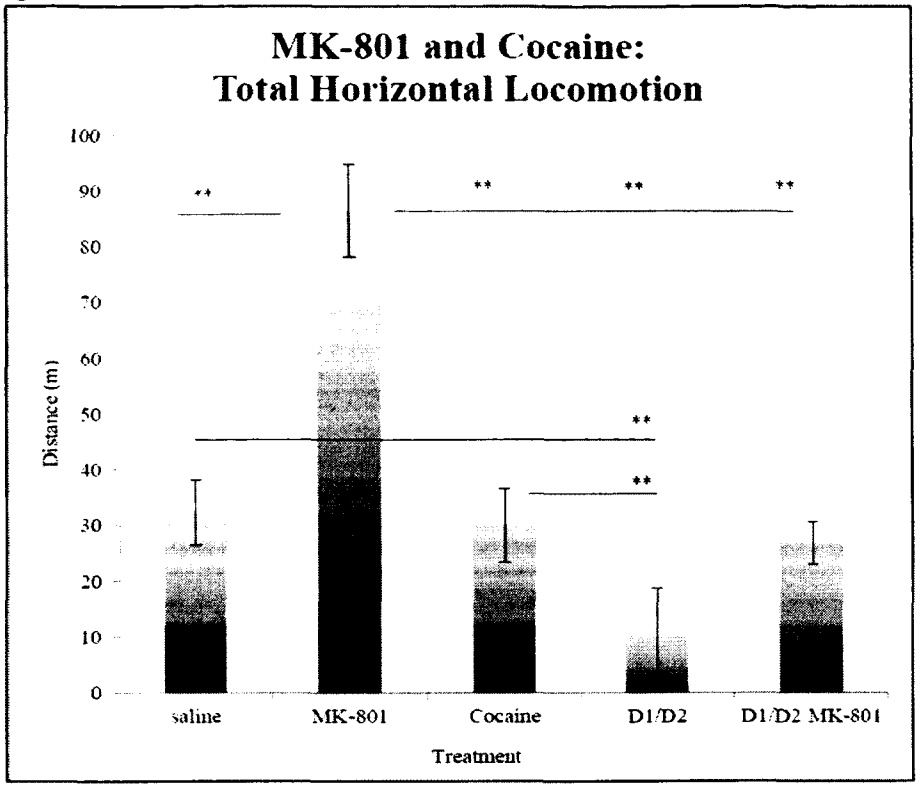


Figure 2: Time bin Locomotion

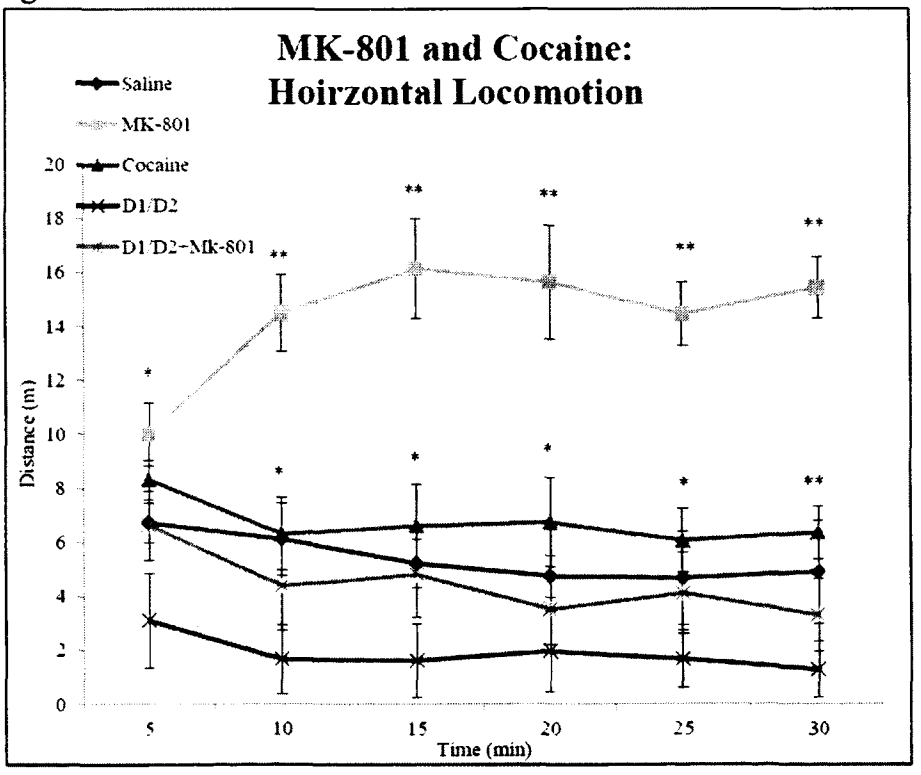


Figure Caption 3: Line graph depicting average total rearing during the 30 minute testing session. MK-801 (0.05mg/kg) and cocaine (10mg/kg) reared significantly more than saline and SCH23390 (D1) (0.01mg/kg) and Haloperidol (D2) (0.1mg/kg) groups, suggesting that these groups were exhibiting potentiated vertical locomotor activity. The D1/D2 and D1/D2 + MK-801 treated animals reared significantly less overall than saline, which may be indicative of a locomotor deficit. (*$p<0.05$, **$p<0.01$)

Figure Caption 4: Line graph depicting the average number times rearing for each 5 minute time interval during the 30 minute locomotor assessment session The results indicated that SCH23390 (D1) (0.01mg/kg) and Haloperidol (D2) (0.1mg/kg) reared significantly less than saline across all time bins and similar findings were observed with D1/D2 + MK-801 treated animals. MK-801 (0.05mg/kg) reared significantly more than saline during the second half of testing. MK-801 also reared significantly more than D1/D2 and D1/D2 + MK-801 treated animals. Similarly, cocaine (10mg/kg) treated animals reared significantly more than saline towards the end of the session. (*$p<0.05$, **$p<0.01$)

Figure 3: Total Rearing

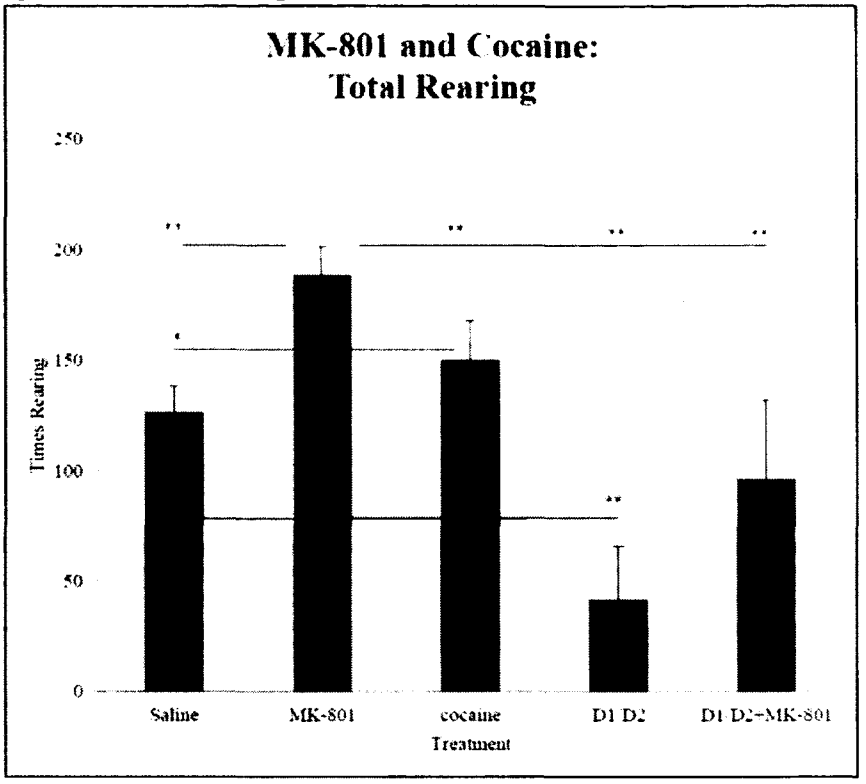
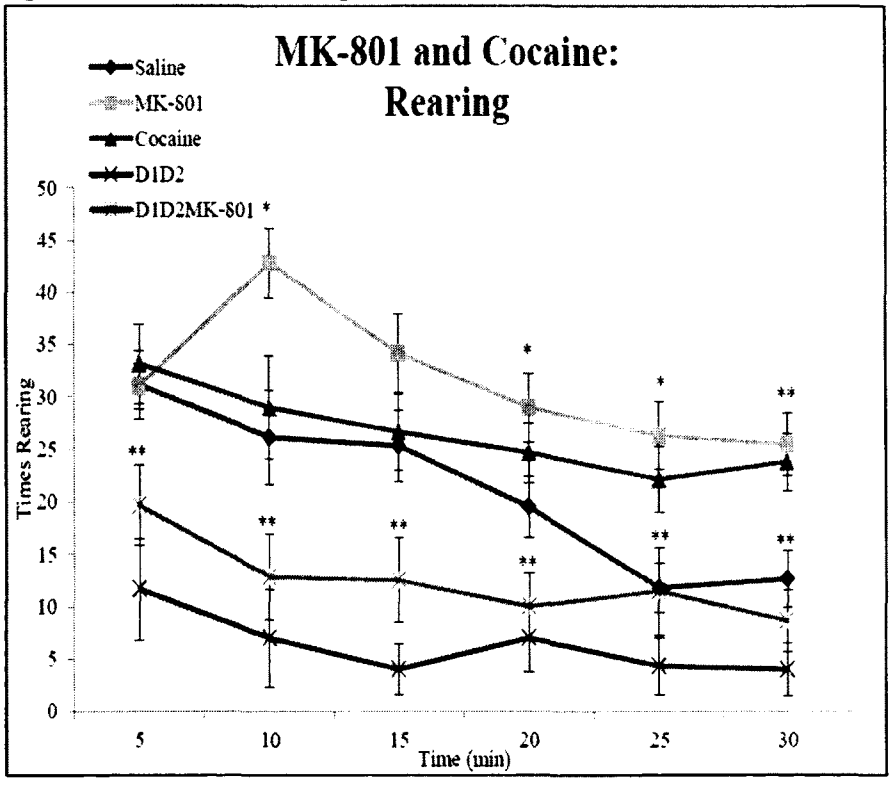


Figure 4: Time Bin Rearing



Appendix 1C: MK-801, Cocaine and D1/D2 receptor antagonists SCH23390 and Haloperidol Phosphorylated Extra Cellular Regulated Kinase Activation

Figure Caption 1: The analysis indicated that the cocaine (10mg/kg) treated animals exhibited significantly more phosphorylated extracellular regulated protein kinase (pERK) labeling than saline, SCH233909 (D1) (0.01mg/kg) and Haloperidol (D2) (0.1mg/kg) and D1/D2 + MK-801 treated animals. MK-801 (0.05mg/kg) had more labeling than D1/D2 and D1/D2 and MK-801 treated animals. There were no other significant differences detected. (*$p<0.05$, **$p<0.01$)

Figure Caption 2: Bar graph depicting the average number of pERK-labeled cells within the core and shell of the Nac. The results indicated that there was significantly more pERK labeling in the core of the Nac of the cocaine (10mg/kg) treated animals compared to saline, SCH233909 (D1) (0.01mg/kg) and Haloperidol (D2) (0.01mg/kg), and D1/D2 + MK-801. There was more pERK labeling in the MK-801 (0.05mg/kg) group, compared to D1/D2 and D1/D2 + MK-801. MK-801 exhibited significantly more pERK labeling than D1/D2 and D1/D2 + MK-801. With regards to differences in core and shell labeling within groups, there was more pERK labeling in the core of the Nac, compared to the shell in the cocaine treated animals. (*$p<0.05$, **$p<0.01$)

Figure 1: Total pERK Labeled Cells

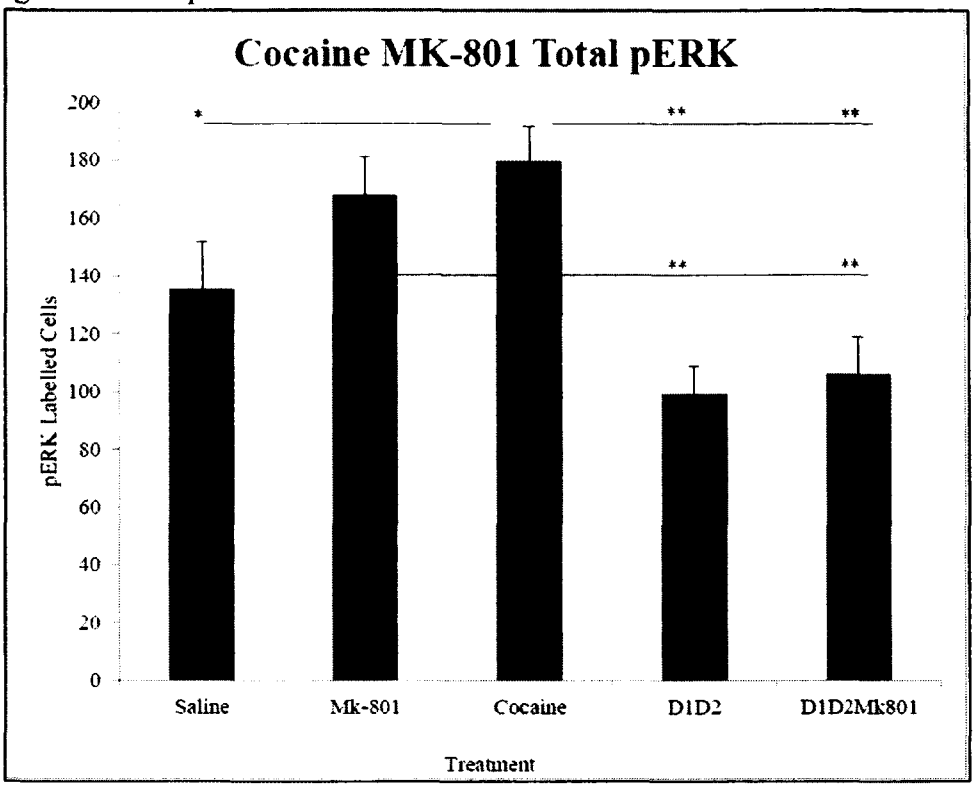
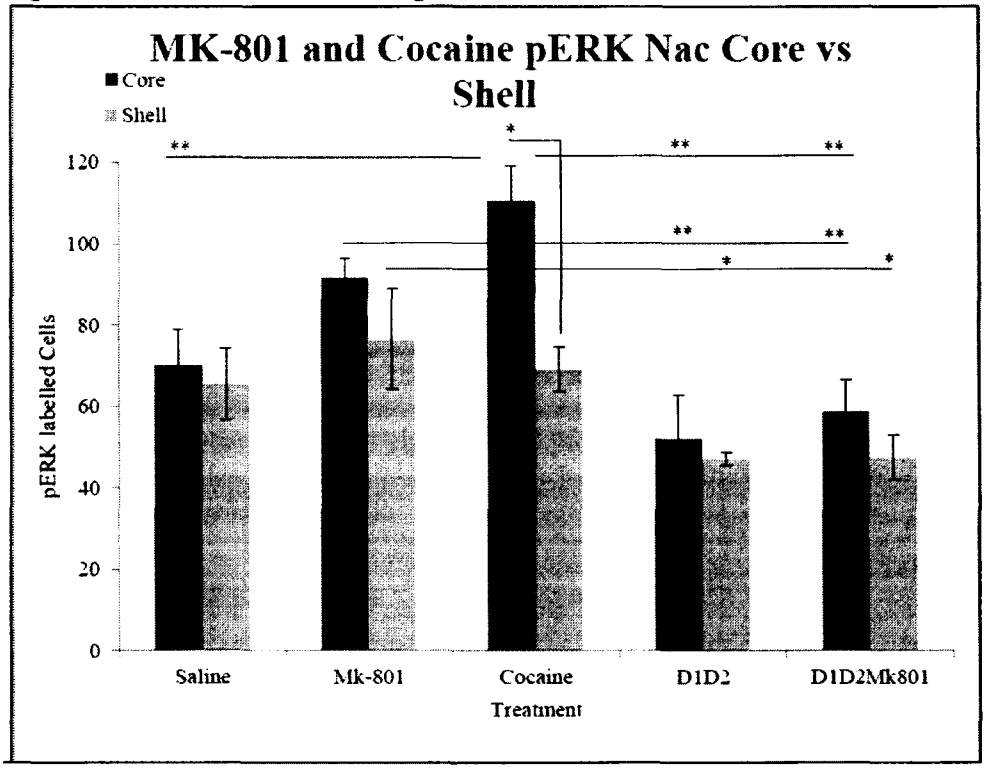
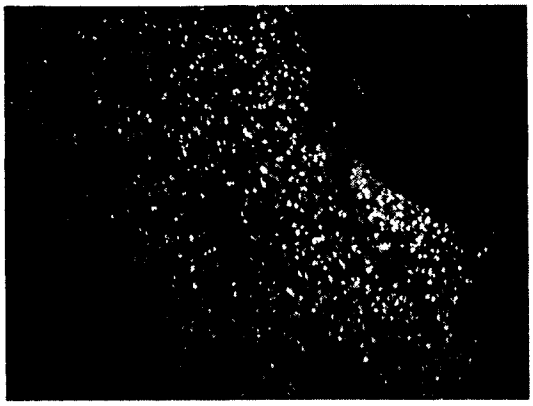


