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Involvement of Mesolimbic D2 Receptors and Accumbal Dopamine Levels in the
Reinstatement of Cocaine Place Preferences in Developing Rats

by

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

To the HPLC: Thank you for cooperating and working flawlessly for about 95% of my dissertation. A special thanks for falling apart in the last month of running my microdialysis samples. Thank you for high pressure, a clogged stator face assembly, scratched stator, the electrode that just stopped working, the empty case that looked like I had a spare stator in it but didn't, drippy needle port, dirty CSF that clogged my column. Nobody ever said getting your PhD would be easy. So thank you for making sure that statement rang true. With that said, thank you for challenging my troubleshooting skills. I can now truthfully say I have conquered the HPLC because I was able to fix every problem you handed me in a quick and efficient manner. Last but not least, thank you for working flawlessly on my last day of running samples. I know that was your way of saying I made it! Thanks again, I could not have made it without you. I know we have bonded over the past few years. We have shared blood, sweat, many tears, and of course a billion sleepless nights. Please do not be sad because I will not be around anymore. No more late nights, no more long weekends. But I am sure you will be revived as soon as you are back to your old tricks (leaks, high pressure, dirty samples)...because a new naïve person is on the way. Thanks again HPLC!!! Don't be to hard on the new technician.

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Involvement of Mesolimbic D2 Receptors and Accumbal Dopamine Levels in the Reinstatement of Cocaine Place Preferences in Developing Rats

Kimberly A. Badanich

ABSTRACT

Psychostimulant-induced reinstatement of place preferences have been used to investigate underlying physiological mechanisms mediating drug-seeking behavior in adolescent and adult rodents; however, it is still unclear how psychostimulant exposure during adolescence affects neuronal communication in the mesolimbic dopamine (DA) pathway and whether these changes would elicit enhanced drug-seeking behavior later in adulthood. The aim of the present study was to investigate the effects of intra-ventral tegmental area (VTA) or intra-nucleus accumbens septi (NAcc) DA D2 receptor antagonist infusions on cocaine-induced reinstatement of cocaine place conditioning in high and low responders for cocaine reward. Adolescent rats were exposed to cocaine place conditioning [postnatal day (PND 28-39)] and divided into high and low responders for cocaine reward based on their place preference expression score. Place preferences were extinguished and guide cannula were implanted into either the VTA or NAcc followed by one of the following: 1) intra-VTA or intra-NAcc infusion of the DA D2 receptor antagonist sulpiride (100 μ M) during a cocaine-primed reinstatement test (10 mg/kg/ip cocaine) or 2) measurement of NAcc DA levels during intra-VTA or intra-NAcc infusion of sulpiride (100 μ M), a cocaine prime (10 mg/kg cocaine) and re-exposure to the cocaine paired chamber. Infusion of sulpiride into the VTA but not the NAcc blocked

reinstatement of cocaine place conditioning in rats exposed to cocaine during adolescence. Furthermore, re-exposure to cocaine-associated cues and simultaneous local infusion of sulpiride into either the VTA or NAcc attenuated cocaine-induced increases in accumbal DA levels for rats pretreated with cocaine during adolescence, regardless of phenotype. These data suggest intrinsic compensatory mechanisms in the mesolimbic DA pathway mediate adolescent behavioral responsivity to cocaine prime-induced reinstatement of cocaine place conditioning later on in adulthood.

Chapter One: Literature Review

For decades, scientists have searched for a biological mediator(s) of drug addiction. In the quest to identify the mediator(s) of drug dependency, several theories have been proposed. One of the earliest theories of addiction focused on the rewarding effects of drugs (Crow, 1970; Rolls et al., 1974; Fibiger and Phillips, 1974). According to the reward theory of addiction, enhanced mesolimbic dopamine (DA) activity mediates pleasure and reward. Findings supporting the reward theory of addiction revealed rats will voluntarily electrically stimulate the medial forebrain bundle (Olds and Milner, 1954) and will further change their response rates for intracranial self-stimulation (ICSS) with the administration of dopaminergic agonists/antagonists suggesting DA is important in reward (Gilliss et al 2002). However, the reward theory of addiction has weaknesses in that it only provides an explanation for the early stages of drug use and poorly addresses the occurrence of chronic drug use. With certain drugs, like cocaine, initial use produces pleasure while later abuse has a diminished rewarding effect. Additionally, addicts repeatedly take drugs and use higher drug doses to achieve the same subjective state as in the first use. Therefore, reward could not be the sole factor driving drug addiction.

An alternative theory, the anhedonia or opponent process theory of addiction (Solomon and Corbit, 1973) provides a better explanation of chronic drug use. This theory suggests addicts chronically use drugs to avoid the negative affect associated with drug use. Although drug use enhances dopaminergic activity of the mesolimbic system, over time drug use induces changes in synaptic transmission causing either

sensitization (e.g. cocaine, amphetamine) or habituation (e.g. alcohol) of the system depending on the type of drug administered. It is suggested by the anhedonia hypothesis that addicts use drugs to avoid these physiological and psychological effects, providing a better explanation for chronic drug use than the reward hypothesis. A limitation of the anhedonia hypothesis is it cannot address the early stages of drug use (Salamone et al, 1997).

The incompleteness of the reward and anhedonia hypotheses led to the development of current theories of drug use. Robinson and Berridge (1993) proposed the incentive salience theory of addiction. Incentive salience is described as “a psychological process that transforms the perception of stimuli, imbuing them with salience, making them attractive, 'wanted', incentive stimuli.” Importantly, Robinson and Berridge stated “the mesolimbic system’s function is to attribute 'incentive salience' to the perception and mental representation of events associated with activation of the system.” Moreover, the function of mesolimbic DA is not simply reward or aversion, but also includes perception of salient stimuli. The incentive salience theory can be applied to many motivationally arousing stimuli. A considerable amount of evidence indicates a much broader range of stimuli affects the activity of the mesolimbic DA pathway. In general, any motivationally significant stimulus, whether it is positive/hedonic or negative/anhedonic, is capable of activating the mesolimbic system (Blackburn et al., 1992). Specifically, increases in mesolimbic DA in the nucleus accumbens (NAcc) of a rodent, as measured by *in vivo* microdialysis, have been reported in response to many motivationally significant stimuli such as food (Martel and Fantino, 1996), sexual activity (Meisel et al., 1993), novelty (Rebec et al., 1997), aversive stimuli such as shock (Morrow et al., 1995) as well as drugs of abuse (Moghaddam and Bunney, 1989; Koob

and Weiss, 1992). Thus, drugs of abuse are only one type of stimuli altering activity of the mesolimbic system.

In the associative learning theory of drug addiction, Di Chiara (1999) proposed drug addiction is manifested by a generalized facilitation of associative learning. In this theory, behavioral control is lost and drug-related cues acquire a motivationally relevant valence that reliably induces drug-seeking behavior. Both the incentive salience and associative learning theories of drug addiction imply environmental and drug-related cues play an integral role in repeated drug use and drug-seeking behaviors; however, these two theories primarily explain the rewarding/hedonic properties associated with drug use and largely ignore the aversive characteristic of addiction.

In the most recent theory of drug addiction, possible mechanisms underlying chronic drug use and the negative effects of withdrawal were attributed to the allostasis theory of addiction (Koob and Le Moal, 2001). The allostasis model of addiction states homeostatic processes during chronic drug use are dysregulated and fail to return to a normal range. This allostatic state drives subsequent drug use and in turn causes a downward spiral of dysregulation. In response to the proposal of these newer theories of addiction, much research has focused on the ability of salient stimuli and homeostatic processes in the mesolimbic DA system to promote compulsive drug use.

Structure and Function of the Mesolimbic DA Pathway

The mesolimbic DA pathway is composed of a group of DA neurons originating in the VTA and project to several brain structures including the NAcc, amygdala, hippocampus, septum, olfactory bulb and the prefrontal cortex (Dahlstrom and Fuxe, 1964). The majority of mesolimbic DA cells are spontaneously active, driven by an

internal pacemaker like stimulus and continuously exhibit background or tonic firing (Chiodo et al, 1984; Grace, 1995). *In vivo* electrophysiological characterization of DA cells showed these cells exhibit a slow depolarizing Na⁺ current followed by a “notch” or break in the depolarizing current, and a prominent afterhyperpolarization (Grace, 1990). The break in depolarizing current is elicited by the difference in time it takes for a depolarizing current to spread between the dendrites and soma (i.e. initial spike vs somatodendritic spike; Grace, 1990). When cell firing is stimulus-induced, cells quickly switch to a burst-firing pattern where action potentials are elicited in groups of 2-3 spikes and several spike groups are included in each burst (Grace, 1995). The inter-spike interval increases and amplitude of each spike group decreases across time and within one burst. Firing rates of mesolimbic DA cells are highly influenced by the activity of DA autoreceptors (Grace, 1995). Mesolimbic DA cell bodies in the VTA expressed D2 autoreceptors that were typically stimulated by somatodendritically released DA. Autoreceptor stimulation induced negative feedback mechanisms such as blockade of DA synthesis and inhibition of mesolimbic DA firing. However, when D2 antagonists were administered locally into the VTA, D2 autoreceptors were blocked, negative feedback mechanisms were inhibited and mesolimbic DA cell firing was disinhibited leading to increased DA cell firing and increased tonic DA release (Kohl et al., 1998). Drugs of abuse have been shown to alter the firing of DA cells by decreasing tonic and increasing bursting or phasic cell firing (Grace, 1995).

The mesolimbic DA pathway's primary terminal region, the NAcc, has been a region of interest for many drug abuse experiments. The majority of NAcc cells are gamma-amino-butyric acid (GABA) containing, quiescent and spontaneously inactive (~90%; O'Donnell and Grace, 1993a; Carelli et al, 1993, Peoples and West, 1996). Those NAcc cells exhibiting spontaneous firing are characterized by specific basal

membrane properties and show a biphasic resting membrane potential. NAcc cells exhibit both a hyperpolarized (~ -90 mV) DOWN state that is mediated by an inward rectifying K^+ current and a depolarized (~ -57 mV) UP state (O'Donnell and Grace, 1995). Activation of hippocampal glutamatergic inputs to NAcc cells induces the UP state and allows cells to fire action potentials. When NAcc cells are in the DOWN state they are unable to fire action potentials thereby showing the hippocampus acts as a "setter" and places NAcc cells into the appropriate manner to receive incoming information from other limbic and cortical inputs (prefrontal cortex, amygdala, ventral tegmental area; O'Donnell and Grace, 1995; Floresco et al, 2001b).

An important issue in studies involving the mesolimbic DA pathway has been the functional and structural heterogeneity of the NAcc. The NAcc is anatomically comprised of two compartments: core and shell. The core encompasses the lateral and posterior portions of the NAcc while the shell encompasses the anteromedial NAcc (Paxinos and Watson, 1983). A common mechanism for defining the core and shell is to identify each regions projection sites. The core has projections to the dorsolateral ventral pallidal subterritory, an area similar in anatomy to the striatopallidal subregion of the caudate putamen while the shell, typically considered the extended amygdala, has projections to the ventromedial portion of the ventral pallidum (Zahm and Heimer, 1990). Although the core and shell subregions both contain GABAergic cells, the structure of these cells is not completely identical. GABAergic cells in the core are densely spinous and primarily contain medium sized soma with three to six dendrites emanating from each soma pole (O'Donnell and Grace, 1993a; O'Donnell and Grace, 1993b). Cells within the shell have similar morphological properties however these cells are less spinous (O'Donnell and Grace, 1993a; O'Donnell and Grace, 1993b). Furthermore, DA terminals synapse onto different sites on NAcc cells depending on whether DA cells are

terminating in the core or shell. DA cells terminating in the core synapse onto GABAergic spines whereas DA cells terminating in the shell synapse onto GABAergic dendritic shafts (O'Donnell and Grace, 1993b).

The functionality of the NAcc in general has been identified as a limbic motor interface due to the fact the NAcc processes information about the probability and magnitude of an expected reward in addition to initiating movement to obtain a reward (Cooper, 2002; Di Ciano and Everitt, 2005; Floresco et al, 2001a). The ability of the NAcc to process motivation and movement information, two very different processes, illustrates the dichotomy of the NAcc. Findings have suggested the shell processes primarily information regarding motivation while the core processes primarily information regarding movement towards a salient stimulus (Ghitza et al, 2003; Di Ciano and Everitt, 2005). Increases in extracellular DA levels in the shell of the NAcc have been found in response to motivationally significant stimuli such as drugs of abuse (Pontieri et al., 1995), food (Tanda and Di Chiara, 1998), aversive stimuli (Kalivas and Duffy, 1995), and novel stimuli (Rebec et al., 1997). Motivation in these studies has been defined by increases in the number of lever presses for a reinforcing stimulus, increases in the amount of time spent in the environment paired with a reinforcing stimulus [i.e., conditioned place preference (CPP)], and increases in the amount of stimulus intake (i.e., greater food intake, greater water intake, greater drug self-administration). However, recent research comparing the functionality of the core and shell suggests the shell mediates some forms of movement such as approach behavior while the core mediates behavior in response to drug-associated cues in a second-order conditioning paradigm (Everitt and Wolf, 2002; Di Ciano and Everitt, 2004). These more recent findings suggest the functionality of the core and shell may not be as “clear cut” as

previous research has indicated. More research is needed to further identify the functional properties of the core and shell subregions of the NAcc.