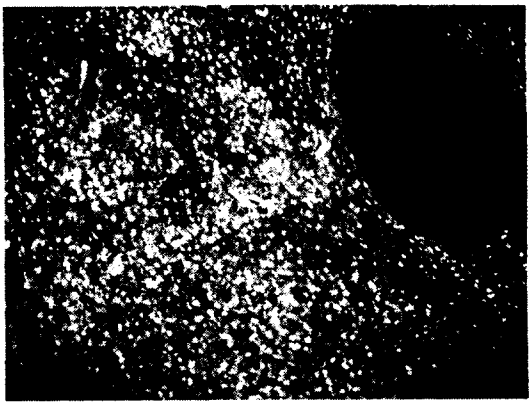
Figure 2: Core vs. Shell Labeling



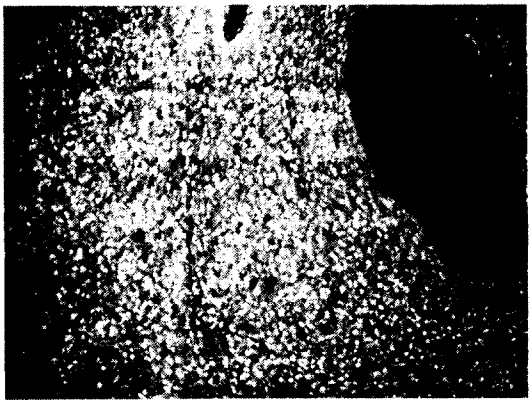
Saline



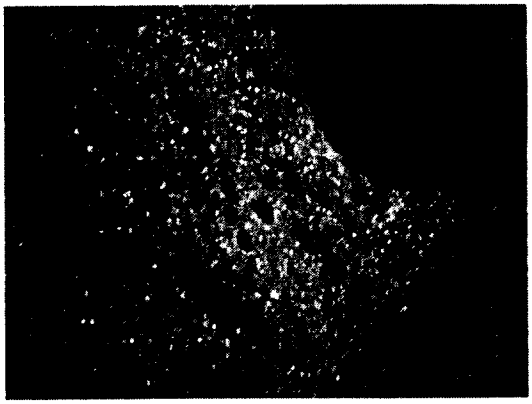
MK-801



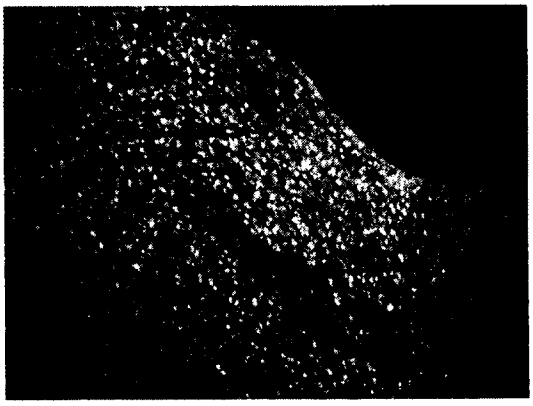
Cocaine



D1/D2



D1/D2 + MK-801



Appendix 2A: Cocaine and Flupenthixol on behavioural extinction, locomotion and pERK expression

Figure Caption 1: A two-way repeated measures ANOVA with group as the between subjects factor and day as the within (repeated) subject factor on the mean number of correct (rewarded) presses revealed that the groups significantly increased lever pressing across acquisition days but there were no effect of group or interactions detected. This indicates that all groups exhibited statistically similar rates of operant responding during the acquisition phase, prior to drug treatment.

Figure 1: Acquisition

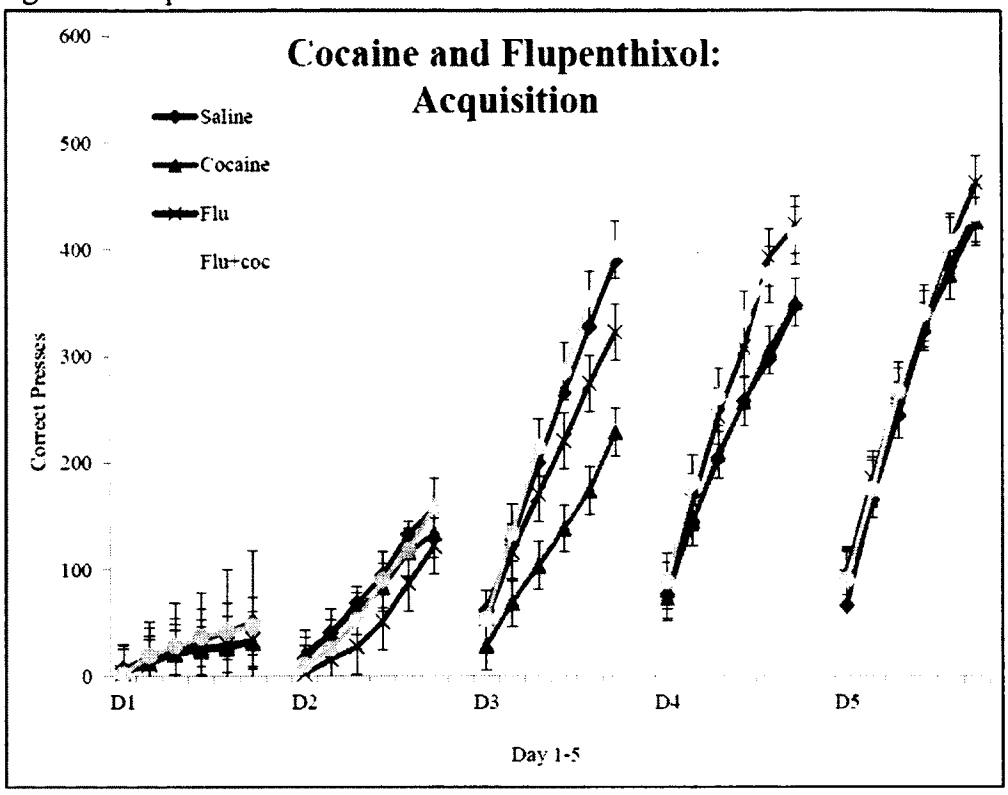


Figure Caption 2: Bar graph depicting the average total number of correct lever presses during the 30 minute extinction session. The analysis identified a significant difference between the cocaine group and the flupenthixol (0.4mg/kg) and cocaine (10mg/kg) group, suggesting that increase cocaine pressing during extinction is DA dependent. (*$p<0.05$, **$p<0.01$)

Figure Caption 3: Line graph depicting average number of correct lever presses during a 30 minute extinction session. During the last ten minutes of testing the cocaine (10mg/kg) group pressed significantly more than saline, suggesting an extinction deficit. Cocaine also exhibited elevated bar pressing throughout the session compared to all other groups. Pre-treatment with the DA antagonist flupenthixol (0.4mg/kg) blocked the cocaine induced extinction deficit, indicating that this deficit is DA dependent. By the end of the first five minutes of testing, the flupenthixol and cocaine treated animals were pressing significantly lower than saline animals, with this groups pressing subsequently increasing to a comparable level to saline for the remainder of testing. Additionally, the flupenthixol treated animals pressed significantly lower than cocaine animals during the twenty five and thirty minute time bins (*$p<0.05$, **$p<0.01$)

Figure 2: Total Correct

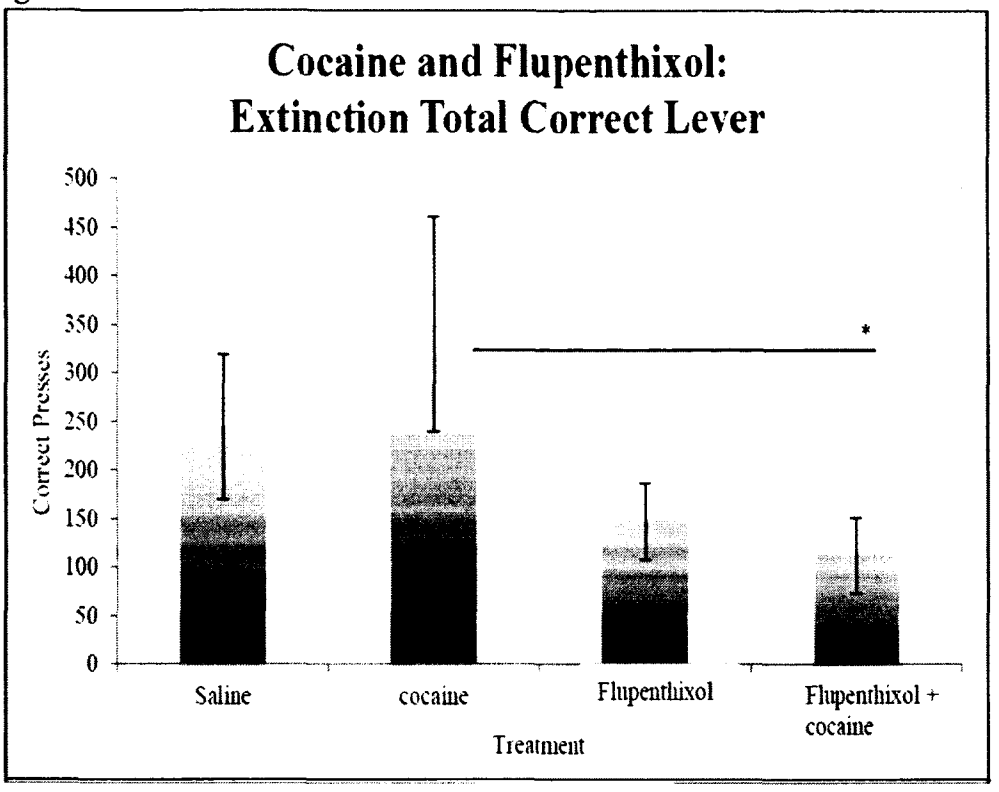


Figure 3: Time Bin Correct

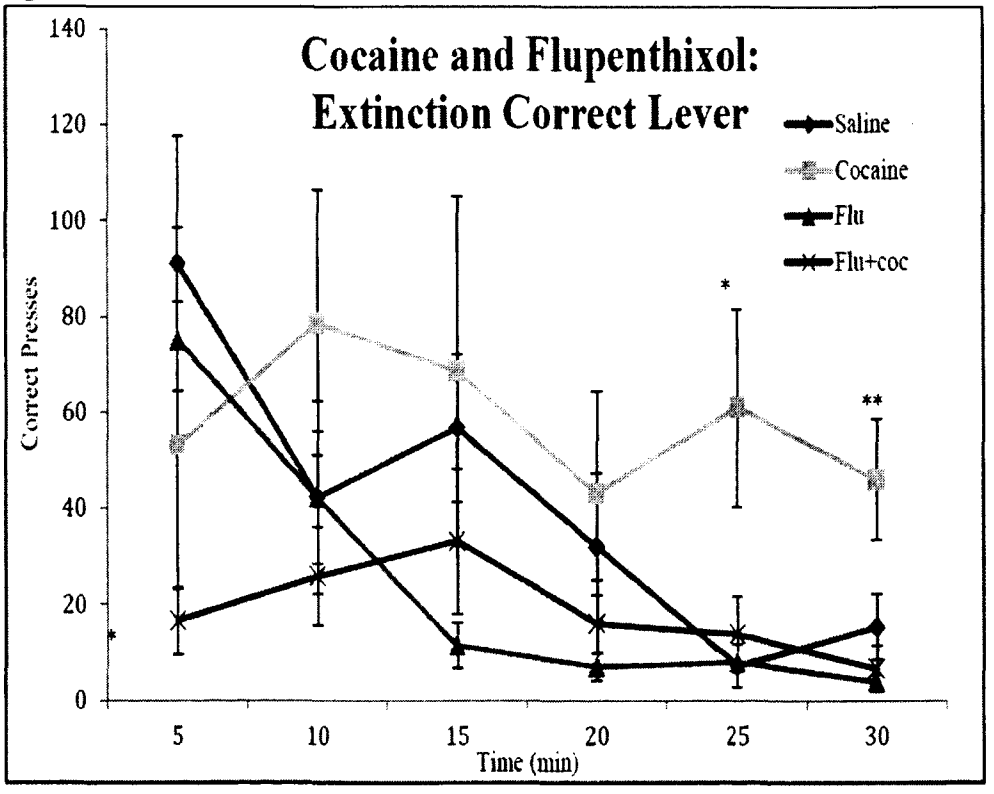


Figure Caption 4: Line graph depicting the average number of incorrect lever presses during a 30 minute extinction session. Flupenthixol (0.4mg/kg) treated animals pressed the incorrect lever significantly less than saline controls during the twenty-five minute time bin. No other significant differences were identified. (*$p<0.05$)

Figure Caption 5: Line graph depicting the average number of nose pokes during the 30 minute extinction session. All of the treatment groups (cocaine (10mg/kg), flupenthixol (0.4mg/kg), flupenthixol and cocaine) exhibited significantly lower nose poke activity than saline controls during the five minutes of testing. Differences reemerged toward the end of testing with the flupenthixol and flupenthixol and cocaine groups displaying lower nose poke activity than the cocaine group, indicating that DA system activation augments nose poke behaviour. (*$p<0.05$)

Figure 4: Time Bin Incorrect

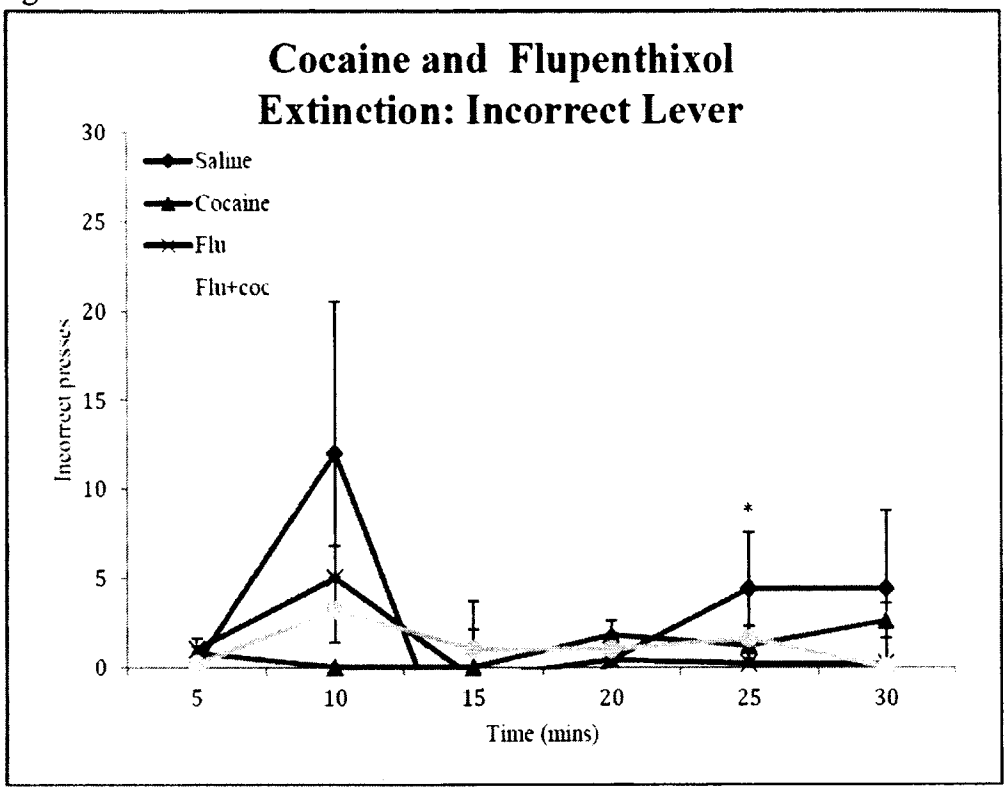
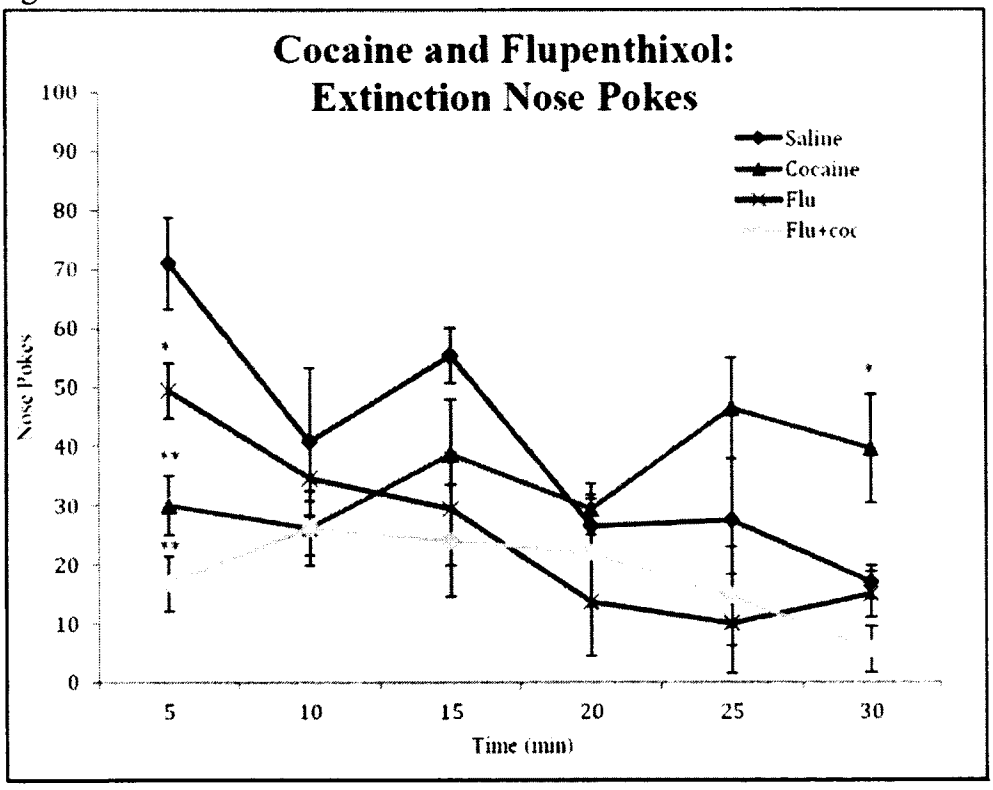


Figure 5: Time Bin Nose Pokes



Appendix 2B: Cocaine and Flupenthixol Locomotion

Figure Caption 1: Bar graph depicting average total horizontal distance moved during the

30 minute testing session. The cocaine (10mg/kg) group exhibited hyperlocomotion throughout the session compared to other treatment groups. Pre-treatment with DA antagonist Flupenthixol (0.4mg/kg) blocked this behaviour, suggesting that hyperlocomotion is DA dependent. (*$p<0.05$, **$p<0.01$)

Figure Caption 2: Line graph depicting the average distance moved across all time bins of the 30 minute testing period. After the first five minutes of testing the saline group was moving less than the cocaine (10mg/kg) and flupenthixol (0.4mg/kg) and cocaine groups. During this time bin saline was also moving less than the flupenthixol group. With exception of the first time bin, cocaine moved significantly more than the flupenthixol group for the duration of testing. The cocaine group continued to move significantly more than saline for the remainder of testing. There were no other significant differences detected. (*$p<0.05$, **$p<0.01$)

Figure 1: Total Locomotion

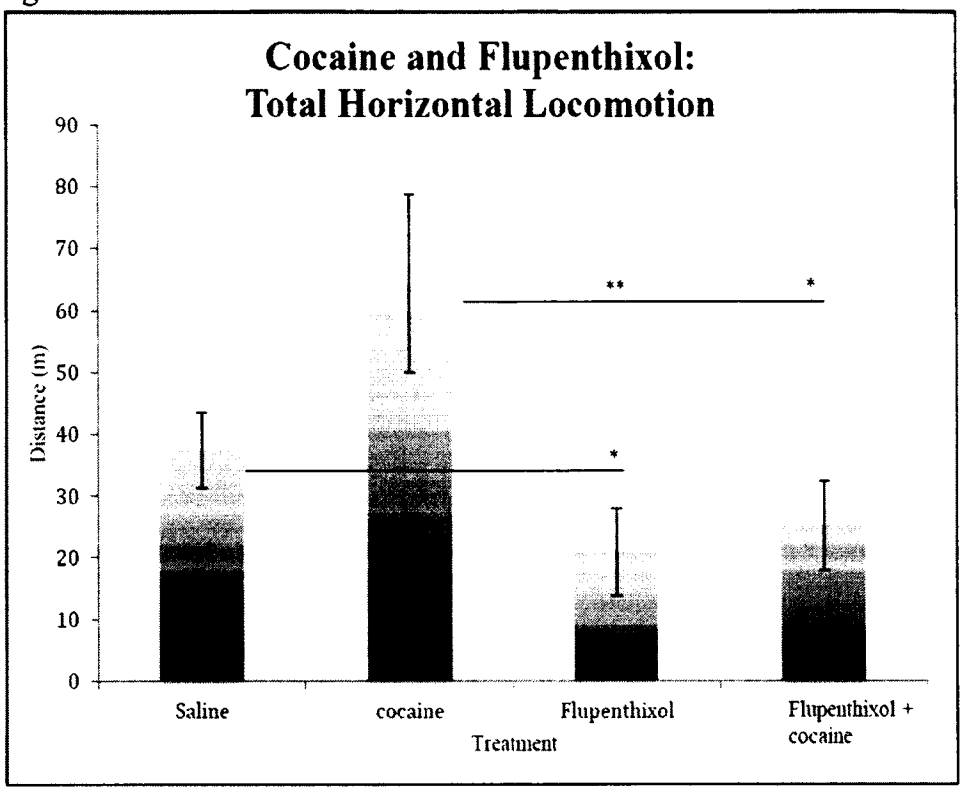


Figure 2: Time Bin Locomotion

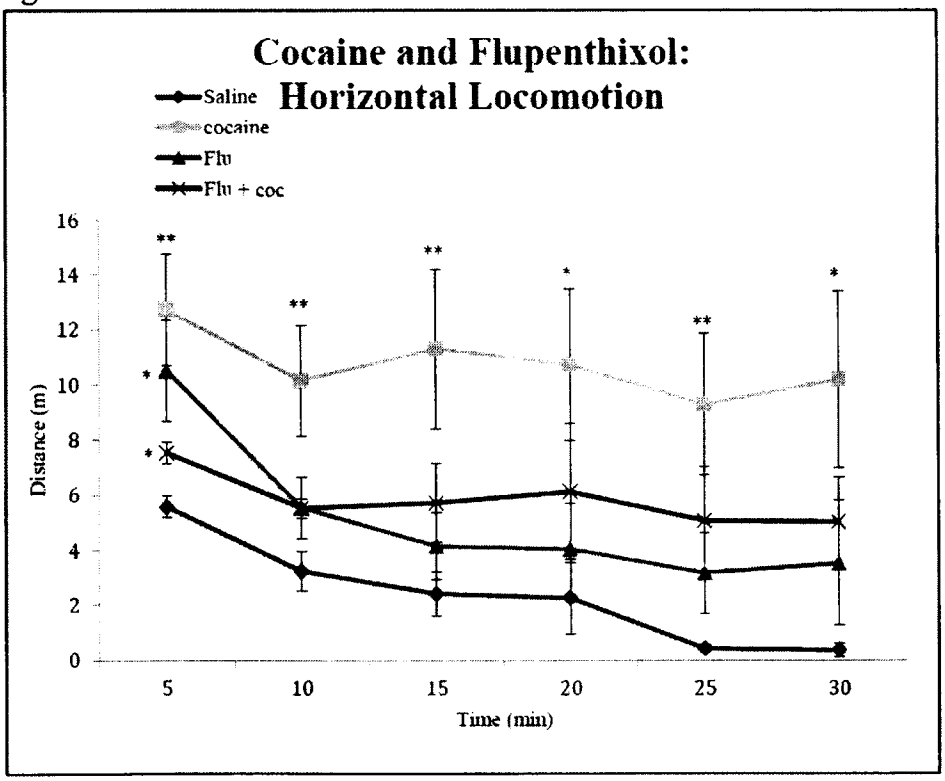


Figure Caption 3: A bar graph depicting the average number of times each group reared during the 30 minute testing session. There were no group differences detected.

Figure Caption 4: Line graph depicting the average number of times rearing for each group across all time bins of testing. The results indicated that the flupenthixol (0.4mg/kg) group reared significantly less than the cocaine (10mg/kg) group at the end of the session. There were no other group differences detected. (*$p<0.05$)

Figure 3: Total Rearing

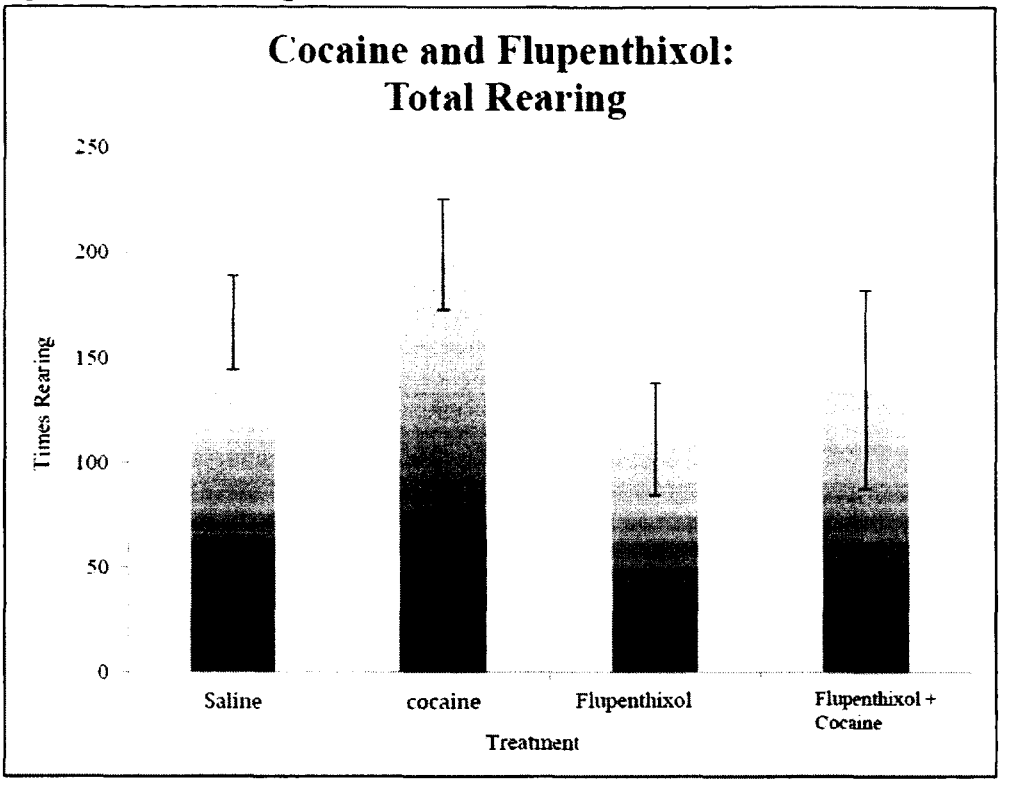
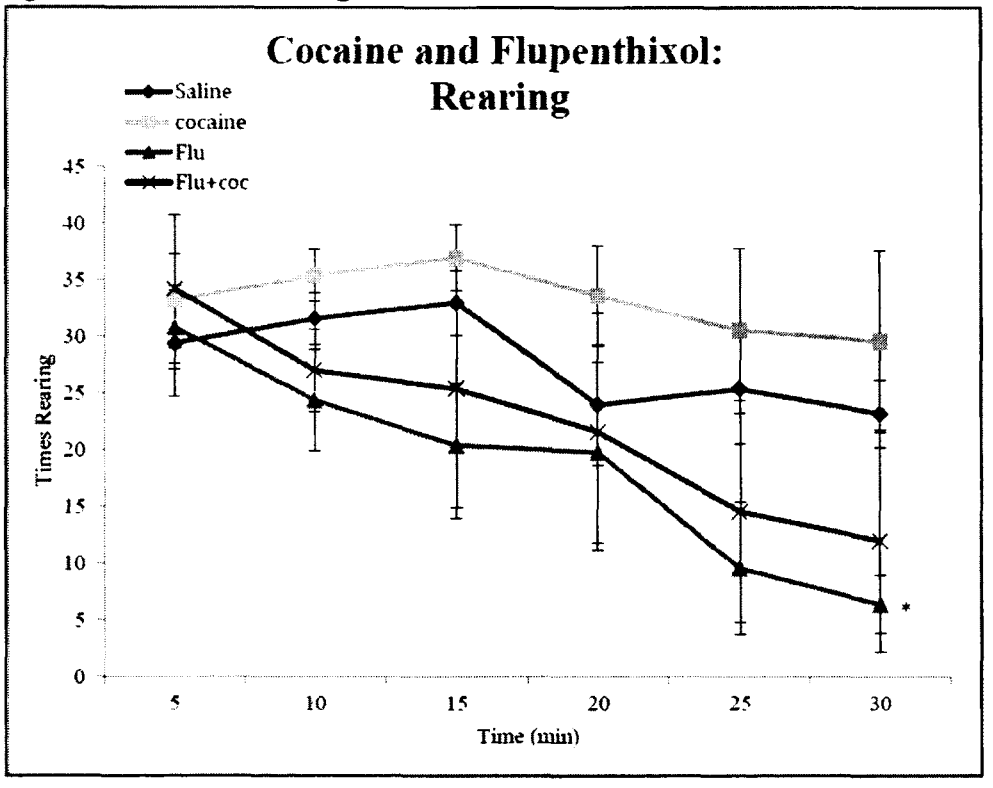