Expectancy Effects and the Mesolimbic DA Pathway

Mesolimbic cell firing has been suggested to play a role in associative learning processes and to act as a “teaching signal” for unexpected or surprising events. Specifically, DA firing patterns are altered in response to reward related cues (Schultz et al, 1993; Schultz et al, 1997; Montague et al, 1996). *In vivo* electrophysiological examination of mesolimbic DA cell firing in monkeys has shown a primary reward, such as juice or a banana, increases DA cell firing. However, if the primary reward is repeatedly paired with a cue immediately prior to reward availability, DA cells begin to fire in response to the cue and no longer fire in response to the primary reward suggesting the reward-related cue acquired the rewarding properties of the primary reward and induced DA cell firing (Schultz et al, 1997). These data can be extended to suggest subjects expected or predicted the primary reward when exposed to the reward-related cue.

DA also plays a role in expectancy-induced behaviors (Schultz, 2006) where subjects have a specific representation of how a specific stimulus should be presented based on the subject’s previous exposures with the stimulus (Goldman et al, 2002). DA cells have been suggested to code for expectancy due to the fact DA cell firing changes in response to altered magnitudes of primary reward and also for timing changes in the onset of reward related cues (Schultz et al, 1993; Schultz et al, 1997). In these experiments, rewards that were greater than expected induced an increase in DA cell firing whereas rewards that were less than expected induced a decrease in DA cell

firing. Rewards that were the same magnitude as expected did not alter DA cell firing implying DA cells fire in response to unpredictable or unexpected stimuli thereby coding for error of discrepancies from the norm (Montague et al, 1996). Further, if a reward-related cue is presented at a different time, DA cells initially fire at the time when the cue was supposed to be presented. If subsequent trials continue to present the reward-related cue at the new time, DA cells update the timing of firing and switch to firing at the new cue presentation time (Schultz, 2006).

These data nicely support learning theories/concepts. For example, DA cell firing was induced when primary reward magnitude differed from what was expected and supports the Rescorla-Wagner model of associative learning. In this model, learning occurs when an event or stimulus is surprising, novel or unexpected, whereas no further learning occurs when the event or stimulus is familiar/expected. Given DA cells fire in response to unexpected or surprising stimuli, it can be suggested DA cells act as a “teaching signal” to induce neuroadaptations and synaptic plasticity necessary for appropriately adapting behavior (Schultz, 2006). DA cells may also act to induce occasion setters. O’Donnell and colleagues have reported in a series of experiments the hippocampus gates information via the mesolimbic DA system (O’Donnell and Grace, 1995; Floresco et al, 2001b). Activation of this gating system is induced by presentation of contextual environmental cues and causes an excitation of mesolimbic DA and NAcc GABAergic cells. Therefore, DA cells may be activated when in the presence of an occasion setter to modulate learning of novel stimuli and events in a particular contextual setting. Importantly, DA has also been suggested to act as a filtering mechanism by strengthening strong synaptic connections and dampening weak synaptic connections in the NAcc. These two neuroadaptations together cause a decrease in background firing and an increase in the signal-to-noise ratio expressed in the NAcc thereby potentiating

information processing of behaviorally relevant stimuli and suppressing extraneous or irrelevant information (Peoples and Cavanaugh, 2003). Taken together these data suggest mesolimbic DA cell firing mediates several aspects of associative learning.

Cell firing in response to “expectancy” and drug related cues have also been investigated in NAcc medium spiny GABAergic cells. Repeated cocaine self-administration sessions have shown NAcc cells develop specific firing patterns in response to cocaine-associated cues (Carelli and Deadwyler, 1994, 1996a, 1996b, 1997 Carelli and Ijames, 2000). In particular, cocaine cues elicit an increase (reinforcement-excitation; type RFe), decrease (reinforcement-inhibition; type RFi), or a biphasic (preresponse + reinforcement type; PR+RF) response pattern of cell firing in the NAcc. Interestingly, all three of these putative cocaine associated cue-induced cell firing patterns were abolished (RFe or PR+RF) or attenuated (RFi) during cue extinction procedures, yet returned during cue re-exposure and cue-induced reinstatement phases (Carelli and Ijames, 2000). These data suggest cue-induced cocaine seeking is mediated by alterations in NAcc cell firing patterns.

Drugs of Abuse and the Mesolimbic DA Pathway

Drugs of abuse have several similarities in common with the salient stimuli discussed above. Rats exhibit place conditioning in response to ethanol (Risinger et al., 2001) heroin (Hand et al., 1989), morphine (Higgins et al., 1992) amphetamine (Lett, 1989; Meyer et al., 2002), methylphenidate (Meririnne et al., 2001) and cocaine (Shippenberg and Heidbreder, 1995; Horan et al., 2000). Rats will also self-administer drugs of abuse such as heroin (Higgins et al., 1994) cocaine (Pudiak and Bozarth, 2002) and ethanol (Tomkins et al., 2002). Drug use is mediated by the mesolimbic DA

pathway and specifically the NAcc. Lesioning DA neurons in the mesolimbic DA pathway with 6-hydroxydopamine (6-OHDA) attenuates drug-induced behavior. For example, rats trained to self-administer nicotine were found to no longer lever press for the drug after infusion of 6-OHDA into the NAcc (Corrigall et al., 1992). Lesioning mesolimbic DA neurons resulted in attenuation of heroin self-administration behavior (Gerrits and Van Ree, 1996). Drug-induced behavior can also be affected by administration of DA agonists/antagonists. Place preference studies reported CPP after administration of SKF38393, a DA D1 agonist (White et al., 1991). However, administration of haloperidol, a DA antagonist significantly decreased CPP scores relative to control rats (Adams et al., 2001). Drugs (amphetamine, alcohol, cocaine) also increase DA in the NAcc as measured by *in vivo* microdialysis (Moghaddam et al., 1989).

Cocaine and the Mesolimbic Dopamine Pathway

Cocaine, like other drugs of abuse, acts on the mesolimbic DA pathway and specifically the NAcc to produce its associated rewarding properties. Cocaine disrupted normal DA transmission by altering DA deactivating processes such as DA reuptake (Cooper et al., 2003; Pontieri et al., 1995; Hitri et al., 1994). In normal functioning DA reuptake, excess extracellular DA in the NAcc binds to the DA transporter (DAT) to be transported back into the presynaptic terminal (Hitri et al., 1994). However, cocaine binds to the DAT in place of DA causing the transporter to be ineffective. Thus, excess extracellular DA was unable to bind to DAT's, reuptake was inhibited and excess DA remained in the extracellular fluid of the NAcc. Inhibition of DA reuptake enhanced accumbal DA and produced the rewarding effects associated with cocaine

administration. Cocaine elevated accumbal DA as measured by *in vivo* microdialysis to approximately 300% of baseline (Chefer and Shippenberg, 2002; Thompson et al., 2000; Camp et al., 1994; Maisonneuve and Kreek, 1994; Parsons and Justice, 1993; Kalivas and Duffy, 1993; Weiss et al., 1992; Hurd et al., 1989). Doses of cocaine ranged from 2-20 mg/kg depending on the method of administration. Hence, the mesolimbic DA pathway is the primary pathway involved in cocaine's rewarding properties.

Cocaine has been shown to alter the firing pattern of GABAergic NAcc cells. Initial reports of the effects of cocaine on NAcc cell firing reported DA application or cocaine exposure decreased NAcc cell firing; however, these initial electrophysiological recordings were conducted in anesthetized rats. In a series of experiments, Carelli and Deadwyler (1994, 1996a, 1996b, 1997-1997) showed repeated cocaine exposure via cocaine self-administration alters the firing pattern of NAcc cells. The experiments showed cells were divided into four firing pattern groups or distinct "neuronal ensembles" (Goto and O'Donnell, 2001). Cocaine induced cells firing patterns included 1) preresponse cell firing (PR) that increased firing immediately prior to the cocaine infusion 2) reinforcement excitation (RFe) that increased firing immediately following cocaine infusion 3) reinforcement inhibition (RFi) that decreased firing during the execution of the lever press for cocaine and 4) preresponse/reinforcement (PR+RF) type cells (PR+RF) that expressed all three of the above firing patterns in one cell (PR, RFe, RFi). Interestingly it was found PR, RFe and RFi also occurred in response to self-administration for natural reinforcers indicating these cells were mediating reinforcement behavior in general. PR+RF cell-firing pattern was only expressed in cocaine self-administering rats suggesting cocaine may cause some PR and RF cells to change to cocaine specific like firing patterns or quiescent cells were solicited and activated by cocaine exposure (Carelli and Deadwyler, 1993). Further RFe, RFi, and PR+RF cells

fired specifically in response to cocaine associated (PR+RF) or natural reward associated (RFe, RFi, PR+RF) cues. These data suggest cocaine mediates alterations in NAcc cell firing patterns.

Models of Cocaine-seeking Behavior

It is important to understand mechanisms mediating cocaine-seeking behavior given that cocaine-seeking and cue-induced cocaine craving are common characteristics shown by cocaine addicts (Foltin and Harney, 2000). Human drug addicts will approach places, people, events and stimuli associated with previous cocaine seeking behavior. In order to investigate underlying mechanisms mediating cocaine-seeking and cue-induced behavior, several behavioral paradigms were developed for experimentation in rodents such as cocaine place conditioning, cocaine self-administration, second order conditioning schedules of reinforcement and reinstatement.

Place conditioning measures the rewarding properties of cocaine, or other drugs of abuse, and the potential for drug administration to be associated with contextual cues (Bardo and Bevins, 2000). A typical place conditioning protocol includes training rodents to associate experimenter-administered cocaine with visual and tactile cues in a conditioning chamber (Spyraki et al, 1982). The conditioning chamber consists of 2 or 3 distinct chambers, typically with black and white patterns on the walls and various types of flooring such as sandpaper, metal bars, wire mesh, or bedding (Tzschentke, 1998). Cocaine (unconditioned stimulus) was administered systemically and the rodent was immediately confined to one conditioning chamber (conditioned stimulus). On the following day, when cocaine effects have dissipated, the same rat was injected with saline as a control and confined to an alternate chamber. After several trials, rodents in

a drug free state were allowed free access to both the cocaine-paired and saline-paired chambers while time in each chamber was assessed. If temporal pairing of cocaine and chamber cues indeed elicited classical conditioning/associative learning processes, cues specifically in the cocaine-paired chamber acquired the rewarding properties of cocaine, initiated drug-seeking behavior via excitation of approach behaviors and increased the amount of time the rodent spent inside the cocaine-paired chamber. Findings suggest cocaine related contextual cues do in fact elicit increased time spent in the cocaine-paired chamber and has been suggested to denote a place preference (Spiraki et al, 1980s; Tzschentke, 1998, Bardo and Bevins, 2000; Bardo et al, 1995).

Cocaine self-administration has been used to investigate cocaine-seeking behavior by training a rodent to lever press for cocaine via operant conditioning (Carelli et al, 1993; Peoples et al, 1997; Di Ciano and Everitt, 2005). Rodents were typically implanted with jugular catheters for intravenous cocaine administration. Self-administration chambers included a house light to cue the start of a conditioning session, a retractable lever that can be pressed by the rodent for cocaine delivery, and a cue light signaling the onset of cocaine availability. Following shaping procedures, rodents readily and consistently lever pressed for cocaine infusions on various schedules of reinforcement such as fixed ratio (FR), progressive ratio (PR) and fixed interval (FI). Findings have been suggested to characterize “cocaine taking” behaviors (Di Ciano and Everitt, 2005). Importantly, the rate of self-administration can be altered by cocaine-associated cues. Tones or lights presented prior to each scheduled cocaine infusion elicited lever-pressing behavior (Carelli and Deadwyler, 1997; Carelli and Ijames, 2000). Additionally, termination of cocaine related cues decreases or ceases lever pressing, incorporating a post-reinforcement pause (Carelli and Ijames, 2000). These findings reveal the rate and pattern of self-administration and cocaine-taking behaviors can be

modulated by exposure to cocaine-related cues present in the self-administration chamber.

Everitt and colleagues (1999, 2005) extended typical self-administration protocols to incorporate second-order schedules of reinforcement in an attempt to more closely model human drug abuse. In general, cocaine relapse in humans was initiated by exposure to cocaine-related cues/contexts that elicits cocaine-seeking followed by cocaine consumption (Goldstein et al, 2007). Second-order schedules of reinforcement allowed researchers to dissociate drug-seeking from drug-taking behaviors in the rodent (Di Ciano and Everitt, 2005). In these procedures, rodents were trained to lever press for cocaine on several increasingly complex schedules of reinforcement. For example, rodents were trained on a FR1 and slowly increased to FR10. Following stable response rates at FR10, a FI15 min reinforcement schedule was added to increase the complexity of the task. Rodents had to wait 15 min before pressing 10 times for cocaine delivery. Data revealed rodents were able to complete this complex task (Everitt et al, 1999). Further, acquisition of the second-order conditioning task was facilitated by cues predicting the end of the 15 min waiting period or by cues predicting the availability of drug. These data eloquently dissociated cue-induced drug-seeking from cue-induced drug-taking behavior due to the fact lever pressing during the first 15 min interval of each daily session denoted drug-seeking in a drug-free rodent whereas lever pressing following the first cocaine infusion denoted drug-taking behavior in a drug treated rodent. These findings suggest the Everitt and colleagues model of second-order conditioning and cocaine reinforcement can be used to further investigate neurochemical mechanisms underlying drug-seeking versus drug-taking behaviors.