Figure 4: Time Bin Rearing



Appendix 2C: Cocaine and Flupenthixol Phosphorylated Extra Cellular Regulated Kinase Activation

Figure Caption 1: Bar graph depicting the average number of phosphorylated extracellular regulated protein kinase (pERK) labeled cells within the Nac. There was significantly more pERK labeling in the cocaine (10mg/kg) group, compared to flupenthixol (0.4mg/kg) and flupenthixol and cocaine groups. There were no other group differences detected. (*$p<0.05$)

Figure Caption 2: Bar graph depicting the average number of pERK labeled cells within the core and shell of the Nac. There was significantly more pERK labeling in the core of Nac following cocaine (10mg/kg) treatment compared to flupenthixol (0.4mg/kg). There was also significantly more pERK labeling in the Nac shell compared to saline and flupenthixol and cocaine animals. There were no other group differences detected. With regard to differences in core and shell labeling within groups, the results indicated that there was significantly more pERK labeling in the core of the Nac, compared to the shell in the saline treated animals. There were no other differences detected in any of the other groups. (*$p<0.05$, **$p<0.01$)

Figure 1: Total pERK Labeled Cells

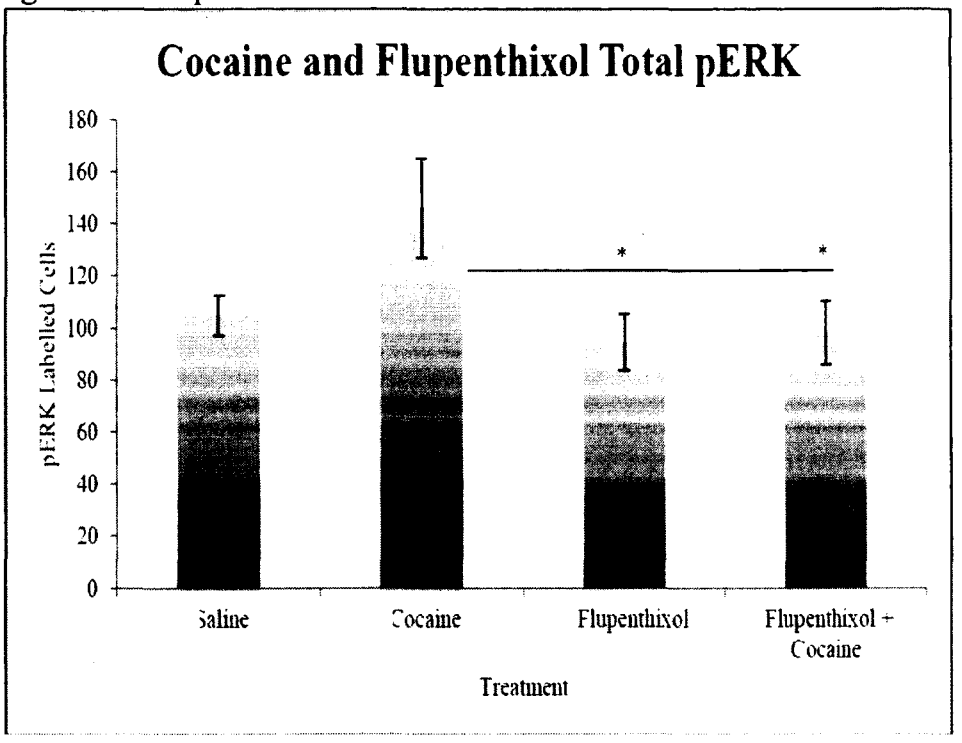
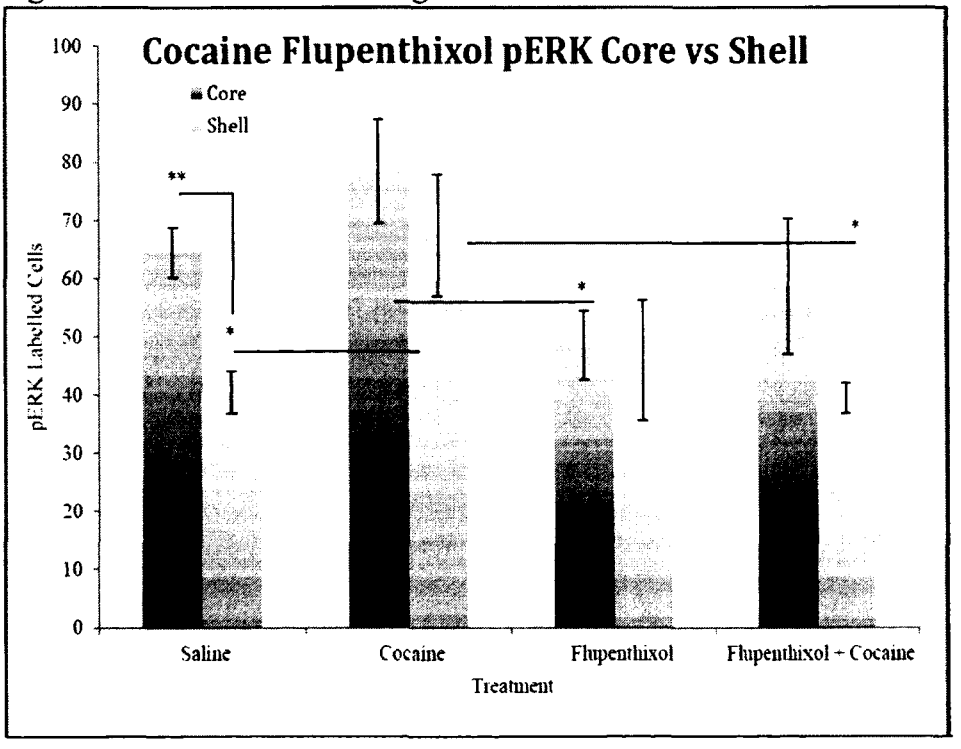
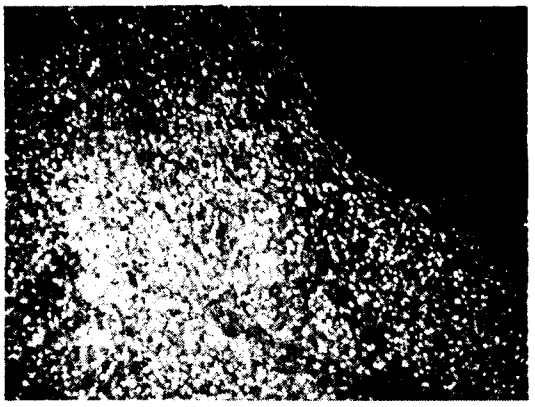


Figure 2: Core vs. Shell Labeling

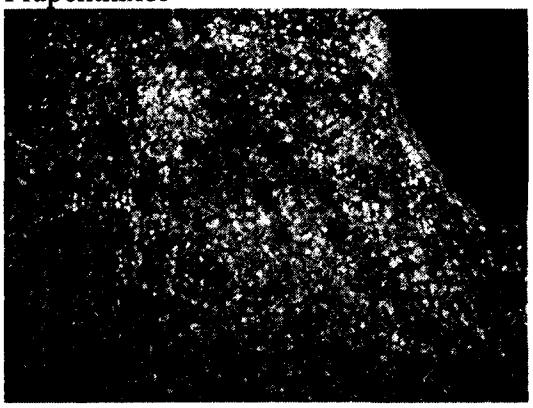


Saline

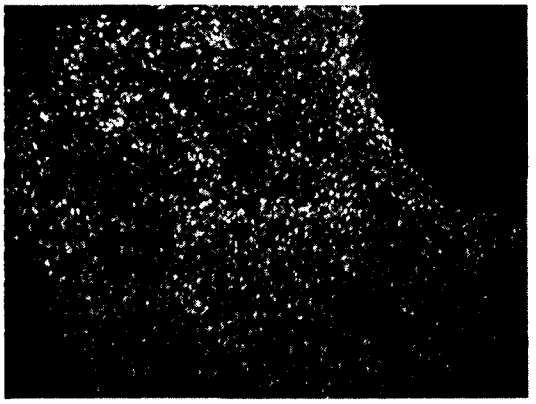
Cocaine



Flupenthixol



Flu + Cocaine



Appendix 3A: GBR12909 dose response on behavioural extinction, locomotion and pERK expression

Figure Caption 1: line graph depicting the average number of correct lever presses during day 1-5 of the acquisition phase. Correct lever pressing for all groups significantly increased across acquisition days but there were no effect of group or interactions detected. This indicates that all groups exhibited statistically similar rates of operant responding during the acquisition phase, prior to drug treatment.

Figure 1: Acquisition

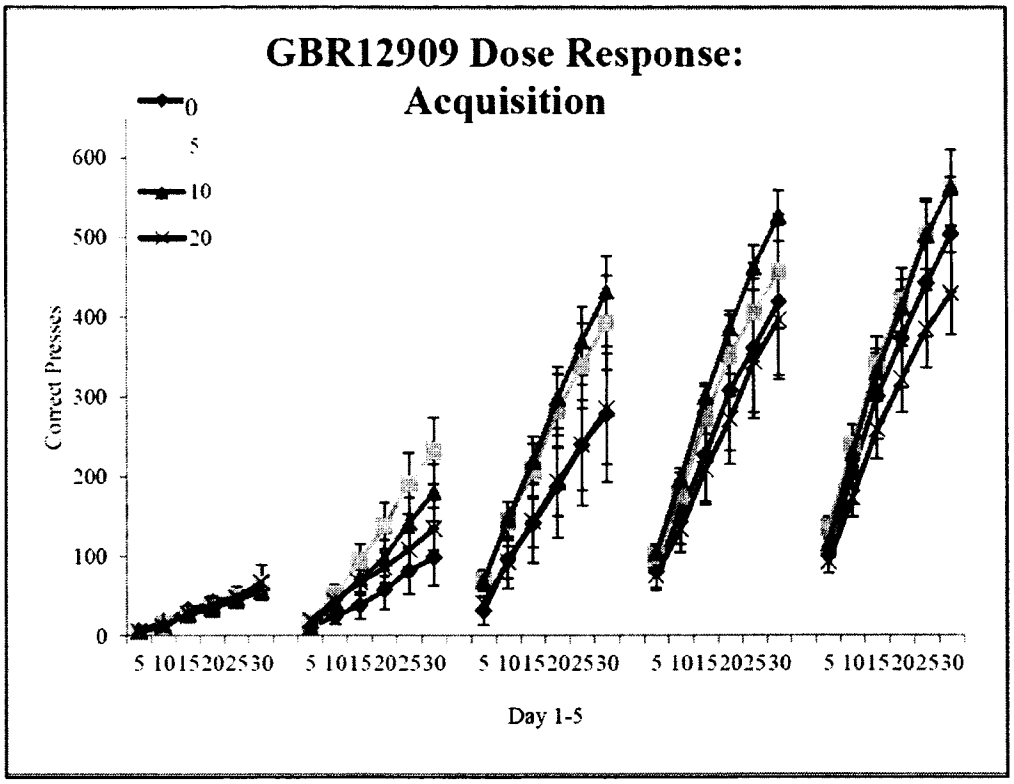


Figure Caption 2: Bar graph depicting average total correct presses during the 30 minute extinction trial. There were no significant differences detected.

Figure Caption 3: GBR-12909 elevates correct lever pressing during extinction.

Average number of correct lever presses per 5-min interval over the 30 minute extinction session.

GBR-12909 (5, 10, 20mg/kg) elevated bar pressing at the end of the session compared to saline + DMSO treated animals, suggesting that an extinction deficit was induced with the highest dose of GBR12909. (*$p<0.05$)

Figure 2: Total Correct Lever

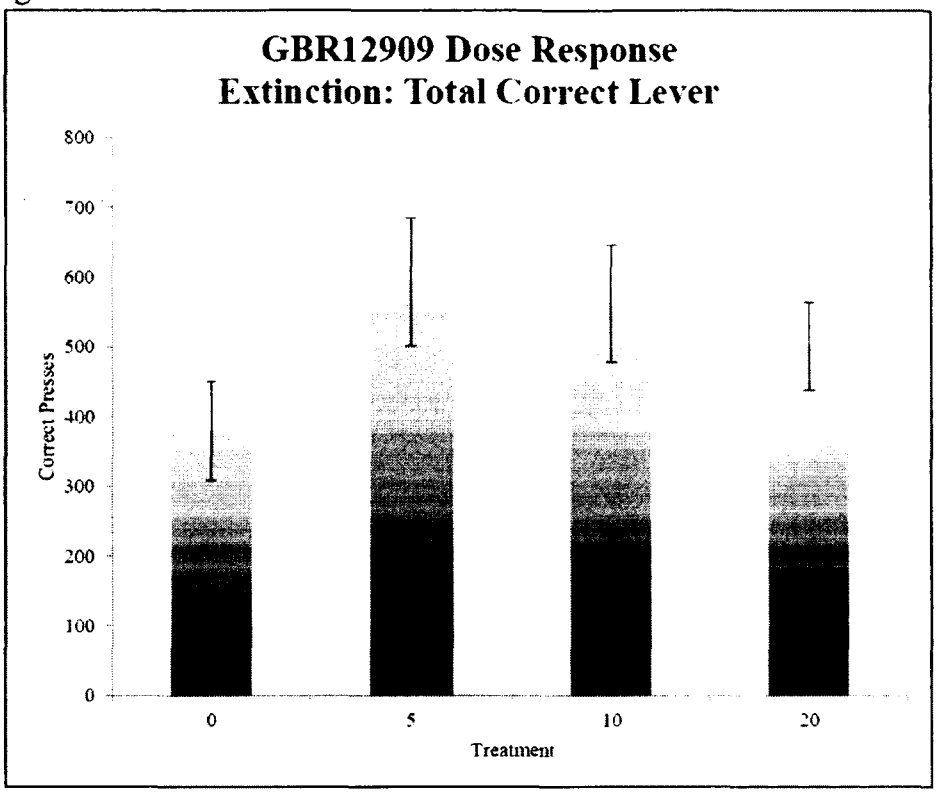


Figure 3: Time Bin Correct Lever

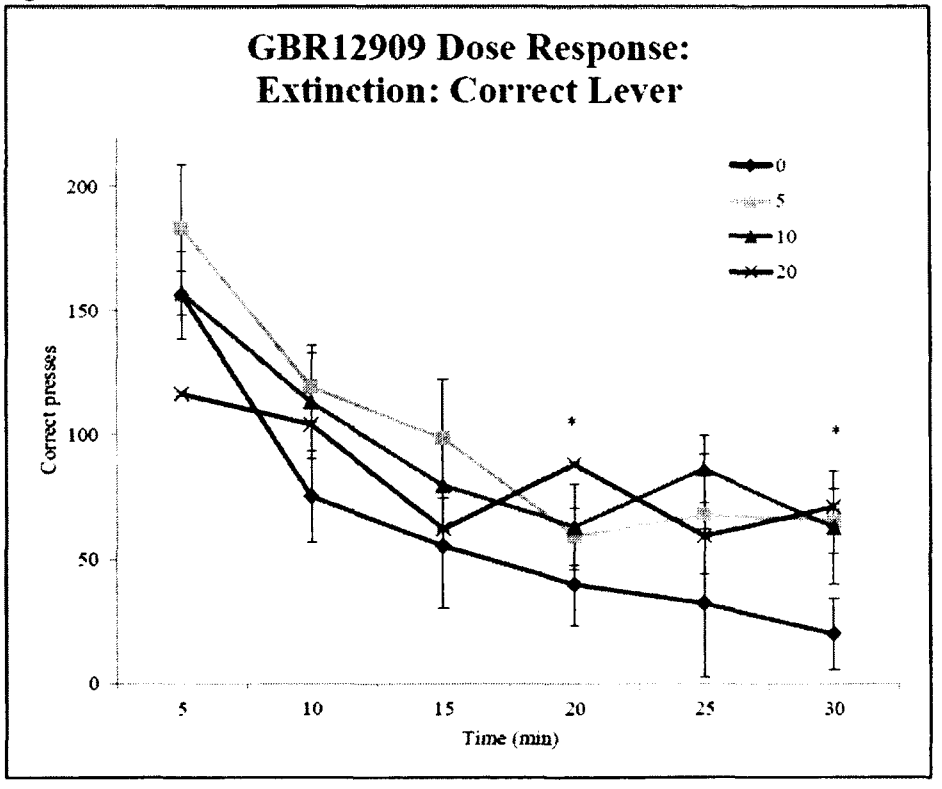


Figure Caption 4: Line graph depicting the average number of incorrect lever presses during the 30 minute extinction session. The 10mg/kg GBR-12909 group pressed significantly more than the 5mg/kg group during the twenty minute time bin of testing and the 20mg/kg group pressed significantly less than saline + DMSO during the twenty minute time bin. There were no other group differences detected. (*$p<0.05$)