Cue- and cocaine-induced reinstatement of cocaine-seeking behaviors aimed to model cocaine cue-induced relapse in humans (Foltin and Harney, 2000; Goldstein et al,

2007). In these procedures, rodents were trained either on a self-administration (FR1) or a place conditioning task as described above to acquire cue-induced drug-seeking behaviors (Erb et al, 2000; Shaham et al, 1998; McFarland et al, 2004). Once stable self-administration levels or a significant place preference was obtained, extinction procedures were used to non-contingently present cocaine in the absence of cocaine-associated cues, decrease self-administration rates and abolish place conditioning. Following extinction, cues were reintroduced into the conditioning chambers. Re-exposure to cocaine-related cues immediately reinstated lever pressing and place preferences (Erb et al, 2000; Shaham et al, 1998; McFarland et al, 2004) suggesting cocaine relapse can be induced by cues/contexts previously associated with cocaine availability providing a competent model for cue-induced drug-seeking and relapse in cocaine addicts.

Individual Differences Predict Drug Use Liability

Animal models of adolescent typical drug-seeking and drug-taking behaviors should investigate whether rodents exhibit individual differences in drug sensitivity given that not all humans respond to drugs in the same way (White et al, 2005) Individual differences have been used as a tool for predicting vulnerability to drug dependency. The most frequently used method for dividing adult rodents into high and low responders was using baseline reactivity to novelty (Hooks et al, 1991, Piazza et al, 1989; Davis et al, 2008; Shimosato and Watanabe, 2003; Mantsch et al, 2001; Gulley, 2007; Klebaur and Bardo, 1999; Chefer et al, 2003; Dietz et al, 2005). Rodents expressing high levels of novel environment-induced activity have greater locomotor activity in response to psychostimulants (Hooks et al, 1991, Piazza et al, 1989; Chefer et al, 2003; Dietz et al,

2005) and a more robust psychostimulant-induced locomotor sensitization (Hooks et al, 1991; Dietz et al, 2005). High responders for novel-environment induced locomotor activity also have higher breakpoints (Gulley, 2007), self-administer a greater number of cocaine infusions (Davis et al, 2008; Mantsch et al, 2001) and more readily initiate low-dose cocaine self-administration (Mantsch et al, 2001; Piazza et al, 2000). Data using individual differences as a predictor for place conditioning have been mixed. High responders for novelty showed more robust psychostimulant-induced place preference (Klebaur and Bardo, 1999) while others showed no effect of phenotype on place conditioning (Gong et al, 1996).

Younger rodents, similar to adults, show individual differences in response to novelty; however, evidence of age effects in novel-environment induced locomotor activity have also been mixed. Some report greater (Lanier and Issacson, 1977; Belluzi et al, 2004; Caster et al, 2005; Stansfield and Kirstein, 2006; Philpot and Wecker, 2008) and others report lower (Laviola et al, 1995; Wooters et al, 2006) levels of novelty-induced activity in adolescent rodents. Lack of agreement for the direction of age effects has been hypothesized to be mediated by length of time exposed to the locomotor activity apparatus (Philpot and Wecker, 2008). Therefore adolescent rodents do exhibit individual differences and these differences can be used to predict drug use liability across development.

Recent findings in the effects of cocaine on multiple neurotransmitters

Multiple neurotransmitters have been reported to mediate cocaine-seeking behavior such as DA, glutamate, GABA, serotonin (5-HT), and norepinephrine (NE). The majority of cocaine research has investigated cocaine's effects on mesolimbic DA.

Cocaine, an indirect DA agonist, blocks reuptake of DA into the presynaptic terminal and subsequently elevates extracellular DA (Pontieri et al, 1995). Systemic and intra-NAcc cocaine administration increased extracellular levels of DA as measured via microdialysis (Smith and Justice, 1994; Parsons et al, 1991a; Shippenberg and Thompson, 1997; Justice, 1993; Chefer and Shippenberg, 2002). Increases in NAcc DA have also been found in response to cocaine-associated cues alone (Di Chiara, 1999) implying cue induced cocaine seeking was mediated by enhanced NAcc DA neurotransmission. Further, DA agonists/antagonists alter cue-induced reinstatement of cocaine seeking. Systemically administered low dose D1 agonists, D3 agonists or local administration of D1 agonists into the NAcc shell reinstate cocaine-seeking whereas systemically administered high dose D1 agonists or D1, D2, D3 antagonists attenuate cocaine seeking behavior (Di Ciano and Everitt, 2005; Spealman et al, 1999).

GABAergic neurotransmission has also been shown to mediate cocaine-seeking behavior via interaction with DA and glutamate (Boudrea and Wolf, 2005). Following cocaine-induced increases in NAcc DA levels, DA binds to postsynaptic D1 receptors located on medium spiny NAcc GABAergic cells. Activation of D1 receptors phosphorylates N-methyl-D-aspartate (NMDA) receptors and activates the retrograde messenger, adenosine, via intracellular signaling cascades in the NAcc GABAergic cell (Snyder et al, 2003). Adenosine, released from NAcc GABAergic cells, activates adenosine A1 receptors located presynaptically on glutamatergic terminals in the NAcc and subsequently inhibits glutamate release. Adenosine induced down-regulation of glutamate release removes a tonic excitation of NAcc GABAergic cells. Deactivated GABAergic cells subsequently show less GABA release in terminal regions such as the ventral pallidum, substantia nigra and associated motor circuits. Together all of these neurotransmitters mediate cocaine seeking behavior.

It should be noted cocaine also binds to serotonin (5-HT) and norepinephrine (NE) transporters to inhibit 5-HT and NE reuptake (Pontieri et al, 1995); however cocaine has a greater affinity for the DA transporter relative to these other two transporters. Additionally the levels of NE and 5-HT in the NAcc are quite low (Shalev et al, 2003; Shaham et al, 1998; Schenk et al, 2002).

Glutamate neurotransmission has been shown to interact with DA in the NAcc to mediate cocaine-seeking behavior. NAcc glutamate levels were decreased following cocaine exposure (Giorgetti et al, 2002). Investigations of the interaction between DA and glutamate in the NAcc have revealed cocaine initially increases NAcc DA and subsequently decreases NAcc glutamate release. Following cocaine blockade of DA reuptake, NAcc DA binds to D1 receptors located presynaptically on corticoaccumbens glutamatergic terminals in the NAcc. Activation of presynaptic D1 receptors decreased release of NAcc glutamate and was associated with an increase in cocaine-seeking behavior (Boudreau and Wolf, 2005). Therefore NAcc glutamatergic neurotransmission plays a major role in mediating cocaine-seeking behaviors. These data were further corroborated by AMPA receptor antagonists blocking cue-induced reinstatement of cocaine seeking.

DA and Glutamate Interactions in the NAcc

It is important to understand the interaction between DA and glutamate in the NAcc given these two neurotransmitters have been suggested to mediate different aspects of drug addiction (Nicola and Malenka, 1997; Beurrier and Malenka, 2002; Kozell and Meshule, 2003; Giorgetti et al, 2002; Brady and O'Donnell, 2004; Kalivas, 2004; Boudreau and Wolf; 2005; Lu et al, 1999). Glutamate modulates DA by increasing

tonic DA release. Furthermore, DA modulates glutamate neurotransmission in the NAcc by decreasing glutamate release, activating intracellular signaling cascades and altering responsiveness of glutamate receptors.

Glutamate increases tonic DA release in the NAcc (Goto and O'Donnell, 2001; Goto and O'Donnell, 2002). Hippocampal, prefrontal and amygdalar glutamatergic projections terminated in the NAcc and synapsed onto mesolimbic DA terminals. Following the release of glutamate from any of these three brain regions, glutamate excited NMDA receptors located presynaptically on mesolimbic DA terminals. DA cell activity was excited via NMDA mediated intracellular signaling mechanisms and subsequently enhanced DA release from mesolimbic DA terminals. Interestingly, DA has the opposite effect on glutamate. DA decreases NAcc glutamate release via a D1 receptor dependent mechanism (Floresco et al, 1998; Beurrier and Malenka, 2002; Kozell and Meshul, 2003; Snyder et al; 2003; White and Cooper, 2001; Wang and O'Donnell, 2001; Dong et al, 2005). DA activates postsynaptic D1 receptors located on NAcc mediums spiny GABAergic cells (Floresco et al, 1998). Activation of D1 receptors stimulated intracellular signaling cascades led to phosphorylation of NMDA receptors (Snyder et al; 2003; White and Cooper, 2001; Wang and O'Donnell, 2001; Dong et al, 2005) and release of the retrograde messenger adenosine (Floresco et al, 1998). Release of adenosine from the postsynaptic membrane of NAcc GABAergic cells stimulated presynaptic adenosine-A1 receptors located on glutamatergic terminals in the NAcc thereby down-regulating glutamate release (Floresco et al, 1998; Beurrier and Malenka, 2002; Kozell and Meshul, 2003; Schmidt et al, 2005).

DA modulates glutamate neurotransmission by activation of postsynaptic D1 receptor dependent intracellular signaling cascades in NAcc GABAergic cells (Snyder et al; 2003; White and Cooper, 2001; Wang and O'Donnell, 2001; Dong et al, 2005). DA-

induced activation of D1 receptors stimulated intracellular G-proteins, adenylate cyclase, cAMP, and protein kinase A. Activation of these signals stimulated calcium- and cAMP-regulated binding protein (CREB) and transcription factors such as delta-fos-B and cyclin dependent kinase-5 (cdk5). Following induction of these factors, DA regulated phosphoprotein (DARPP-32) is phosphorylated at threonine residue #34, leading to an inhibition of phosphoprotein-1 (PP1) and alterations in various ion channel activities and receptor trafficking to the cell membrane. DA activation of D1 receptor dependent signaling cascades elicited a decrease in NAcc GABAergic cell firing by decreasing Na⁺ and Ca⁺ channel currents and increasing K⁺ channel currents (O'Donnell and Grace, 1993a).

Activation of D1 or D2 receptor signaling pathways was repeatedly shown to alter glutamate receptor responsivity (O'Donnell et al, 1999; Floresco et al, 1998; Wolf et al, 1994; Nicola and Malenka, 1997; Kalivas and Duffy, 1997; Tseng and O'Donnell, 2004). DA application enhances NMDA responsivity (Wolf et al, 1994; Nicola and Malenka, 1997; Kalivas and Duffy, 1997). D1 receptor agonists increased (O'Donnell et al, 1999; Floresco et al, 1998) while D2 receptor agonists decreased (Tseng and O'Donnell, 2004) glutamate receptor responsivity. Changes in glutamate receptor responsivity were elicited by D1 and D2 receptor signaling cascades that phosphorylated or dephosphorylated NMDA receptors, respectively (Snyder et al, 2003; White and Cooper, 2001; Wang and O'Donnell, 2001; Dong et al, 2005). Together these data show DA modulates glutamate neurotransmission in the NAcc by decreasing glutamate release, activating intracellular signaling cascades and altering responsivity of glutamate receptors.

Investigations of repeated psychostimulant exposure and behavioral sensitization has shown drug-induced changes in several neuronal mechanisms such as changes in

DA, glutamate, receptor surface expression and intracellular signaling (Nicola and Malenka, 1997; Beurrier and Malenka, 2002; Kozell and Meshul, 2003; Schmidt et al; 2005; Giorgetti et al, 2002; Brady et al, 2005; Kalivas et al; 2004; Boudreau and Wolf; 2005; Lu et al, 1999). Decreased NAcc glutamate correlated well with the expression of amphetamine and cocaine induced behavioral sensitization (Beurrier and Malenka, 2002; Kozell and Meshul, 2003; Schmidt et al, 2005) and may be mediated by the removal of tonic glutamate excitation of NAcc medium spiny GABAergic cells thereby inhibiting NAcc cell firing (Nicola and Malenka; 1997). Further, psychostimulant-induced behavioral sensitization was correlated with an increased glutamatergic drive to the VTA from the basolateral amygdala and prefrontal cortex (Beurrier and Malenka, 1997; Brady et al, 2005; Giorgetti et al, 2002; Kalivas, 2004). Enhanced glutamatergic activity may be the mechanism initiating several psychostimulant-induced neuroadaptive changes in the mesolimbic DA pathway and correlate well with the expression of behavioral sensitization including subsensitivity of DA autoreceptors and supersensitivity of NAcc postsynaptic D1 receptors (Giorgetti et al, 2002). Additionally, repeated exposure to psychostimulants increased surface expression of GluR1 and GluR2/3 subunits of glutamate AMPA receptors whereas GluR4, NMDAR1, and mGluR1 subunits of glutamate AMPA, NMDA and metabotropic receptors, respectively, showed increased internalization (Boudreau et al, 2005; Lu et al, 1999; Kozell and Meshul, 2003). Taken together these data show the interaction between NAcc DA and glutamate neurotransmission plays a major role in the expression of psychostimulant-induced behavioral sensitization.