Figure Caption 5: Line graph depicting the average number of incorrect lever presses during extinction across all time bins. The 20mg/kg dosed animals exhibited a significant increase in nose poke activity compared to saline + DMSO, 5mg/kg and 10mg/kg treated animals by the end of the first five minutes of testing. These differences did not persist and by the end of the fifteen minute time bin all doses of GBR12909 exhibited similar nose poke activity. At the end of testing the 10 and 20mg/kg groups exhibiting higher nose poke activity than saline + DMSO, which is indicative of increase reward seeking. There were no other differences detected. (*$p<0.05$, **$p<0.01$) Figure 4: Time Bin Incorrect

Extinction Nose Pokes

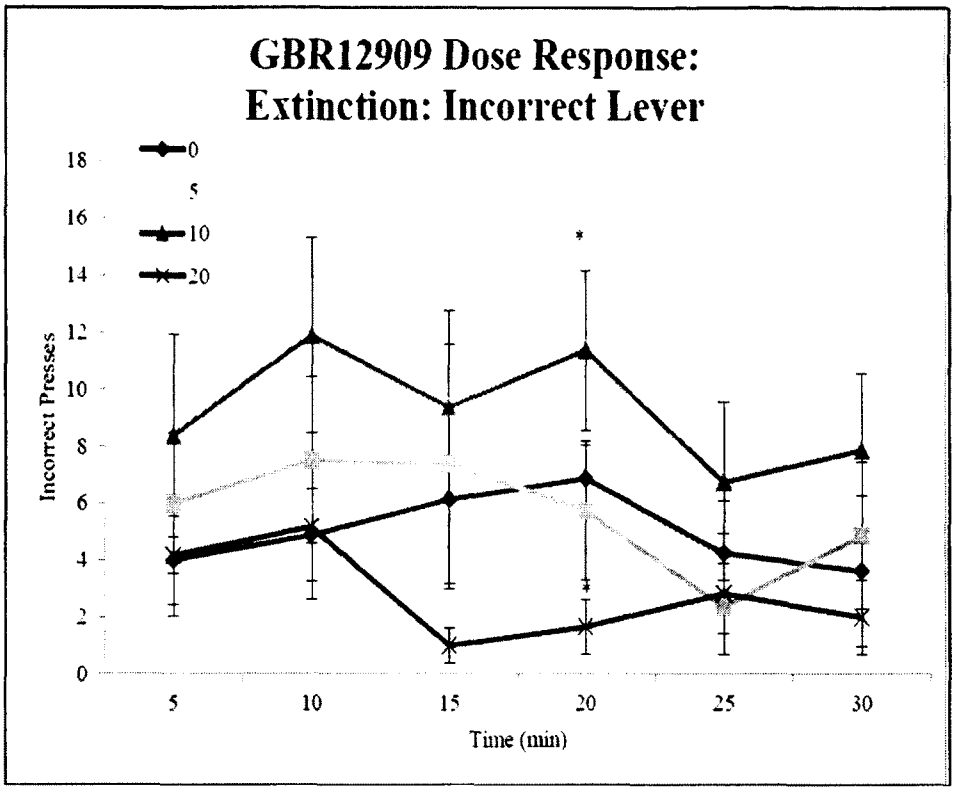
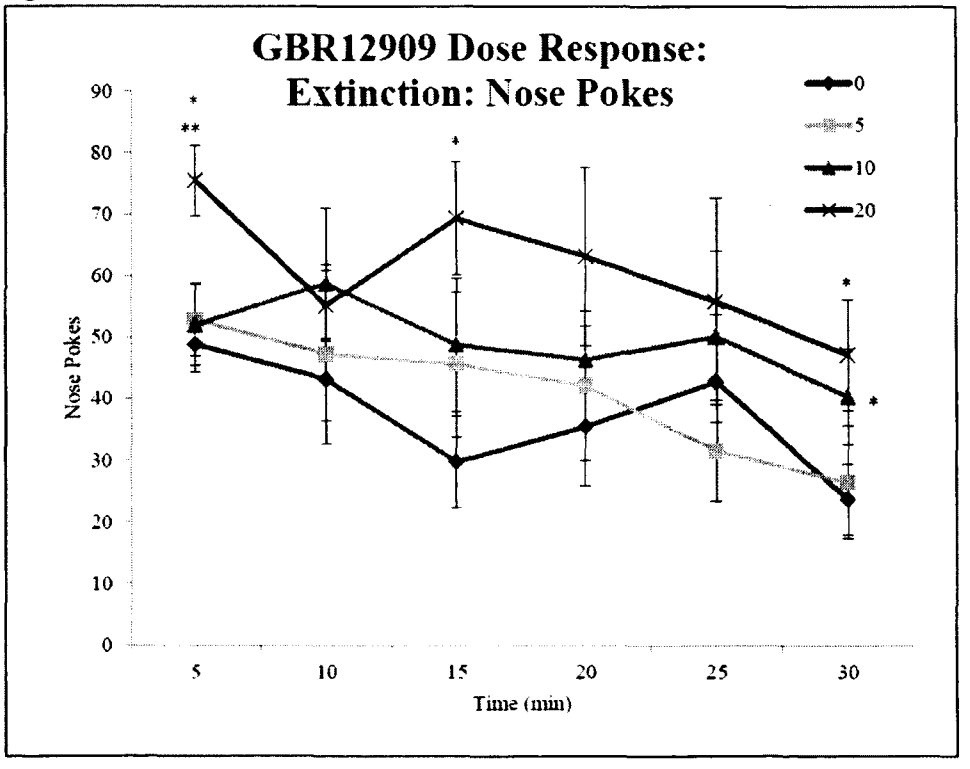


Figure 5: Time Bin



Appendix 3B: GBR12909 Locomotion

Figure Caption 1: Bar graph depicting average total distance moved. There were no significant differences detected.

Figure Caption 2: Line graph depicting vertical and horizontal locomotion during the 30 minute testing session. The 20mg/kg group exhibited significantly higher horizontal locomotion than saline + DMSO during the first 5 minutes of testing. There were no other group differences detected

Figure 1: Total Horizontal Locomotion

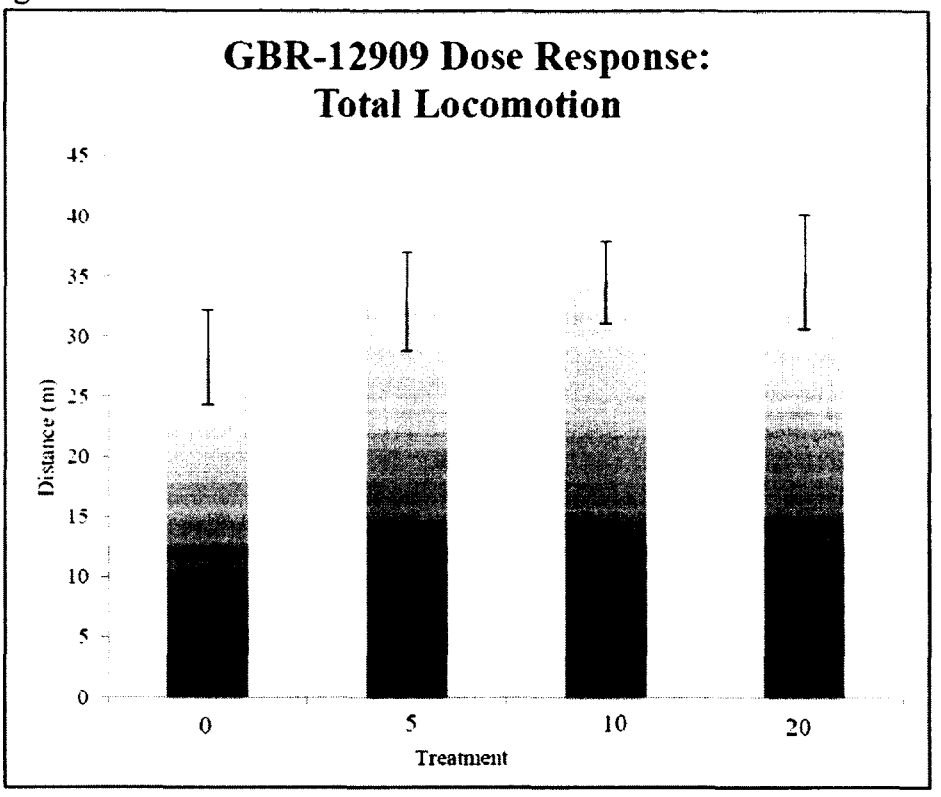


Figure 2: Time Bin Horizontal Locomotion

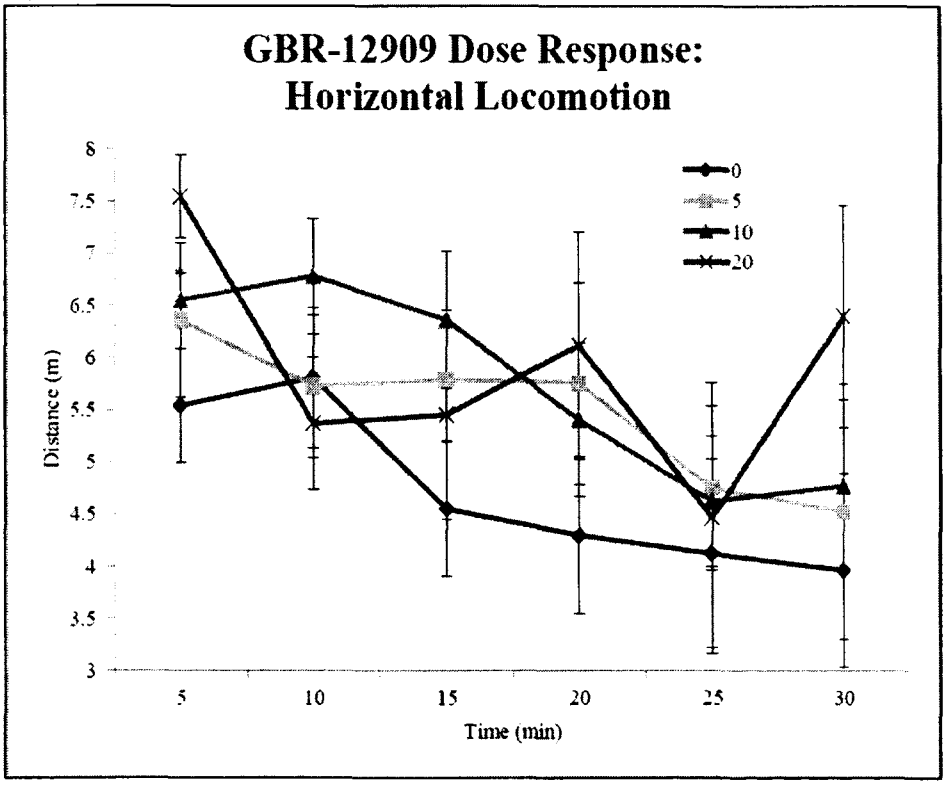


Figure Caption 3: Bar graph depicting the average number of times rearing during the 30 minute testing session. There were no significant differences detected between any of the treatment groups.

Figure Caption 4: The 5mg/kg group reared significantly more than saline + DMSO during the five, fifteen and twenty minute time bins. There were no other group differences detected. (*$p<0.05$)