Recent Findings in Reward and Reinstatement-related Neurocircuitry

As indicated above (see structure and function of the mesolimbic DA pathway section), initial investigations of drug use suggested the mesolimbic DA pathway was the primary physiological mediator of compulsive drug use. Current research supports these original findings but has further examined the role of other brain regions that have primary connections with the mesolimbic DA pathway. The neurocircuitry underlying reinstatement has been identified via local infusions of pharmacological manipulations, lesions or by reversibly inactivating various brain regions with GABA agonists and subsequently measuring the impact on cue-induced reinstatement (see Dopamine and Glutamate Interactions in the NAcc section immediately above for details; Kalivas and McFarland, 2003; Shalev et al, 2003; Shaham et al, 1998; Di Ciano and Everitt, 2005). A priming injection of cocaine has been shown to activate cocaine-prime induced reinstatement circuitry beginning in the VTA (McFarland and Kalivas, 2003). DA cells from the VTA project to the prefrontal cortex, particularly the anterior cingulate and prelimbic cortices where cocaine prime information is processed via DA induced inhibition of glutamatergic cells. Tonic excitation of core GABAergic cells by the prefrontal cortex was removed and GABAergic core cell inhibition of downstream motor circuitry was dampened allowing increased approach behavior towards a drug-conditioned stimulus (Di Ciano and Everitt, 2004). Activation of the motor circuit increases execution of behavioral responses mediating cocaine-seeking behavior (McFarland et al, 2003).

Adolescence

The primary focus of projects in our laboratory has been to elucidate the behavioral and neurochemical factors mediating enhanced vulnerability to drug addiction in adolescents using a rodent model of adolescent drug exposure. The focus of these projects have stemmed from the fact drug use during early adolescence is associated with rapid escalation from casual to daily substance abuse (Estroff et al., 1989). Human adolescents, who repeatedly use drugs, show greater substance consumption as adults (Taioli and Wynder, 1991; Chen and Millar, 1998). A higher rate of substance dependence is found in those initiating use during adolescence. For example, adolescents exhibit a rapid escalation from initiation to dependence (Anthony and Petronis, 1995; Clark et al. 1998) and more difficulty quitting (Khuder et al., 1999; Chen and Millar, 1998). Even when substance consumption is controlled, adolescents show a higher prevalence of dependence than adults (Kandel and Chen, 2000) suggesting increased risk of adolescent substance abuse is not a consequence of greater total consumption for early users; but relates to accelerated progression of dependence. The combination of adolescent neural developmental and drug-induced synaptic change may likely potentiate the onset and severity of adult drug dependency. As a result of these findings, experimental research in our lab has focused on identifying the behavioral and neurochemical mechanisms underlying adolescent vulnerability to addiction. It is important to investigate the behavioral and neurochemical correlates of drug use in subjects exposed to drugs during adolescence. The aim of the present research was to identify neurochemical correlates mediating cocaine-seeking behaviors in adolescent rats.

Summary

According to the above literature review, DA in the NAcc is one of the critical factors needed for the expression of various drug-seeking behaviors. Dopamine permitted processing of salient and behaviorally relevant information in addition to promoting compulsive drug-taking behaviors. Investigations of DAergic activity during various models of cocaine seeking behavior such as place conditioning, self-administration and cue- and cocaine-induced reinstatement have been used to isolate the underlying physiological mechanisms inducing compulsive drug-seeking/taking behaviors. As a result, specific neuronal pathways have been attributed to mediating the effects of cue-induced drug-seeking behaviors. However, the majority of drug abuse research has been primarily conducted in adult rodents (Spear, 2000). Investigating drug use during adolescence is imperative given drug use in middle and high school students is prevalent and has been suggested to potentiate the likelihood of becoming drug dependent during adulthood (Taioli and Wynder, 1991; Chen and Millar, 1998). It is the aim of the paper to further identify the behavioral and neurophysiological mechanisms underlying adolescent vulnerability to drug addiction.

Chapter Two: Involvement of Mesolimbic D2 Receptors and Accumbal Dopamine Levels in the Reinstatement of Cocaine Place Preferences in Developing Rats

Introduction

Animal models of drug use have recently been extended to investigate the impact of drugs on adolescent behavior and brain functioning. Specifically, place conditioning and cocaine-induced reinstatement paradigms provided a measure of drug reward by assessing an animal's ability to associate drug-induced effects with environmental cues. In adult rats, a cocaine place preference was effectively established for 20 mg/kg cocaine (Spyraki et al., 1982; Bardo et al., 1986). In young rodents, the expression of cocaine place conditioning was shown for both pre-adolescent and adolescent rodents (Laviola et al., 1992; Pruitt et al., 1995; Campbell et al., 2000; Schramm-Sapyta et al., 2004; Badanich et al., 2006; Balda et al., 2006). Given clinical studies have shown early drug use, such as use during the adolescent period, was highly associated with chronic and more severe drug use as an adult (Estroff et al., 1989; Anthony and Petronis, 1995; Clark et al., 1998), it would be expected preclinical studies would show adolescents find drugs of abuse more rewarding than adult comparisons. Nicotine and alcohol have elicited greater place preferences in adolescents relative to adult rats (Vastola et al., 2002; Belluzzi et al., 2004; Philpot et al., 2003; Torrella et al., 2004), while mixed results have been found for amphetamine (Adriani and Laviola, 2003; Badanich and Kirstein, *under revision*) with greater place preferences in adults relative to adolescent rats. Similar responses for cocaine place

conditioning have been shown for adolescent and adult rats at standard cocaine doses (Campbell et al., 2000; Schramm-Sapyta et al., 2004) suggesting adolescents were as vulnerable as adults to the rewarding properties of high dose cocaine. However, when lower doses were tested, a different pattern emerged. Early adolescents demonstrated place preferences for lower doses of cocaine than late adolescent and adult rats indicating early adolescent rats were more responsive to the rewarding properties of low dose cocaine than older rats (Badanich et al, 2006). Furthermore, Balda and colleagues (2006) were the first to demonstrate adolescent mice were able to express cocaine-induced reinstatement of cocaine place conditioning. Although expression and reinstatement of cocaine place preferences were comparable in adolescent and adult male mice, a more recent report suggested age effects for cocaine-induced reinstatement of place conditioning were dose dependent (Brenhouse and Andersen, 2008). Adolescents exhibited a more robust reinstatement than adults following a low dose cocaine prime as compared to a higher cocaine dose (20 mg/kg/ip cocaine; Brenhouse and Andersen, 2008). In general, these data revealed greater sensitivity to low dose cocaine reward during adolescence (Badanich et al, 2006; Brenhouse and Andersen, 2008) and indicate associative learning mechanisms and/or the ability of cues to reinstate cocaine-seeking behavior differs between adolescent and adult male rodents.

Neurochemical research in adolescence has focused on the development of the mesolimbic pathway, a dopaminergic projection from the VTA to the NAcc (Dahlstrom and Fuxe, 1964). A fine tuning of neuronal anatomy occurs during adolescence including a general heightened neural activity (Chugani et al., 1987), and changes in receptor density. During adolescence, mesolimbic DA receptors and DA transporters are overproduced and subsequently pruned back (Lidow et al., 1991; Teicher et al.,

1995; Tarazi et al., 1998a; Tarazi et al., 1998b; Tarazi et al., 1999). DA autoreceptors were found to be supersensitive in adolescents in comparison to adult rodents (Andersen et al, 1997) in addition to an age-dependent difference in the rate of DA synthesis (Shalaby et al, 1981). Age-related differences in DA degradation were reported including an adult specific cocaine-induced DA transporter upregulation that was not expressed in adolescents (Collins and Izenwasser, 2002) and age differences in enzymatic degradation of DA in the striatum (Nakano and Mizuno, 1996) and the NAcc (Philpot and Kirstein, 2004). Furthermore, early adolescents have lower and late adolescents have greater basal DA levels in the NAcc (Badanich et al., 2006); however, others did not show an age effect for NAcc basal DA (Frantz et al., 2007). A recent report addressed development of neuronal circuitry during development and found reorganization of critical neuronal circuitry occurred during adolescence (Brenhouse et al, 2008). This report eloquently demonstrated the density of glutamatergic projection cells from the PFC to the NAcc core progressively increased across age with adults having greater cortical innervation of the NAcc than younger rats. Additionally, D1 receptor expression on these glutamatergic projection cells peaked during adolescence as compared to younger and older rats (Brenhouse et al, 2008). These findings suggest the mesolimbic and mesocortical DA pathways undergo marked changes during adolescence.

Cue- and cocaine-induced reinstatement of cocaine seeking behaviors have been used to model relapse in humans (Foltin and Harney, 2000; Goldstein et al, 2007). In these procedures, rodents were trained either on a self-administration or a place conditioning task to acquire cue-induced drug seeking behaviors (Erb et al, 2000; Shaham et al, 1998; McFarland et al, 2004). Once stable self-administration levels or a significant place preference was obtained, extinction procedures were used to non-

contingently present cocaine in the absence of cocaine-associated cues, decrease self-administration rates and abolish place conditioning. Following extinction, cues were reintroduced into the conditioning chambers. Re-exposure to cocaine-related cues immediately reinstated lever pressing and place preferences (Erb et al, 2000; Shaham et al, 1998; McFarland et al, 2004) suggesting cocaine relapse can be induced by cues/contexts previously associated with cocaine availability providing a competent model for cue-induced drug seeking and relapse in cocaine addicts. Further, DA agonists/antagonists alter cue-induced reinstatement of cocaine seeking. Systemically administered low dose D1 agonists, D3 agonists or local administration of D1 agonists into the NAcc shell reinstated cocaine-seeking whereas systemically administered high dose D1 agonists or D1, D2, D3 antagonists attenuated cocaine seeking behavior (Di Ciano and Everitt, 2005; Spealman et al, 1999).

Specific neuronal circuitry including the mesolimbic and mesocortical pathways have been suggested to mediate cocaine-induced reinstatement (Kalivas and McFarland, 2003; Cornish and Kalivas, 1999; De Vries et al, 1999; Capriles et al, 2002; McLaughlin and See, 2003; Everitt and Wolf, 2002). Specifically the VTA, NAcc core, and PFC have been shown to mediate cocaine-induced reinstatement in adult rodents by pharmacologically manipulating or reversibly inactivating target brain regions (McFarland and Kalivas, 2003; Di Ciano and Everitt, 2004; See, 2005). Inactivation of the dPFC, VTA or NAcc core blocked reinstatement of drug seeking behaviors (McFarland and Kalivas, 2001; Di Ciano and Everitt, 2004). There was no effect on reinstatement following inactivation of the basolateral amygdala (BLA), vPFC, NAcc shell, substantia nigra, striatum or mediodorsal nucleus of the thalamus (McFarland and Kalivas, 2001; Di Ciano and Everitt, 2004; See, 2005).

To further support the involvement of the VTA, PFC and NAcc in cocaine-seeking behaviors, cocaine has been shown to alter both DA and glutamate levels in the NAcc following exposure to cocaine (Kozell and Meshul, 2003; McFarland et al, 2003; Hotsenpiller et al, 2001; Chefer and Shippenberg, 2002; Thompson et al., 2000; Camp et al., 1994; Maisonneuve and Kreek, 1994; Parsons and Justice, 1993; Kalivas and Duffy, 1993; Weiss et al., 1992; Hurd et al., 1989). Much research has been conducted on the involvement of DA receptors and transporters in both basal and cocaine-induced DA levels for adult rodents (Beyer and Steketee, 2000; Chen and Pan, 2000). Under drug naïve conditions, intra-VTA or intra-NAcc D2/D3 agonist infusions decreased DA in the VTA and NAcc (Kohl et al, 1998; Rahman and McBride, 2001; Parsons et al, 1996; Zapata and Shippenberg, 2002) while intra-VTA D2 antagonist infusions increased DA levels in the VTA, NAcc and PFC (Chen and Pan, 2000; Kohl et al, 1998). However, when psychostimulants were present, intra-VTA infusions of D1 antagonists or systemic D2 antagonists blocked psychostimulant-induced increases in NAcc DA (Veznia, 1996; Parsons et al, 1993). Some have shown intra-VTA or intra-NAcc D2 antagonist infusions increased cocaine-induced DA levels (Yan, 2003; Tanabe et al, 2004) while others showed a blockade of accumbal DA levels during co-infusion of D2 antagonists and another DAT blocker, GBR12909 (Rahman et al, 2001).

Quantitative microdialysis, a variant of conventional microdialysis, has been used used quantify basal DA levels (Parsons et al, 1991a; Smith and Justice, 1994; Chefer et al, 2003). In this procedure, rats were typically perfused with a single concentration of DA after which perfusate and brain DA concentrations equilibrate. Basal extracellular DA was calculated ($DA_{concentration\ in\ the\ perfusate} - DA_{concentration\ in\ the\ sample}$; $DA_{in} - DA_{out}$) and the net difference was averaged and plotted against C_{in} to form a linear regression line. The x-intercept represented the extracellular DA concentration

while the slope of the regression line represented the extraction fraction (E_d) and provided a measure of DA recovery and an indirect measure of DA reuptake. Quantitative microdialysis studies of basal DA have reported ~ 4nM DA in the NAcc (Parsons et al, 1991a).

Recently *in vivo* microdialysis has been used to investigate the effects of various drugs of abuse on DA in the NAcc of young rodents [Badanich and Kirstein, 2004; Badanich et al, 2006; Camarini et al, 2008; Frantz et al, 2007; Kuczenski and Segal, 2002; Philpot and Kirstein, 1998; Philpot and Kirstein, 1999; Stansfield and Kirstein, 2005]. Repeated exposure to cocaine increased DA in the NAcc of preadolescent rats [Philpot and Kirstein, 1999]. For adolescent rats, psychostimulants increased DA in the NAcc [Frantz et al, 2007; Stansfield and Kirstein, 2005] and repeated exposure to low or moderate dose cocaine induced a leftward shift in the peak onset of DA in adolescent but not adult rats [Badanich et al, 2006; Camarini et al, 2008]. These data indicate microdialysis is an effective technique for measuring drug-induced changes in DA levels in young rodents. Given that the VTA, dPFC and NAcc core mediate cocaine-induced reinstatement (Kalivas and McFarland, 2003; Cornish and Kalivas, 1999; De Vries et al, 1999; Capriles et al, 2002; McLaughlin and See, 2003; Everitt and Wolf, 2002) and it is these same brain regions undergoing developmental transitions during adolescence (Brenhouse et al, 2008), investigating the involvement of these brain regions on reinstatement in the developing rodent was warranted.

It is important animal models of adolescent typical drug-seeking and drug-taking behaviors investigate whether rodents exhibit individual differences in drug sensitivity given that not all humans respond to drugs in the same way (White et al, 2006) Individual differences have been used as a tool for predicting vulnerability to drug dependency. The most frequently used method for dividing adult rodents into high and

low responders was using baseline reactivity to novelty (Hooks et al, 1991, Piazza et al, 1989; Davis et al, 2008; Shimosato and Watanabe, 2003; Mantsch et al, 2001; Gulley, 2007; Klebaur and Bardo, 1999; Chefer et al, 2003; Dietz et al, 2005). Rodents expressing high levels of novel environment-induced activity have greater locomotor activity in response to psychostimulants (Hooks et al, 1991, Piazza et al, 1989; Chefer et al, 2003; Dietz et al, 2005) and a more robust psychostimulant-induced locomotor sensitization (Hooks et al, 1991; Dietz et al, 2005). High responders for novel-environment induced locomotor activity also have higher breakpoints (Gulley, 2007), self-administer a greater number of cocaine infusions (Davis et al, 2008; Mantsch et al, 2001) and more readily initiate low-dose cocaine self-administration (Mantsch et al, 2001; Piazza et al, 2000). Data using individual differences as a predictor for place conditioning have been mixed. High responders for novelty showed more robust psychostimulant-induced place preference (Klebaur and Bardo, 1999) while others showed no effect of phenotype on place conditioning (Gong et al, 1996).

Younger rodents, similar to adults, show individual differences in response to novelty; however, evidence of age effects in novel-environment induced locomotor activity have also been mixed. Some report greater (Lanier and Issacson, 1977; Belluzi et al, 2004; Caster et al, 2005; Stansfield and Kirstein, 2006; Philpot and Wecker, 2008) and others report lower (Laviola et al, 1995; Wooters et al, 2006) levels of novelty-induced activity in adolescent rodents. Lack of agreement for the direction of age effects has been hypothesized to be mediated by length of time exposed to the locomotor activity apparatus (Philpot and Wecker, 2008). Therefore adolescent rodents do exhibit individual differences and these differences can be used to predict drug use liability across development.

Objectives

Homeostatic and compensatory mechanisms in the mesolimbic DA pathway may be the underlying factor(s) mediating enhanced responsivity to psychostimulants in adolescent rats. Cocaine-seeking experiments in adolescent rodents are needed to investigate whether basal differences in compensatory mechanisms in the mesolimbic DA pathway exist such as differences in DA cell firing and mesolimbic DA autoreceptor sensitivity. The present paper examined compensatory mechanisms such as blockade of DA autoreceptors by infusing a DAergic D2 antagonist, sulpiride, into the VTA or NAcc and altering the responsivity of DAergic negative feedback mechanisms of developing rats. It was also of interest to investigate individual differences in cocaine reward to see if specific phenotypic behavior could be used to predict future drug use liability in developing rodents. Therefore, the present study investigated individual differences in 1) cocaine-induced place conditioning during adolescence 2) the effects of intra-VTA or intra-NAcc D2 DA autoreceptor antagonist infusions on cocaine-induced reinstatement of cocaine place conditioning later during adulthood 3) the effects of cocaine exposure during adolescence on basal DA levels in the NAcc later during adulthood and 4) the effects of intra-VTA or intra-NAcc D2 DA autoreceptor antagonist infusions on cocaine-induced increases in accumbal DA during re-exposure to the cocaine paired chamber.

Hypotheses

1. There will be individual differences in cocaine place conditioning.

2. High responders for cocaine reward will be more likely than low responders to show cocaine-induced reinstatement of cocaine place conditioning later on in adulthood.
3. Rats expressing a cocaine place preference during adolescence will demonstrate **potentiated** cocaine-induced reinstatement of a cocaine place preference following local administration of sulpiride, a D2 antagonist, into the **VTA**.
4. Rats expressing a cocaine place preference during adolescence will demonstrate an **attenuation** of cocaine-induced reinstatement of a cocaine place preference following local administration of sulpiride, a D2 antagonist, into the **NAcc**.
5. Quantitative microdialysis will show rats exposed to cocaine during adolescence will demonstrate lower basal DA levels in the NAcc relative to rats who were not treated with cocaine during adolescence.
6. Cocaine-induced increases in NAcc DA levels will be potentiated in high responders for cocaine reward in comparison to low responders.
7. Cocaine-induced increases in NAcc DA levels will be **potentiated** following local administration of sulpiride, D2 antagonist, into the **VTA** relative to cocaine-treated rats without exposure to sulpiride.
8. Cocaine-induced increases in NAcc DA levels will be **attenuated** following local administration of sulpiride, D2 antagonist, into the **NAcc** relative to cocaine-treated rats without exposure to sulpiride.

Methods and Materials

Subjects

One hundred fifty-nine male Sprague-Dawley rats derived from established breeding pairs at the University of South Florida, Tampa (Harlan Laboratories, IN), were used in the present study. The day of birth was designated as PND 0 and litters were sexed and culled to 10 pups per litter on PND 1. Pups remained housed with their respective dams in a temperature and humidity-controlled vivarium on a 12:12-hour light/dark cycle (lights on from 0700 to 1900 hours). On PND 21, pups were weaned and housed in groups of two or three with same-sex littermates. Adolescence in rodents is encompassed within approximately PND 28 to PND 46 and is marked by several developmental events including the onset of puberty and changes in neuroendocrine systems in addition to increased socialization and exploratory behaviors (Spear, 2000; Tirelli et al, 2003). Therefore, to investigate the effects of cocaine on adolescent behavior and neurochemistry, rats in the present study began training on PND 28 (adolescence). Observed body weight for PND 28 rats bred in our vivarium typically averages 91 g. To eliminate the potential confound of litter effects, no more than one pup per litter was used for any given condition and remaining pups were used for other ongoing laboratory experiments. In all respects, the maintenance and treatment of rats were within the guidelines for animal care as approved by the University of South Florida's Institutional Animal Care and Use Committee and the National Institutes of Health.

Apparatus

The place conditioning apparatus was a single runway comprised of acrylic. A removable acrylic door was used to separate the apparatus into two equal sized chambers. Each chamber (21 x 24.5 x 20.5 cm) was comprised of removable visual and tactile cues. One chamber had black and white horizontal striped (1 inch thick) walls with a grey sandpaper floor while the alternate chamber had black and white vertical striped walls (1 inch thick) with a wire-mesh floor. A 2-chamber apparatus, rather than a 3-chamber, was used to eliminate age-related differences in novelty-induced exploration (Stansfield and Kirstein, 2006) that may likely be induced by a less familiar central choice chamber, which is typically incorporated in the 3-chamber CPP paradigm. The place conditioning apparatus was used during place conditioning acquisition, extinction and cocaine-induced reinstatement tests.

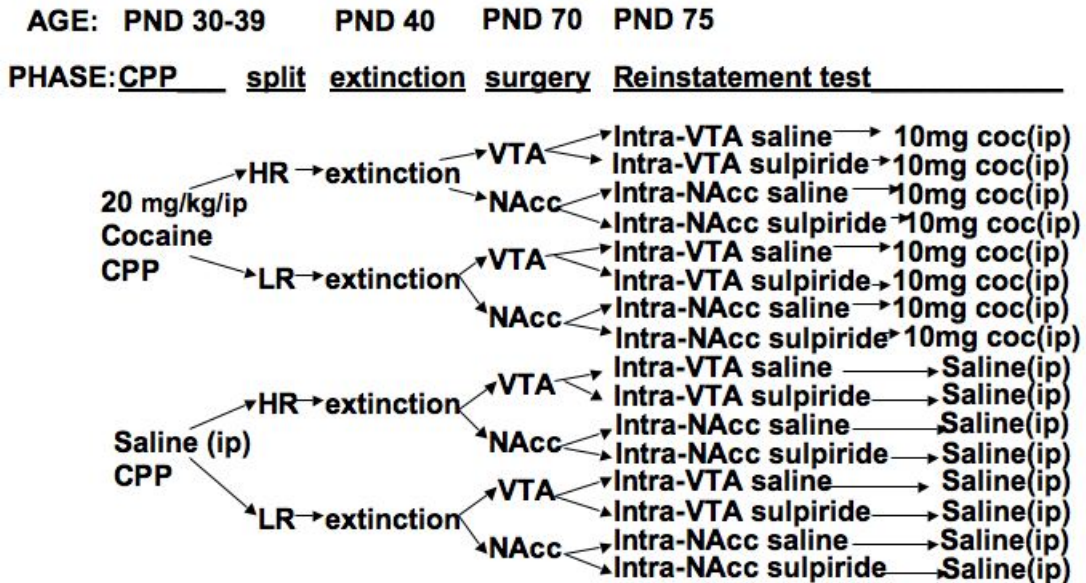
During *in vivo* microdialysis experiments, rats were initially housed in a large, round, clear plastic bowl mounted onto a swiveling Ratum table (Bioanalytical Systems Inc., Indianapolis, IN). The swiveling table was designed to allow the rat to freely move without tangling/disrupting microdialysis tubing emanating from the rat's surgically implanted *in vivo* microdialysis probe. The bowl was lined with bedding and equipped with a water bottle and rat chow. Rats were housed in the Ratum bowl overnight and during quantitative microdialysis. For experiments in which cocaine-induced DA was measured, the paired chamber from the conditioning apparatus, identical to the one described above, was mounted on the swiveling Ratum table in place of the plastic bowl. Mounting paired chamber on the swiveling Ratum table ensured the rat could freely explore the cued chamber without tangling/disrupting microdialysis tubing.

Experiment One Procedure: The effects of intra-VTA or intra-NAcc D2 antagonist infusions on cocaine- induced reinstatement of a conditioned place preference

Cocaine place conditioning and local microinjections was used to investigate the underlying physiological mechanisms inducing cocaine-induced reinstatement of drug seeking behaviors. Experiment One consisted of five phases including 1) place conditioning 2) division of rats into high and low responders, 3) extinction, 4) surgery, and 5) local infusion and reinstatement test. For all rats, the experiment began on PND 28 and each rat was investigated at all five phases. Procedures for each of the five phases are described below and illustrated in Figure 1.

Figure 1: Experiment 1 Procedures

REINSTATEMENT METHODS



Phase 1. On days one and two (PND 28-29), rats were wheeled on a cart into the room where conditioning took place and gently handled for three minutes. Handling occurred once a day so rats became used to the experimenter. The place conditioning procedure was then used to allow rats to establish an association between the effects of cocaine and cocaine-paired environmental cues. Place conditioning was conducted on days three through twelve (PND 30-39) and consisted of three phases: baseline (day three), drug conditioning (days four-eleven) and a CPP expression test (day twelve). On day three (PND 30), a biased design was used to determine baseline chamber preferences. Naïve-rats were placed in the center of the two cued chambers described above (see apparatus section) in a dimly lit room and given free access to the entire apparatus for fifteen minutes. Time (sec) spent in each chamber was recorded. A camera was suspended above the cued apparatus to record behavior. The camera signal was digitized and sent to a computer (Dell OptiPlex GX110) for analysis. Once data are received, movement was analyzed by distinguishing the tracked object (e.g., Sprague-Dawley rat) from the black background (Ethovision video tracking system, Noldus, Netherlands). The chamber in which each animal spent the least amount of time at baseline was designated as the cocaine-paired chamber for future trials. Starting on the morning of day four (PND 31), rats were injected with 20 mg/kg cocaine intraperitoneally and confined to the cocaine-paired chamber for fifteen minutes. The 20 mg/kg cocaine dose has been previously shown to effectively establish a CPP in adolescent rats (Badanich et al, 2006). On the following day, once the effects of cocaine have dissipated, rats were injected with saline and confined to the alternate cued chamber for fifteen minutes. Control rats received saline injections in both chambers. Place conditioning occurred once a day over eight consecutive days (PND 31-38) for a

total of four cocaine and four saline chamber exposures. The apparatus was cleaned with Quatricide (Pharmacal Research Laboratories Incorporated) and ethanol prior to each trial to remove odors. On day twelve (PND 39), the conditioned effects of cocaine was tested by administration of a CPP expression test. Rats were administered a saline injection and tested drug-free in the same manner as at baseline (day three). A saline injection was given during the CPP expression test to control for any associations the injection may have acquired during CPP acquisition. Locomotor activity was also measured inside the conditioning apparatus for each rat to determine whether place conditioning effects were related to changes in locomotor activity.