Figure 3: Total Rearing

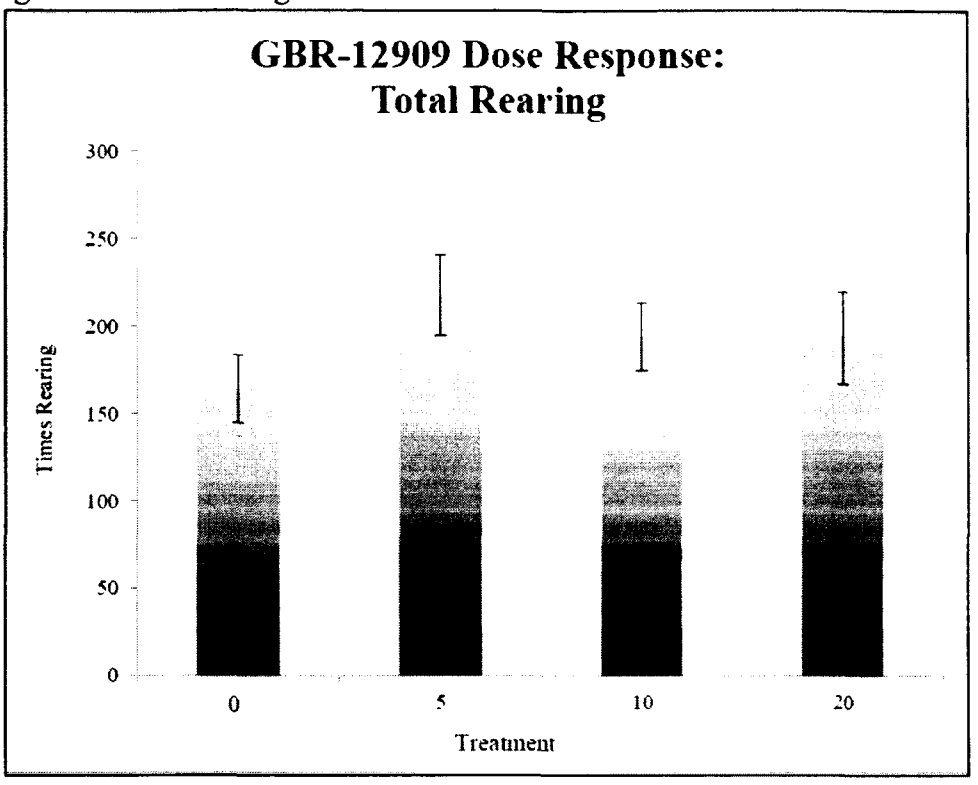
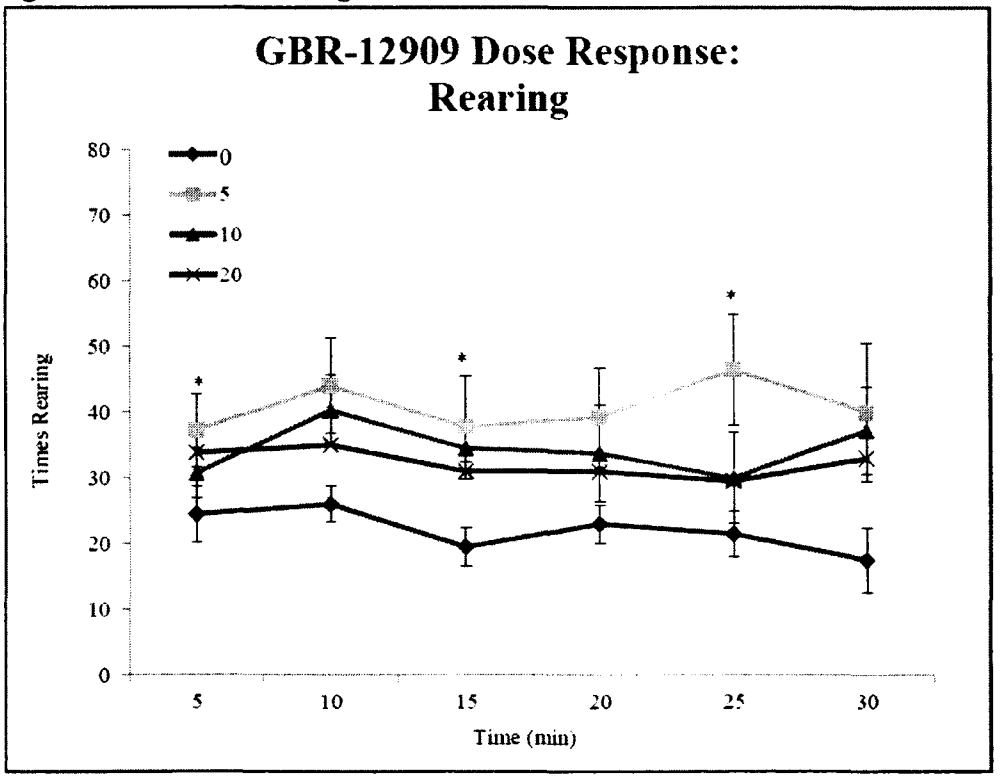


Figure 4: Time Bin Rearing



Appendix 3C: GBR12909 Phosphorylated Extra Cellular Regulated Kinase Activation

Figure Caption 1: Bar graph depicting the average number of phosphorylated extracellular regulated protein kinase (pERK) labeled cells within the Nac. The 20mg/kg treated animals had significantly more pERK labeled cells than all other groups. There were no other group differences detected. (*$p<0.05$, **$p<0.01$)

Figure Caption 2: Bar graph depicting the average number of pERK labeled cells within the core and shell of the Nac. The increase in pERK labeled cells in the 20mg/kg was confined to the core of the Nac, where this group had significantly more labeled cells than saline + DMSO, 5mg/kg and 10mg/kg, whereas there were no significant differences in the shell between any of the groups. With regards to differences in core and shell labeling within groups, the 5mg/kg group exhibited significantly more pERK labeled cells in the core of the Nac. Similarly, the 20mg/kg group exhibited significantly more pERK staining in the core of the Nac. (*$p<0.05$, **$p<0.01$)

Figure 1: Total pERK

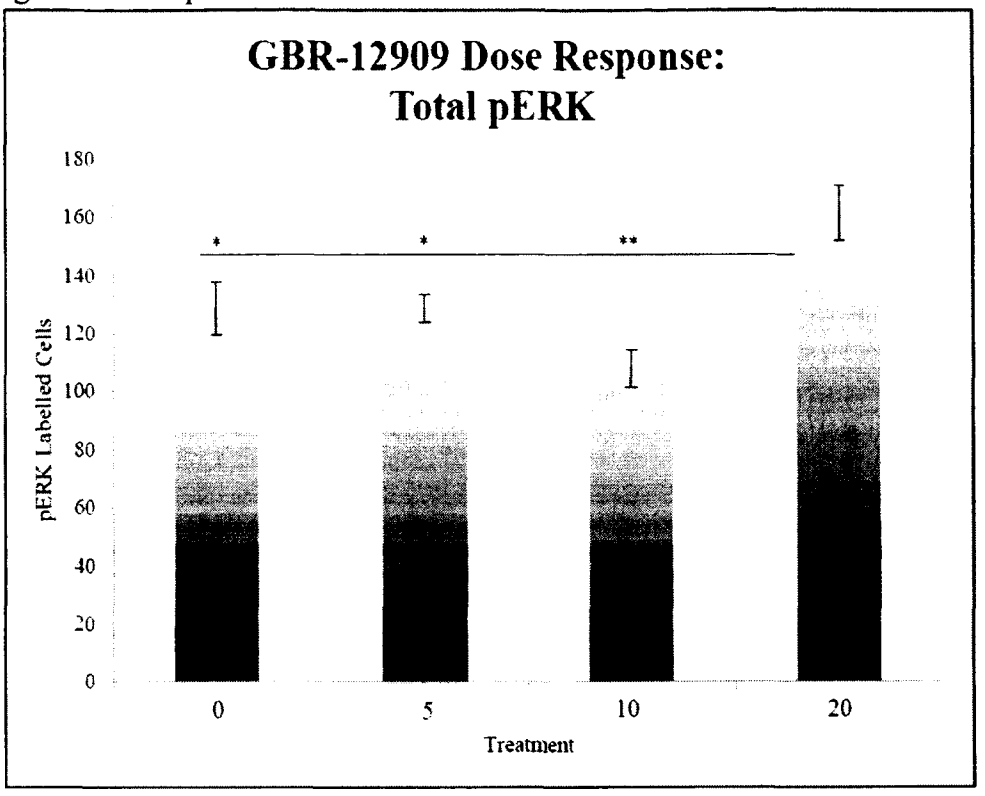
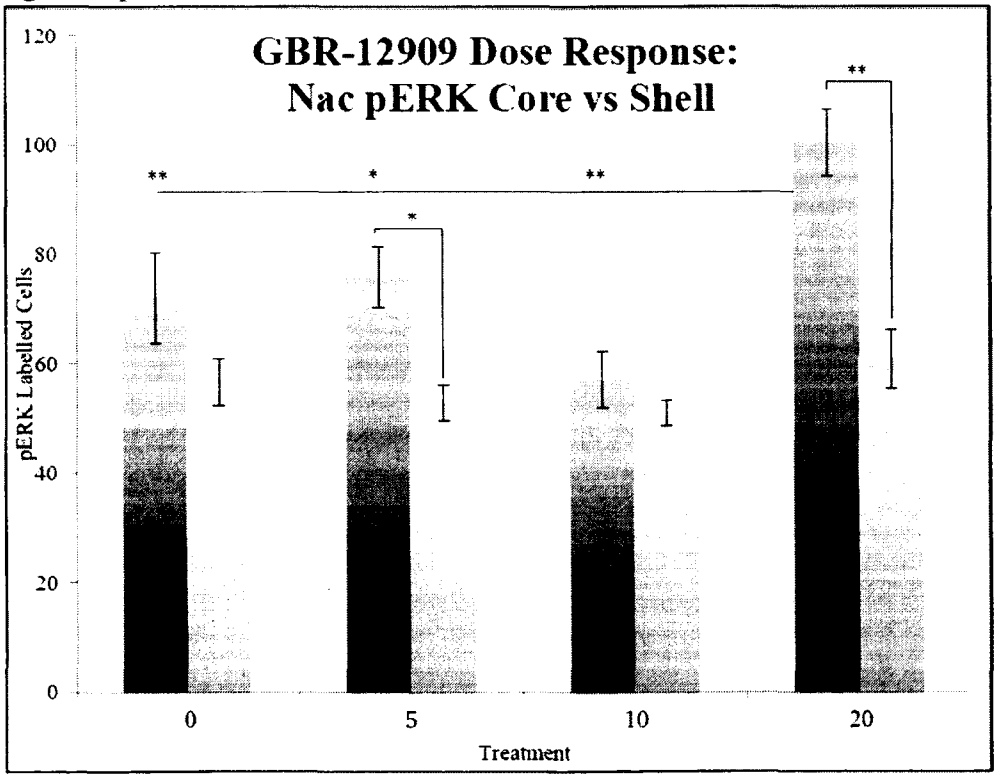
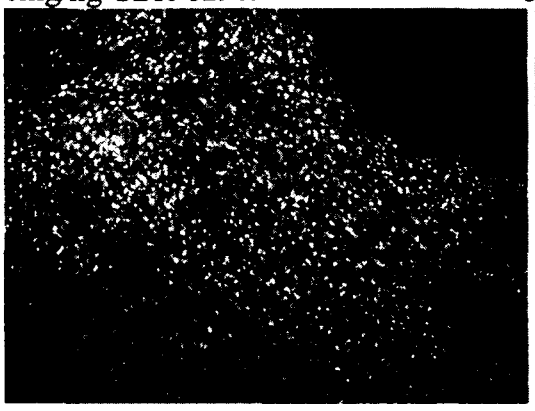


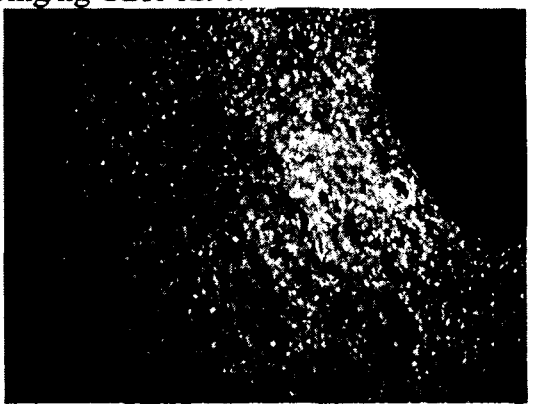
Figure 2: pERK Core vs. Shell



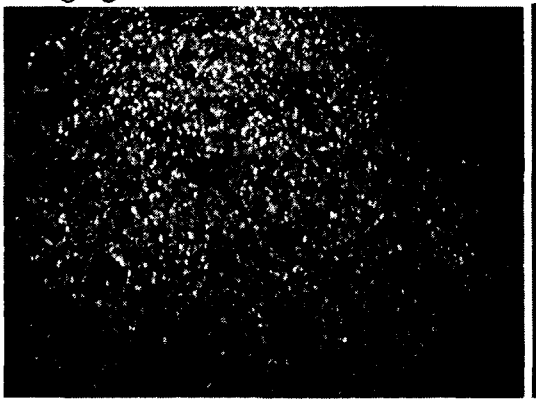
0mg/kg GBR-12909



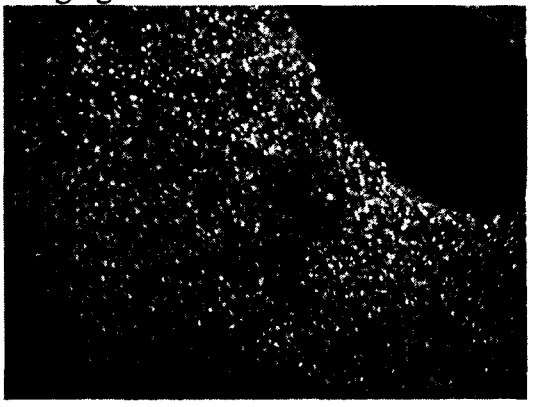
5mg/kg GBR-12909



10mg/kg GBR-12909



20mg/kg GBR-12909



Appendix 4A: Apomorphine dose response on behavioural extinction, locomotion and pERK expression

Figure Caption 1: A line graph depicting the average number of correct lever presses during acquisition days 1-5 for all treatment groups. The groups significantly increased lever pressing across acquisition days but there were no effect of group or interactions detected. This indicates that all groups exhibited statistically similar rates of operant responding during the acquisition phase, prior to drug treatment.

Figure 1: Acquisition

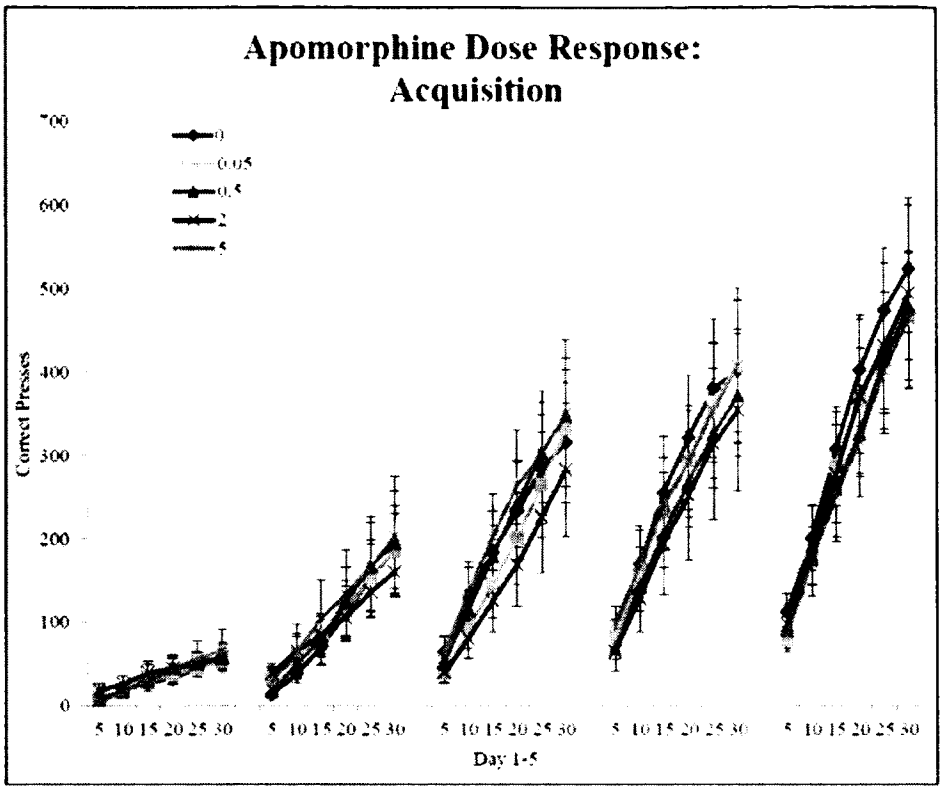


Figure Caption 2: A line graph depicting the average number of correct lever presses during the 30 minute extinction session. Apomorphine dose dependently reduces correct lever pressing during extinction. The 0.5, 2 and 5mg/kg groups pressed significantly lower than saline. The 2 and 5mg/kg groups pressed significantly lower than all other groups, suggesting that these higher doses impair extinction behaviour, either by decreasing reward seeking, or by impairing locomotor ability. (*$p<0.05$, **$p<0.01$, ***$p<0.001$)

Figure Caption 3: Line graph depicting the average number of correct lever presses during the 30 minute extinction session. Apomorphine dose dependently reduces correct lever pressing during extinction. The 2 and 5mg/kg treated animals pressed significantly lower than saline and 0.05APO treated groups. The 0.5mg/kg group began the session with a low rate of pressing that began to increase in the middle of the session and eventually was comparable to saline by the end of testing. (*$p<0.05$, **$p<0.01$, ***$p<0.001$)