Phase 2. Following the place conditioning expression test (PND 39), rats within each condition were rank ordered according to their place preference scores. Each experimental condition underwent a median split and the top half of each condition was labeled as high responders for cocaine reward. The bottom half of each condition was labeled as low responders for cocaine reward. Subjects remained classified as high and low responders for the duration of the experiment.

Phase 3. In order to extinguish the association between cocaine and the chamber cues, rats were repeatedly tested in the same manner as during the CPP expression test (day twelve; PND 40). Each rat was tested daily for extinction until the subject spent relatively equal amounts of time in the paired and unpaired chambers. Extinction was defined as rats no longer expressing a preference for either chamber (i.e., 44-55% of entire trial spent in one chamber). The apparatus, identical to the one used during CPP acquisition and expression tests was cleaned with Quatricide (Pharmacal Research Laboratories Incorporated) and ethanol prior to each trial to

remove odors. Locomotor activity was also measured inside the conditioning apparatus for each rat to determine whether extinction effects were related to changes in locomotor activity.

Phase 4. Four weeks following the first extinction trial, rats were anesthetized using a ketamine/xylazine cocktail (1.0 and 0.15 mg/kg/ip). An incision was made over the skull and the rat was mounted on a stereotaxic instrument for surgery. Two holes for skull screws and one for the guide cannula were drilled in the skull. A cannula equipped with a dummy cannula (for CMA 11 probes; outer diameter 0.6 mm) was lowered into the brain to a site just above the VTA (P: -3.5; L: +1.0; V: -8.0) or the anterior NAcc shell (A: +2.375; L: +0.730; V: -6.695) and affixed to the skull with cranioplast. The dorsal-ventral coordinate was measured from the skull surface and the incisor bar was set to zero. Rats were implanted with only one bilateral cannula (VTA or NAcc). Typical cannula placements for the VTA and NAcc are illustrated in Figure 2. One ketamine/xylazine cocktail booster was given to rats if needed. Rats were singly housed in the home cage and allowed at least 4 days for recovery.

Figure 2: Dropsites for VTA and NAcc Cannula

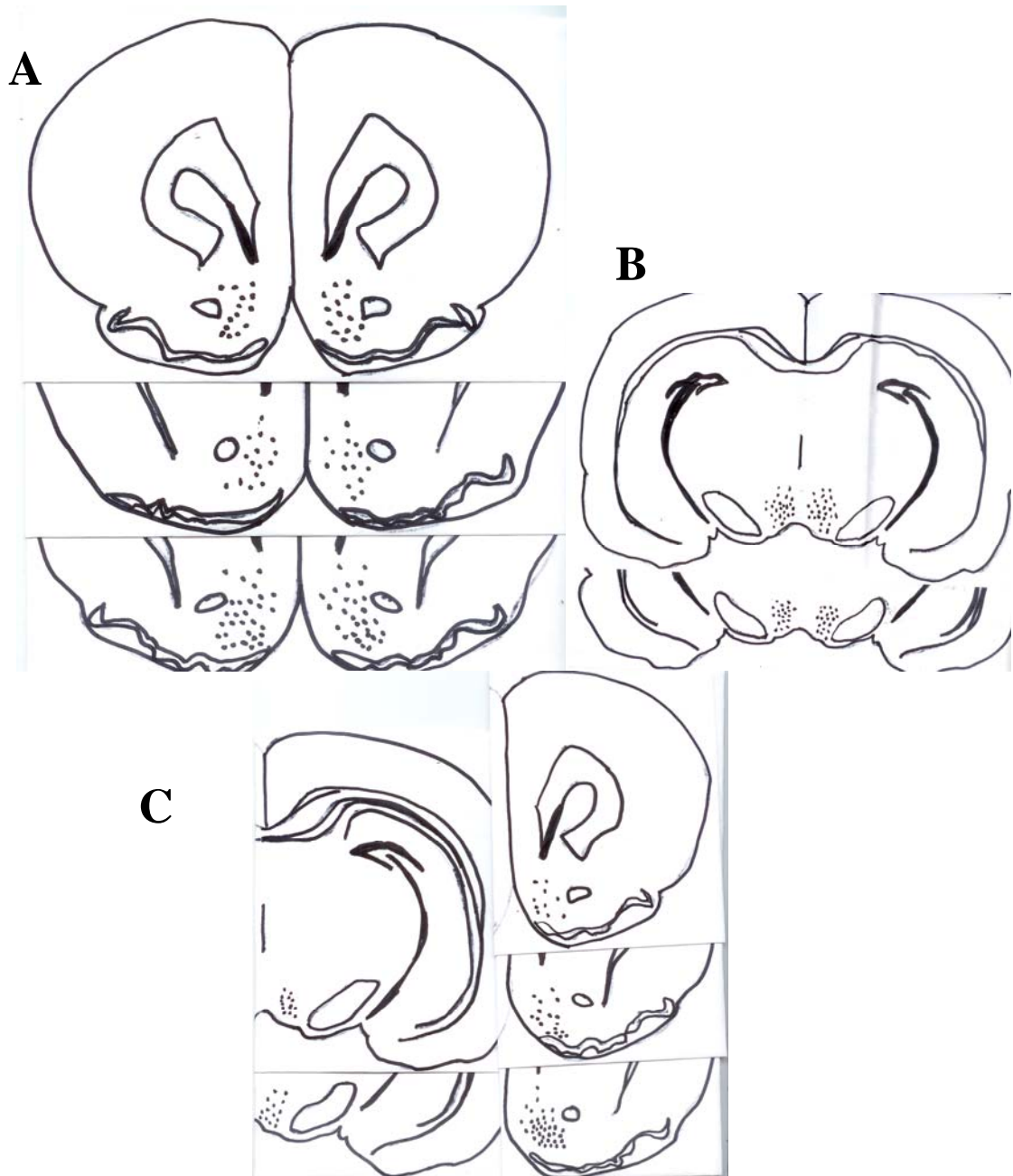


Figure 3: Placement of cannula and microdialysis probes into the NAcc and VTA. A) NAcc cannula placement in rats tested for reinstatement. B) VTA cannula placement in rats tested for reinstatement. C) Microdialysis probe placement in the NAcc (right side) and cannula placement in the VTA (left side) for dialysis experiments.

Phase 5. On reinstatement test day, the dummy cannula was removed and an injection cannula was inserted into the brain region of interest (VTA, NAcc). The

injection cannula was connected via dialysis tubing to a syringe pump set to a flow rate of 0.5 μ L/min. The syringe was filled with either saline alone or saline + 100 μ M sulpiride (Kohl, et al, 1998). Each perfusate was injected into the appropriate brain region for 1 min, followed by turning the syringe pump off. The injector cannula remained inserted for an additional 2 min to facilitate diffusion of the perfusate into the brain tissue and to prevent the perfusate from drawing up into the cannula shaft. Following local perfusions, the injector cannula was removed and replaced with the dummy cannula. These procedures have been used before to successfully infuse drugs into NAcc (Liao et al, 2000; Delfs et al, 1990). Rats were placed back into their homecage for 30 min to allow infusions to have a maximal effect on accumbal DA (Kohl et al, 1998) and to allow time before a subsequent cocaine-induced reinstatement test.

Cocaine-induced reinstatement of cocaine place preferences were investigated by reintroducing rats to cocaine associated cues in the conditioning apparatus immediately following a systemic cocaine prime. Immediately following the local infusion of sulpiride and the 30 min waiting period, rats were tested for cocaine prime-induced reinstatement by administering a priming injection of cocaine (10 mg/kg/ip) and reintroducing rats to the cocaine-paired cues by placing them inside the place conditioning apparatus. Once inside the place conditioning chamber, all rats were tested in the same manner as during the CPP expression test (phase 1, day twelve). Locomotor activity was measured inside the conditioning apparatus for each rat to determine whether reinstatement effects were related to changes in locomotor activity.

Immediately following the reinstatement test, rats were euthanized and brains were removed to histologically verify cannula were accurately placed in the VTA or NAcc. Histological verification was conducted by removing and freezing the brain and subsequently cutting the frozen tissue into 40 μ m sections using a cryostat. Brain slices

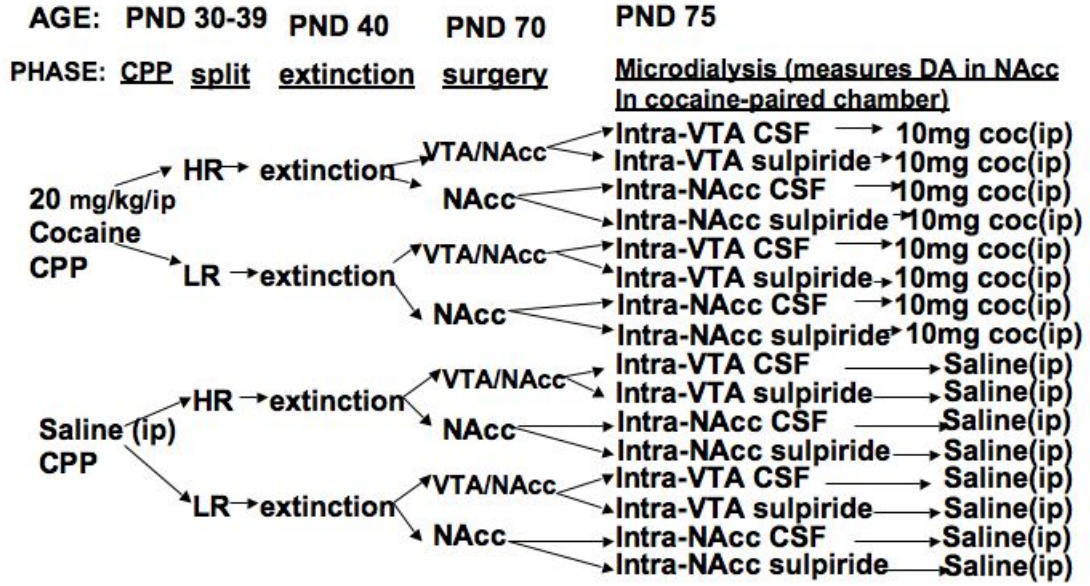
were mounted on slides, stained with cresyl violet and examined with brightfield microscopy. Accurate placement of cannula tracts were identified by comparing each slice to Paxinos and Watsons (1986) rat brain atlas.

Experiment 2 Procedure: The effects of intra-VTA or intra-NAcc D2 antagonist infusions on cocaine-induced dopamine in the nucleus accumbens septi.

In vivo microdialysis and local microinjections were used to investigate the underlying physiological mechanisms mediating cocaine-induced effects on DA in the NAcc. Procedures for Experiment Two were very similar to those in Experiment 1 up to and including surgery and the 4 days of recovery (See Figure 2 for illustration). Rats were trained according to the procedures outline above for 1) acquisition and expression of cocaine place conditioning, 2) divided into high and low responders for cocaine reward, 3) extinction, and 4) surgery. One major difference between Experiment 1 and 2 was the placement of the cannula. Half of the rats in Experiment 2 were implanted with only one single guide cannula into the NAcc so local perfusions and microdialysis could be conducted through the same guide. The remaining rats in Experiment 2 were implanted with two separate single guided cannula. In these rats, one cannula was placed into the VTA for local perfusions and a second cannula was implanted into the ipsilateral NAcc for microdialysis sampling. Following surgery and at least four days of recovery, all rats received 1) local infusions and 2) conventional microdialysis of DA in the NAcc during a cocaine-induced reinstatement test. A subset of rats were used to measure basal DA levels using quantitative microdialysis. Procedures for quantitative microdialysis, local infusions and conventional microdialysis are described below and illustrated in Figure 3.

Figure 3: Experiment 2 Procedures

MICRODIALYSIS METHODS



Quantitative microdialysis procedures. Following recovery from surgery, a subset of rats were housed in large plastic bowls positioned on the Raturon swivel system (see apparatus section above). Probes [CMA 11, 2 mm membrane, 240 mm ODS, 6 kDa MW cutoff] were immediately inserted and perfused continuously with artificial cerebrospinal fluid (136 mM NaCl, 3.7 mM KCl, 1.0 mM MgCl₂, 1.2 mM CaCl₂, 10mM NaHCO₃ pH = 7.2) for at least twelve hours prior to the start of sampling at a flow rate of 0.1 μ L/min. Brain microdialysis probes were connected through the use of a liquid switch to a 500 μ L Hamilton gastight syringe and a Bioanalytical Systems syringe pump. The following morning, the flow rate was increased to 0.5 μ L/min. DA solutions were prepared fresh from a 3 μ M stock solution in artificial cerebrospinal fluid (aCSF) to 1, 5 or 20 nM DA concentrations. The perfusion medium in the syringe pump was changed to one of the following DA concentrations: 0, 1, 5 or 20 nM DA. After an equilibration period of 20 min, dialysates were collected at 20 min intervals into microcentrifuge tubes containing 1.0 μ l of diluted 12N hydrochloric acid to prevent enzymatic breakdown. Three samples were taken before switching to the next DA perfusion concentration. For each subject, DA perfusates were administered in a random sequence to avoid a potential order effect. Data from this portion of the experiment determined basal DA levels in the NAcc for rats exposed to cocaine during adolescence in comparison to untreated rats.

Conventional microdialysis and local perfusion procedures. At the beginning of the microdialysis experimental phase, the large plastic bowl on the swiveling Raturon was replaced with each subject's respective cocaine-paired chamber as designated during

the place conditioning phase. Rats were injected with 5 mg/kg/ip cocaine and then placed inside the cocaine-paired chamber. Once rats were inside the cocaine-paired chamber, collection of dialysates began. Dialysates were collected at 10 min intervals for 40 min into microcentrifuge tubes containing 1.0 μ l of diluted 12N hydrochloric acid to prevent enzymatic breakdown. Following the 40 min baseline exposure to the paired chamber, rats were immediately injected with 10 mg/kg/ip cocaine (or saline for saline pretreated controls) and simultaneously perfused with either aCSF alone or aCSF + 100 mM sulpiride into their respective brain region of interest (VTA or NAcc). Infusions were conducted in the same manner as described above in Experiment 1 except the infusion period lasted for the entire duration of the microdialysis sampling period (70 min) so time course effects of sulpiride on DA could be observed. Additionally, intra-NAcc infusions for rats in Experiment 2 were made via the microdialysis probe and the process of reverse microdialysis. Following the systemic injection and local perfusion, microdialysis sampling continued for an additional 70 min for a assessment of sulpiride on cocaine-induced increases in accumbal DA levels. At the end of the sampling phase, rats were euthanized and brains were removed to histologically verify cannula were accurately placed in the VTA or NAcc according to the histological verification procedures outlined in Experiment 1.

Neurochemical analyses. All dialysate samples and DA-containing perfusates were analyzed by high performance liquid chromatography (HPLC) with electrochemical detection set to oxidize DA at 700 mV (Bioanalytical Systems, IN). A digital detector (Epsilon, Bioanalytical Systems, IN, USA) was used with a radial flow carbon working electrode, referenced to an Ag/AgCl electrode. DA was eluted with a mobile phase consisting of 75 mM sodium phosphate, 1.4 mM octane sulfonic acid, 1 mM EDTA and

10% v/v acetonitrile with a pH = 2.9 and set at a flow rate of 60 μ l/min. Dialysate samples (6 μ l) were injected onto a C-18 microbore column, 100 x 1 mm, 3 μ m ODS for peak separation (Bioanalytical Systems, IN, USA). The HPLC was calibrated with a standard curve consisting of 20 to 0.1 nM DA standards. The range of detection was 2 nA and the average retention time for DA was 4.5 min. Data were recorded and quantified by Chromgraph on a Dell Dimension 2100.

Design and analyses. It was the aim of the present study to determine whether cocaine-induced reinstatement of cocaine place conditioning was mediated by D2 receptors in the VTA and NAcc. For Experiment 1, variables manipulated were Session (baseline vs expression), Pretreatment (saline vs cocaine), Phenotype (high responders vs low responders) and Group (sal/sal; sal/sul; coc/sal; coc/sul). The dependent measure for reinstatement was time spent in the paired chamber expressed as a difference score (sec in paired chamber – sec in unpaired chamber) for all reinstatement procedures. Total distance moved in centimeters (cm) was used as the dependent measure for locomotor activity. Separate two-way analyses of variance (ANOVA) were used at each phase (mixed model: Session x Pretreatment at place conditioning; between subjects: Phenotype x Pretreatment at extinction). One way-between subjects ANOVA of Group was used at the reinstatement phase. When appropriate, post hoc analyses such as simple effects and Fisher's least significant difference were used to isolate Session, Pretreatment, Phenotype and Group effects. All statistical analyses were determined significant at the 0.05 alpha level.

Quantitative microdialysis samples were analyzed by the equation $DA_{in} - DA_{out} = \text{net DA}$, where DA_{in} was the amount of DA perfused through the brain and DA_{out} was the amount of DA obtained in the dialysate (Parsons et al, 1991a; Parsons et al, 1991b).

Net DA was analyzed by linear regression and solved for basal extracellular DA. The slope of each regression line was equal to the recovery of DA and yielded an indirect measure of DA reuptake, E_d . A between subjects two-way ANOVA for Phenotype (high responder vs low responder) and Pretreatment (saline vs cocaine) was used to analyze for basal DA and E_d . All statistical analyses were determined significant at the 0.05 alpha level.

Microdialysis samples collected during exposure to cocaine and sulpiride were presented as percent change from baseline to control for possible group differences in basal DA. Separate two-way mixed model ANOVA with Group (sal/CSF; sal/sul; coc/CSF; coc/sul) and Time (ten DA samples) as the repeated measure were used to analyze DA during exposure to systemic cocaine, locally infused sulpiride and re-exposure to the paired chamber. When appropriate, post hoc analyses such as simple effects and Fishers least significant difference were used to isolate Group and Time effects. All statistical analyses were determined significant at the 0.05 alpha level.

It should be noted three way ANOVAs were originally used and incorporated Phenotype into the statistical analyses; however, because of the opposite effects in high and low responders, most of the effects washed when high and low responders were analyzed together. Given the clear divergent patterns in high and low responders (for behavior at least), two way ANOVAs were instead used at each Phenotype when appropriate.

Results

Experiment 1: Effects of D2 antagonist on the Reinstatement of Cocaine Place Preferences

There were individual differences in cocaine place conditioning for adolescent rats (Figure 4). Half of the rats tested during adolescence showed a clear preference for the cocaine-paired chamber (Figure 4a). For these rats, a significant effect of Pretreatment x Session was found [$F(1, 91) = 7.12, P < 0.05$]. Post hoc analyses of Session revealed time spent in the paired chamber was greater during expression than at baseline regardless of Pretreatment group (indicated by *); however, post hoc analyses of Pretreatment revealed rats pretreated with 20 mg/kg cocaine spent more time in the paired chamber than saline-pretreated rats (indicated by **). Therefore, for these rats, cocaine-treated rats showed a place preference and were labeled as high responders for the duration of the experiment. The remaining rats tested during adolescence did not show a preference for the cocaine-paired chamber (Figure 4b). For these rats, there was no effect of Pretreatment x Session [$F(1, 102) = 0.37, P > 0.05$] and were labeled as low responders for the duration of the experiment.

Figure 4: Place Conditioning

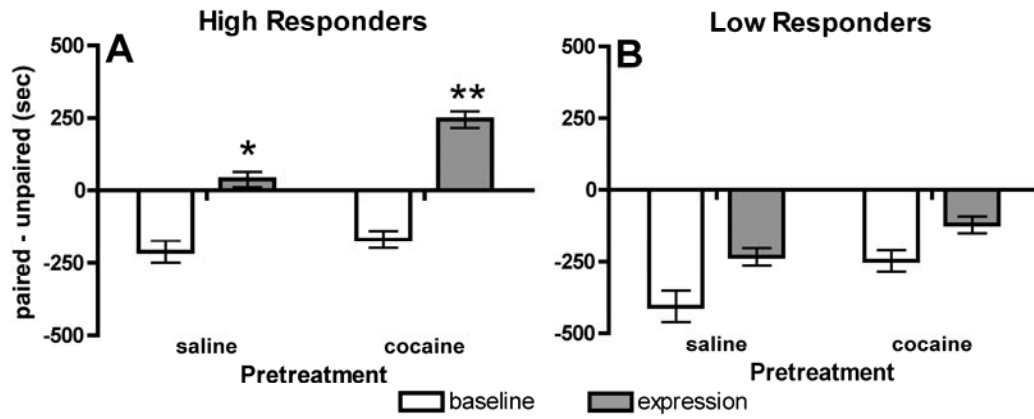


Figure 4: Individual differences in cocaine place conditioning. High responders (A) showed a place preference (indicated by **) while low responders (B) did not.

There was no effect of Pretreatment x Phenotype for the last extinction trial [Figure 5; $F(1, 96) = 0.06, P > 0.05$]. Furthermore, there was no effect of Phenotype x Pretreatment for number of days it takes to extinguish [Figure 6; $F(1, 98) = 1.09, P > 0.05$]

Figure 5: Extinction



Figure 5: All rats extinguish. There were no effects of Phenotype or Pretreatment on extinction

Figure 6: Rate of Extinction

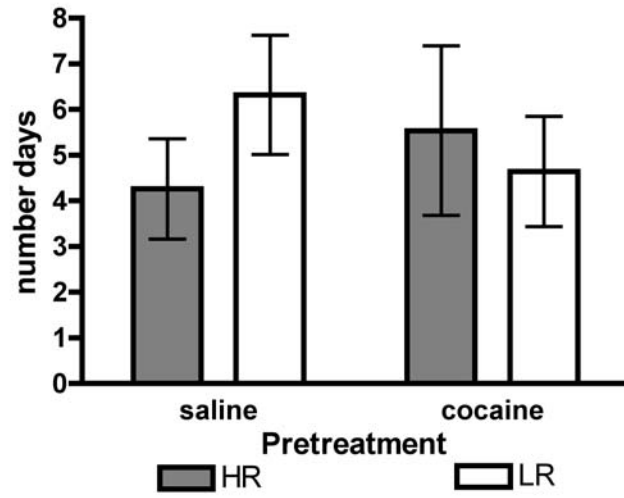


Figure 6: There were no differences in the amount of time it took to extinguish for any of the experimental groups (Pretreatment; Phenotype)

High responders for cocaine place preferences had a significant effect of Group when tested for reinstatement later on in adulthood [Figure 7a; Group: $F(3, 21) = 3.46$, $P < 0.05$]. Post hoc analyses revealed coc/sal and coc/sul treated groups spent more time on the paired chamber than sal/sal and sal/sul groups (indicated by *). These data illustrate a priming dose of cocaine (10 mg/kg/ip) and an intra-NAcc infusion of saline reinstated a cocaine place preference as shown by increased time spent in the paired chamber for the coc/sal group (Figure 7a; grey bars). Reinstatement of cocaine place preference was not altered by intra-NAcc injections of sulpiride as shown by a similar increase in time spent in the paired chamber for the coc/sul group (Figure 7a; grey striped bars). Low responders for cocaine place preferences did not show an effect of reinstatement or sulpiride on time spent in the paired chamber [Figure 7b; Group: $F(3, 27) = 1.00$, $P > 0.05$].

Figure 7: Effects of Intra-NAcc Sulpiride on Reinstatement

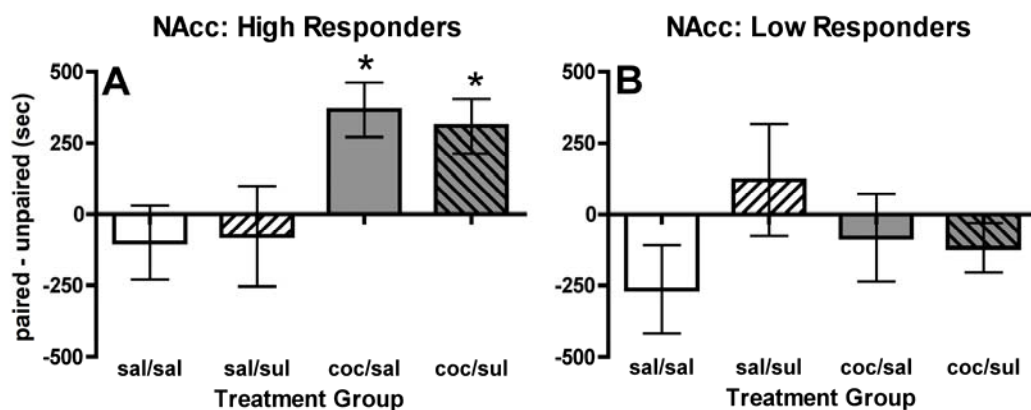


Figure 7: Individual differences in reinstatement occurred. (A) High responders for cocaine reward showed cocaine induced reinstatement of cocaine place conditioning (grey bars; indicated by *). There was no effect of intra-NAcc sulpiride on cocaine-

*induced reinstatement of cocaine place conditioning (grey striped bars; indicated by *).*
(B) There were no effects of cocaine or sulpiride on reinstatement in low responders for cocaine reward

High responders for cocaine place preferences had a significant effect of Group when tested for reinstatement later on in adulthood [Figure 8a; group: $F(3, 22) = 5.22$, $P < 0.05$]. Post hoc analyses revealed the coc/sal treated group spent more time on the paired chamber than sal/sal, sal/sul and coc/sul groups (indicated by #). These data illustrate a priming dose of cocaine (10 mg/kg/ip) and an intra-VTA infusion of saline reinstated a cocaine place preference as shown by increased time spent in the paired chamber for the coc/sal group (Figure 8a; grey bars). Reinstatement of cocaine place preference was blocked by intra-VTA injections of sulpiride as shown by a lack of increased time spent in the paired chamber for the coc/sul group (Figure 8a; grey striped bars). Low responders for cocaine place preferences did not show an effect of reinstatement or sulpiride on time spent in the paired chamber [Figure 8b; Group: $F(3, 21) = 1.65$, $P > 0.05$].