Figure 2: Total Correct Lever

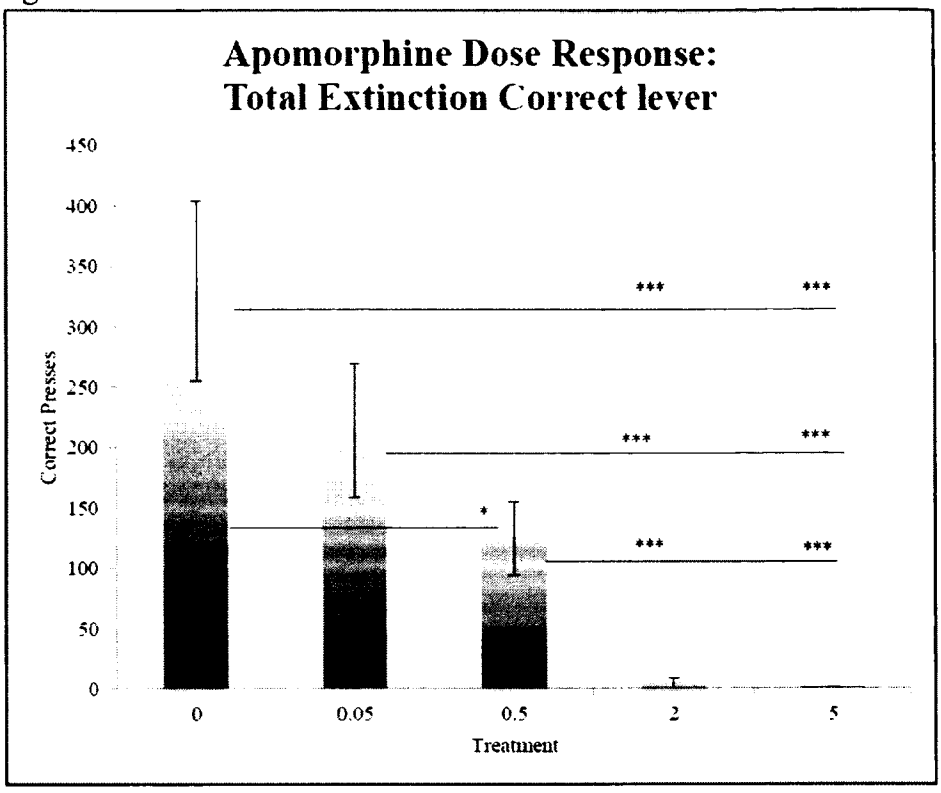


Figure 3: Time Bin Correct Lever

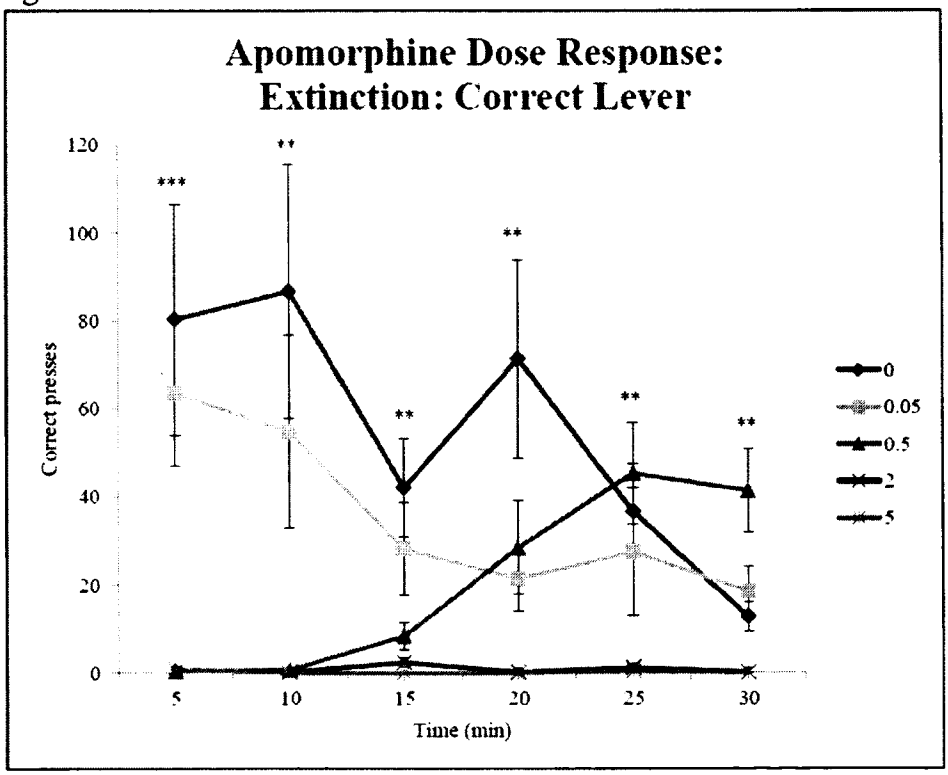


Figure Caption 4: Line graph depicting the average number of incorrect lever presses

during the 30 minute extinction session. The 0.5mg/kg group pressed significantly lower than saline during the five minute time bin. During the last ten minutes of testing only the 2.0 and 5.0mg.kg dosed animals were pressing significantly lower than saline ($p<0.016$; $p<0.021$). There were no other group differences in incorrect lever pressing identified. (*$p<0.05$, **$p<0.01$)

Figure Caption 5: Line graph depicting the average number of nose pokes for each group across all time bins of the 30 minute extinction session. The 0.5, 2.0 and 5.0mg/kg APO groups exhibited significantly less nose poke activity than saline at the beginning of testing. The 0.05APO group also exhibited higher nose poke activity then the 0.5, 2.0 and 5.0 APO dosed animals. For the 2.0 and 5.0 APO groups, nose poke activity was low for the majority of testing. (*$p<0.05$, **$p<0.01$, ***$p<0.001$)

Figure 4: Time Bin Incorrect

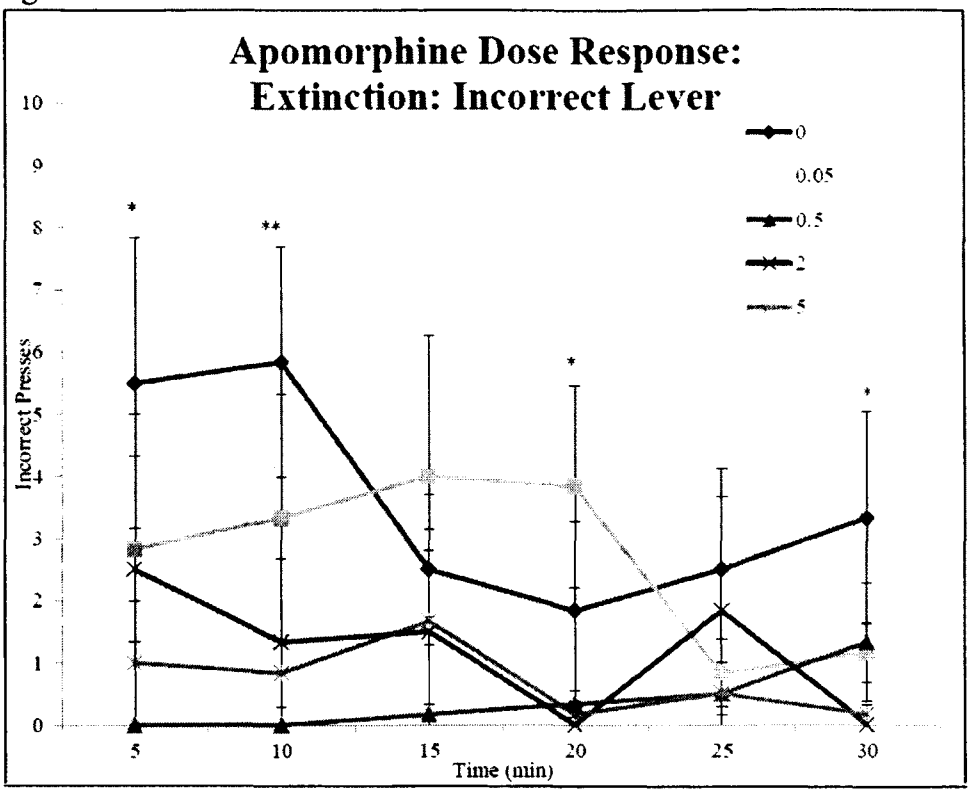
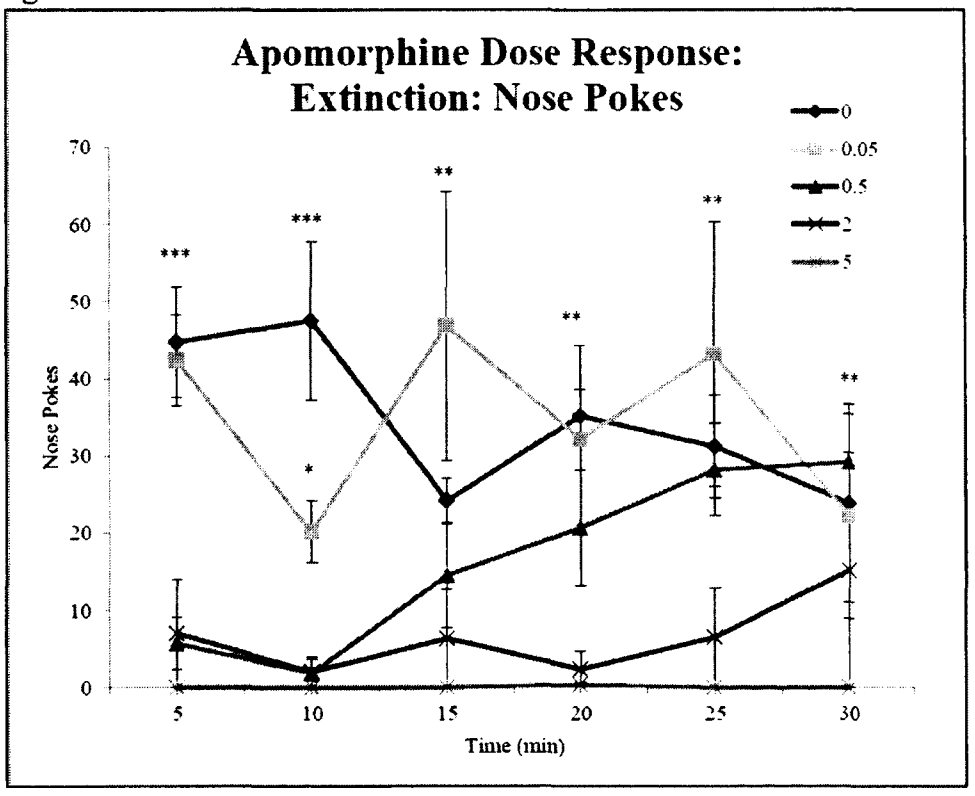


Figure 5: Time Bin Nose Pokes



Appendix 4B: Apomorphine Locomotion

Figure Caption 1: Bar graph depicting average distance moved during the entire 30 minute testing session. APO dose dependently produced hypolocomotion, with the higher doses (2, 5mg/kg) producing the greatest locomotor deficit. 2.0 and 5.0mg/kg APO groups moved significantly less than saline and 0.05 APO. The 5.0 APO group also moved significantly less overall than the 0.5mg/kg APO group, suggesting a dose dependent motor impairment in the high dose groups. (*$p<0.05$, **$p<0.01$)

Figure Caption 2: Line graph depicting horizontal locomotion across time bins of the 30 minute testing session. The animals that received the highest doses of APO (2, 5mg/kg) displayed a marked reduction in locomotor activity throughout testing. The 0.5mg/kg APO group began the session moving significantly lower than saline, but increased across time and became statistically similar to both saline and 0.05mg/kg treated animals toward the end of the session. (*$p<0.05$, **$p<0.01$, ***$p<0.001$)

Figure 1: Total Horizontal Locomotion

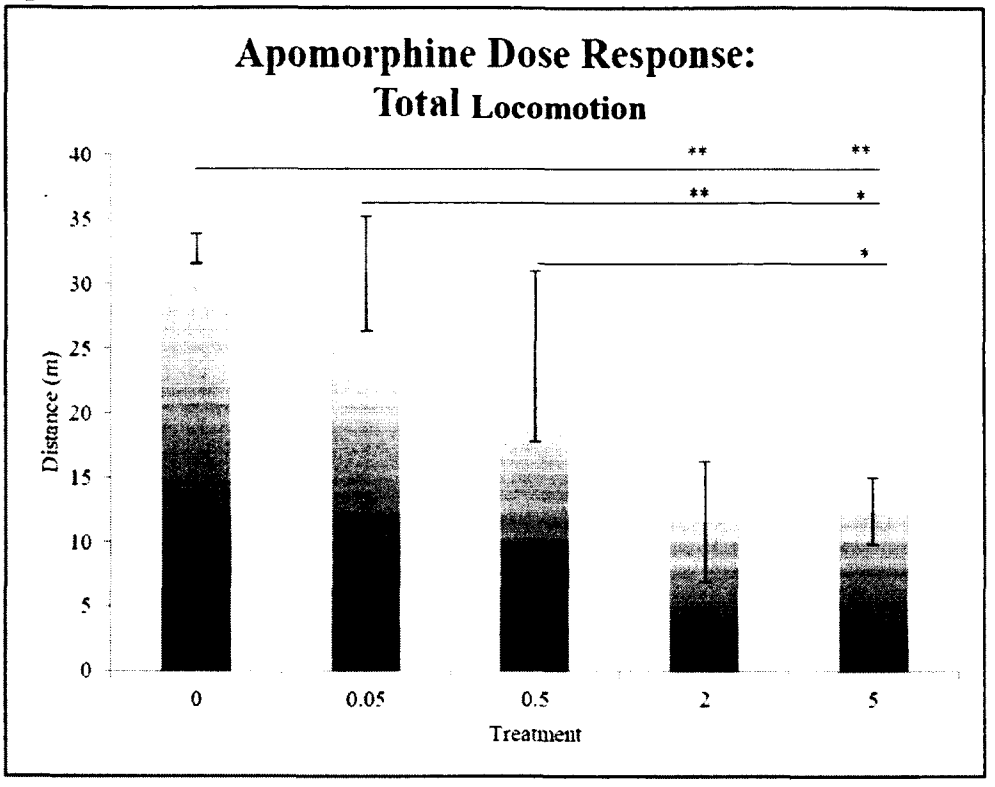


Figure 2: Time Bin Horizontal Locomotion

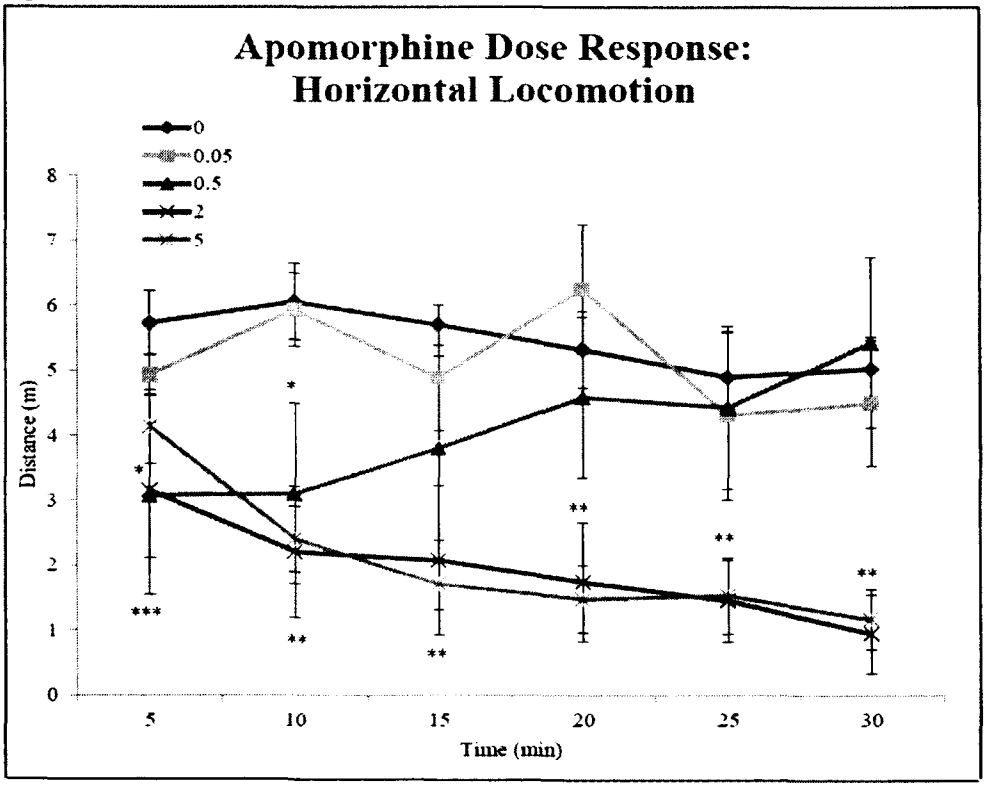


Figure Caption 2: Line graph depicting the total number of times rearing. The results indicated that the 0.5, 2.0 and 5.0 APO groups reared significantly less than saline overall ($p<0.002$; $p<0.001$; $p<0.001$). They were also lower than the 0.05APO group ($p<0.004$; $p<0.001$; $p<0.001$).

Figure Caption 3: Line graph depicting rearing across time bins of the 30 minute testing session. The animals that received the highest doses of APO (2, 5mg/kg) displayed a marked reduction in rearing activity throughout testing. The 0.5 APO group began the session moving significantly lower than saline, but increased across time and reared at a statistically similar rate to saline during the second half of the session. (*$p<0.05$, **$p<0.01$, ***$p<0.001$)

Figure 2: Total Rearing

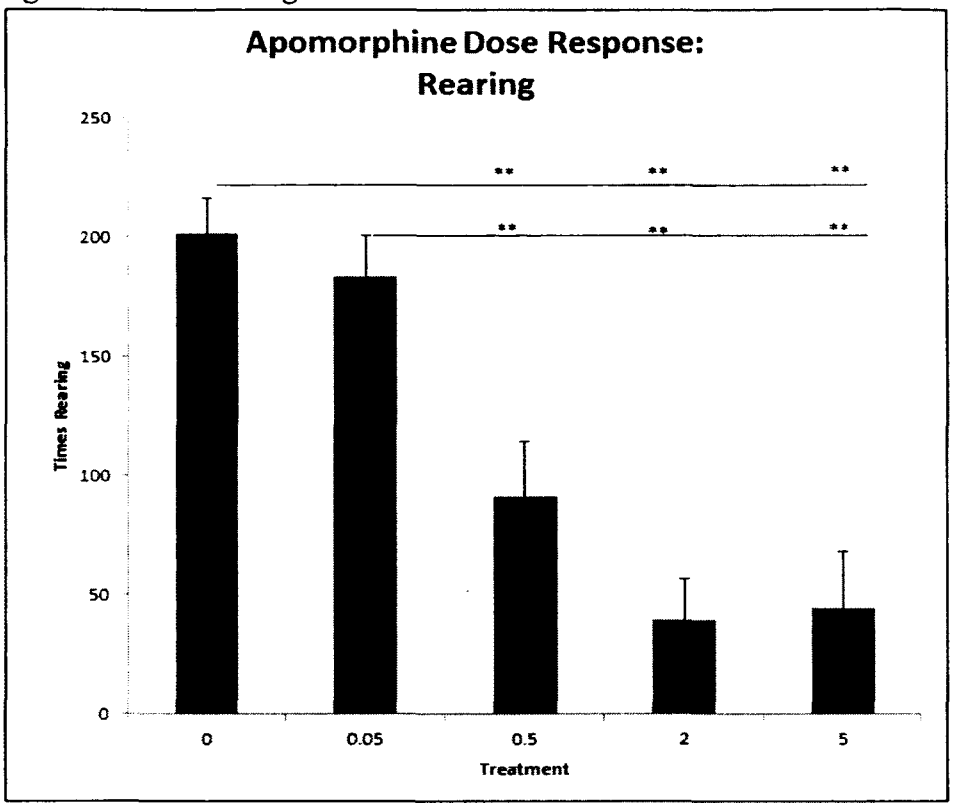
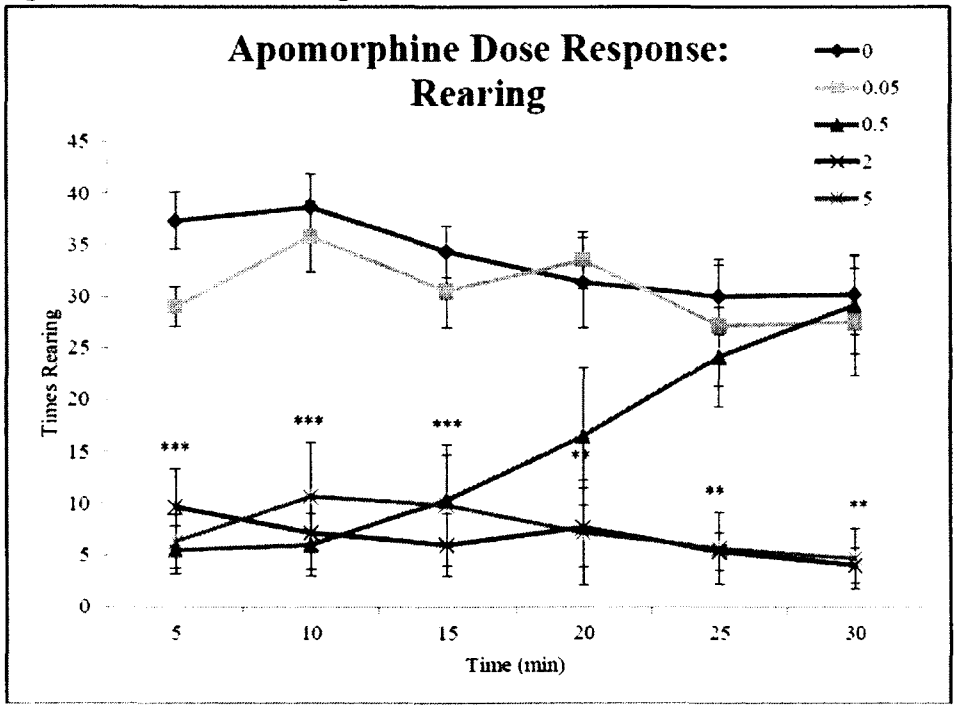


Figure 3: Time Bin Rearing



Appendix 4C: Apomorphine Phosphorylated Extra Cellular Regulated Kinase Activation

Figure Caption 1: Bar graph depicting the average number of phosphorylated extracellular regulated protein kinase (pERK) labeled cells within the Nac. There were no significant differences detected.

Figure Caption 2: Bar graph depicting the average number of pERK labeled cells within the core and shell of the Nac. There were no significant differences detected across groups for either region of the Nac. The saline, 0.05, 2 and 5mg/kg groups exhibited significantly more pERK labeling in the core of the Nac than the shell. (*$p<0.05$, **$p<0.01$)

Figure 1: Total pERK Labeled Cells

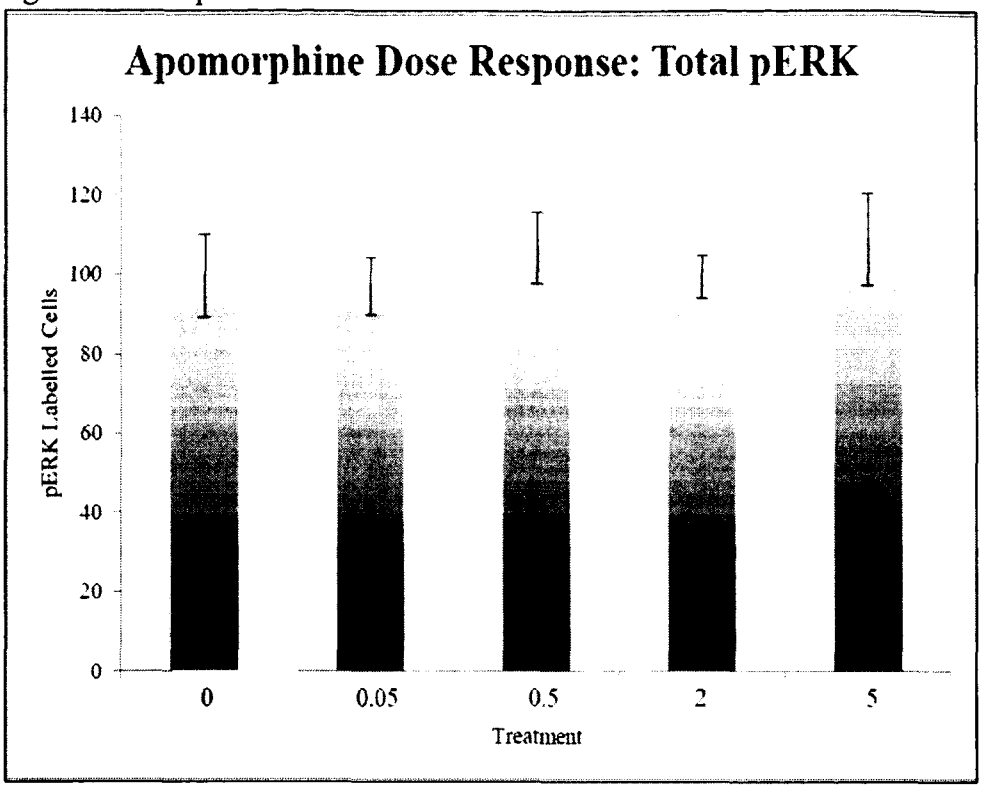
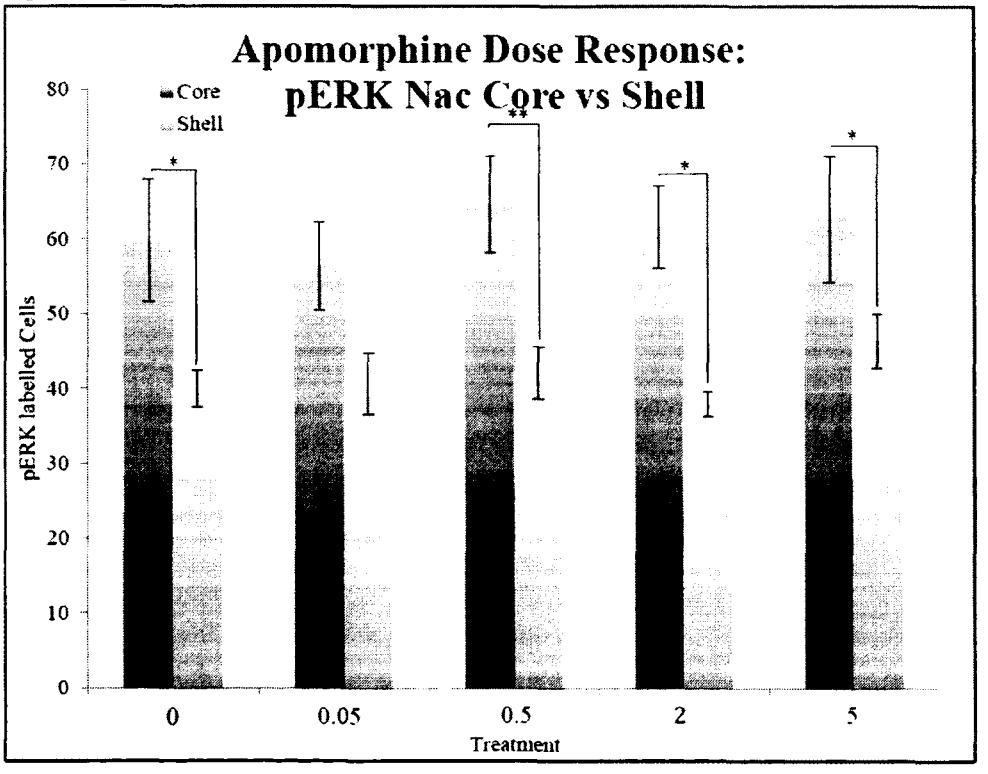
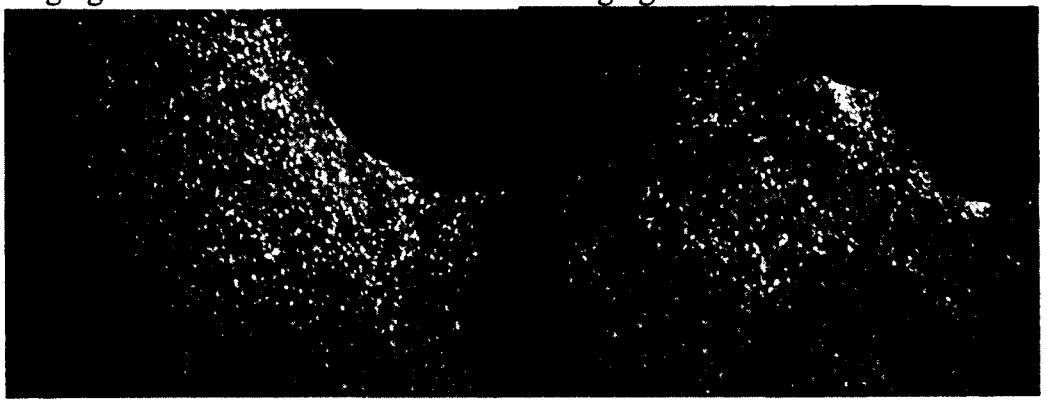


Figure 2: pERK Core vs. Shell

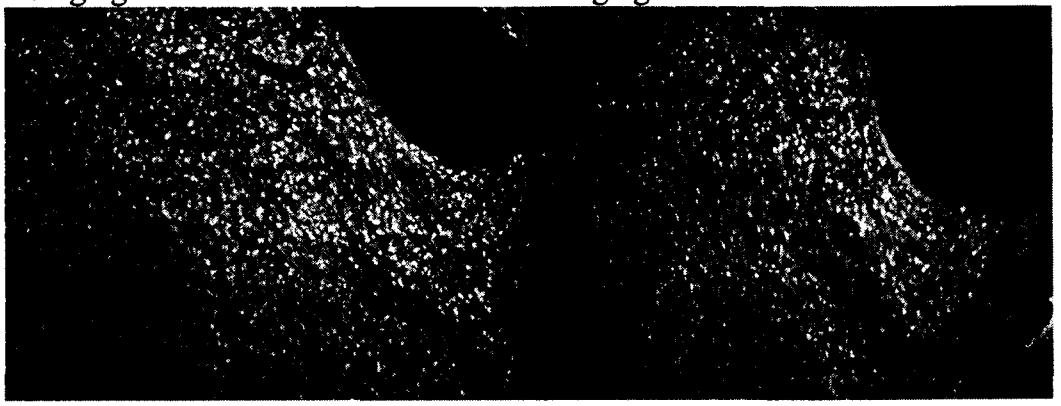


0mg/kg APO



0.05mg/kg APO

0.5mg/kg APO



2.0mg/kg APO

5.0mg/kg APO