Figure 8: Effects of Intra-VTA Sulpiride on Reinstatement

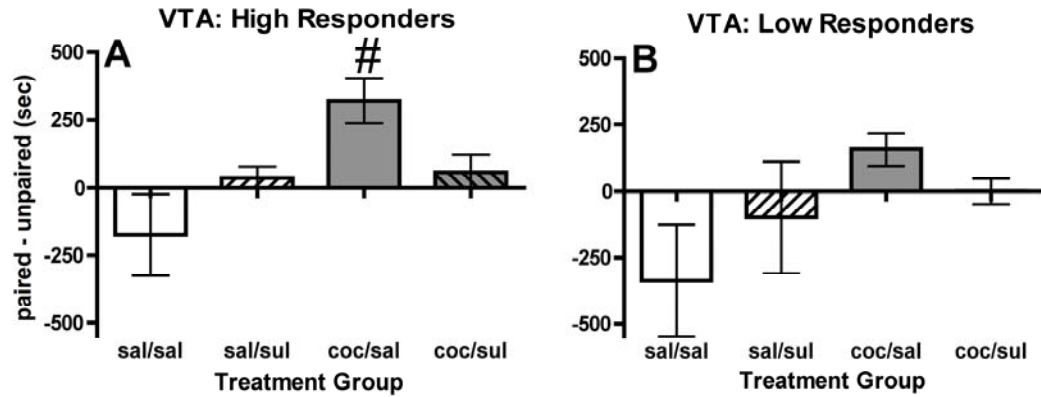


Figure 8: Individual differences in reinstatement occurred. (A) High responders for cocaine reward showed cocaine induced reinstatement of cocaine place conditioning (grey bars; indicated by #). Intra-NAcc sulpiride blocked cocaine-induced reinstatement of cocaine place conditioning (grey striped bars). (B) There were no effects of cocaine or sulpiride on reinstatement in low responders for cocaine reward

For high responders, there was not a significant interaction between Pretreatment x Session for cue-induced locomotor activity [Figure 9a; $F(1, 91) = 1.77, P > 0.05$]; however, there was a main effect of Session [Figure 9a; $F(1, 91) = 22.49, P < 0.05$] suggesting high responders moved a greater distance in the paired chamber during the expression test than at baseline, regardless of Pretreatment exposure. Furthermore, there was a main effect of Pretreatment [Figure 9a; $F(1, 91) = 6.11, P < 0.05$] suggesting cocaine pretreated rats moved a greater distance in the paired chamber than saline controls, regardless of Session. For low responders, there was not a significant interaction between Pretreatment x Session for cue-induced locomotor activity [Figure 9b; $F(1, 101) = 0.32, P > 0.05$]; however, there was a main effect of Pretreatment [Figure 9b; $F(1, 101) = 14.16, P < 0.05$] suggesting cocaine pretreated rats moved a greater distance in the paired chamber than saline controls, regardless of Session.

Figure 9: Cue-induced Locomotor Activity

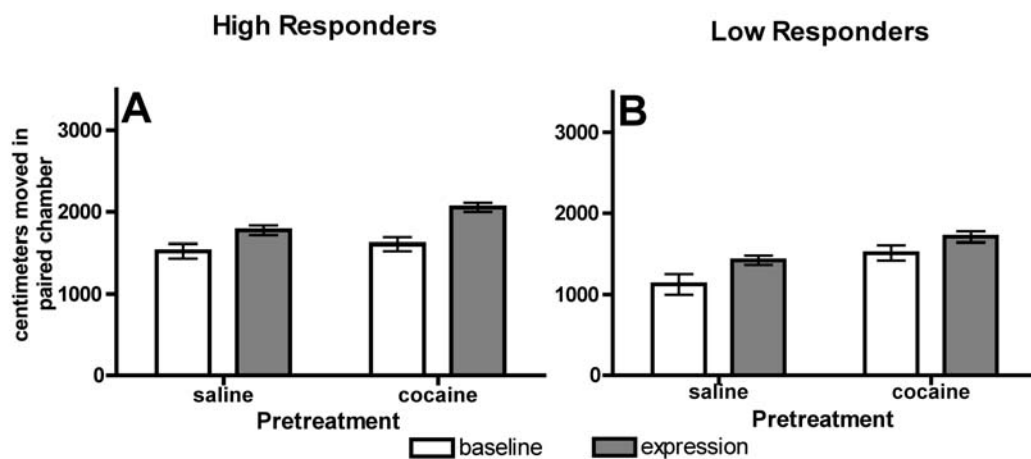


Figure 9: There was no significant interaction between Session and Pretreatment for (A) high responders or (B) low responders for cocaine reward

There was no effect of Pretreatment x Phenotype for the last extinction trial [Figure 10; $F(1, 97) = 1.83, P > 0.05$].

Figure 10: Extinction of Locomotor Activity

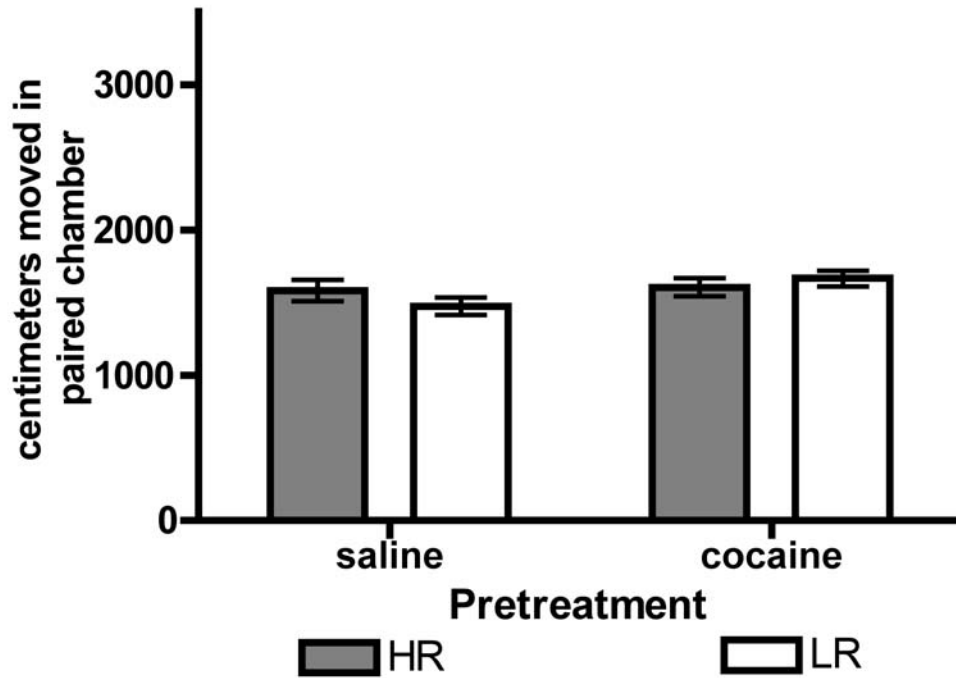


Figure 10: There was no effect of Phenotype or Pretreatment during extinction

High responders had a significant effect of Group when tested for cocaine-induced locomotor activity later on in adulthood [Figure 11a; group: $F(3, 21) = 9.14, P < 0.05$]. Post hoc analyses revealed the coc/sal and coc/sul treated groups moved a greater distance in the paired chamber than sal/sal and sal/sul groups (indicated by **). These data illustrate a priming dose of cocaine (10 mg/kg/ip) and an intra-NAcc infusion of saline increased locomotor activity as shown by increased distance moved in the paired chamber for the coc/sal group (Figure 11a; grey bars). Cocaine-induced locomotor activity in the paired chamber was not altered by intra-NAcc injections of sulpiride as shown by a similar increase in time spent in the paired chamber for the coc/sul group (Figure 11a; grey striped bars). Low responders did not show an effect of increased locomotor activity or sulpiride on distance moved in the paired chamber [Figure 11b; Post-treatment: $F(3, 24) = 2.14, P > 0.05$].

Figure 11: Effects of Intra-NAcc Sulpiride on Locomotor Activity

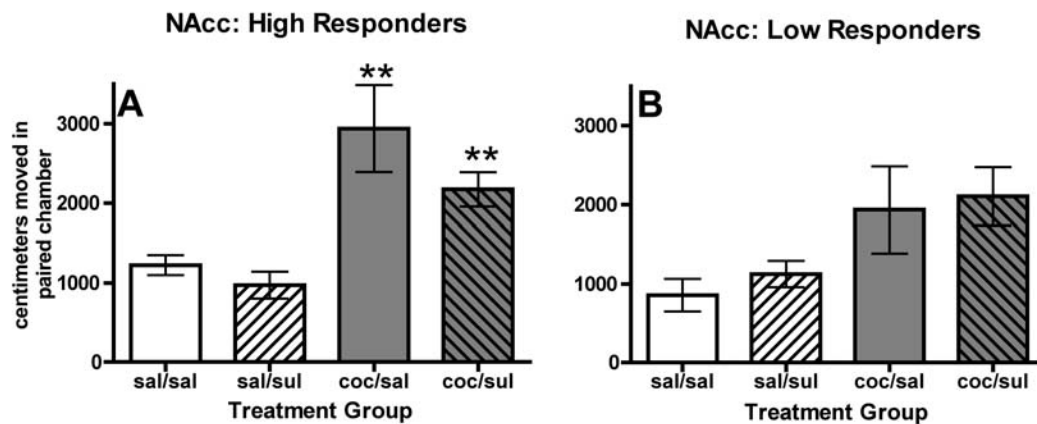


Figure 11: Individual differences occurred for locomotor activity during the reinstatement test. (A) High responders had increased locomotor activity following a cocaine prime injections during the reinstatement test (grey bars; indicated by **). Intra-NAcc sulpiride had no effect on cocaine-induced locomotor activity. (B) There were no significant effects of cocaine or sulpiride in low responders.

High responders had a significant effect of Group when tested for cocaine-induced locomotor activity later on in adulthood [Figure 12a; Group: $F(3, 20) = 5.44, P < 0.05$]. Post hoc analyses revealed the coc/sal treated group moved a greater distance in the paired chamber than the sal/sal group (indicated by *) while the coc/sul group moved a greater distance in the paired chamber than the sal/sal and sal/sul groups (indicated by **). These data illustrate a priming dose of cocaine (10 mg/kg/ip) and an intra-VTA infusion of saline increased locomotor activity as shown by increased distance moved in the paired chamber for the coc/sal group (Figure 12a; grey bars). Cocaine-induced locomotor activity in the paired chamber was not altered by intra-VTA injections of sulpiride as shown by a similar increase in distance moved in the paired chamber for the coc/sul group (Figure 12a; grey striped bars). Low Responders had a significant effect of Group when tested for cocaine-induced locomotor activity later on in adulthood [Figure 12b; $F(3, 20) = 4.78, P < 0.05$]. Post hoc analyses revealed the coc/sal treated group moved a greater distance in the paired chamber than the sal/sul group (indicated by #) while the coc/sul group moved a greater distance in the paired chamber than the sal/sal and sal/sul groups (indicated by **).

Figure 12: Effects of Intra-VTA Sulpiride on Locomotor Activity

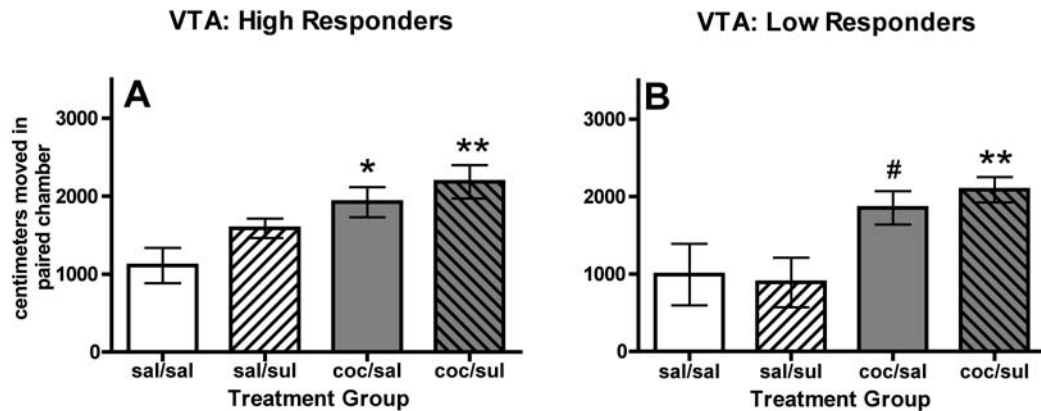


Figure 12: Individual differences in cocaine-induced locomotor activity during the reinstatement test. (A) High responders showed increased locomotor activity following a cocaine prime and during the reinstatement test (grey bars; indicated by *). Intra-VTA sulpiride did not alter cocaine-induced locomotor activity during the reinstatement test. (B) Low responders only showed increased locomotor activity during the reinstatement test following a cocaine prime and intra-VTA sulpiride (grey striped bars; indicated by **). The coc/sul group was only different from sal.su group (grey bars; indicated by #).

Experiment 2: Effects of repeated Cocaine Exposure during Adolescence on Basal DA levels in the Adult NAcc

There were no differences in basal DA for any of the treatment groups nor was there an effect of Phenotype [Figure 13 and 14a; Pretreatment x Phenotype: $F(1, 24) = 0.48$, $P > 0.05$]. DA levels in Figure 13 were equal to the x-intercept of each regression line as indicated by quantitative microdialysis. Mean levels of basal DA for each group were 5.3 nM for saline pretreated high responders, 3.9 nM for saline pretreated low responders, 3.9 nM for cocaine pretreated high responders, and 3.0 nM for cocaine pretreated low responders. Data were collapsed across groups to see if significant effects of either Phenotype or Pretreatment would become evident. After collapsing across Phenotype, there were no differences between saline and cocaine pretreated rats [Figure 14b; $t(26) = 1.17$, $P > 0.05$]. No differences were found between high and low responders after collapsing across Pretreatment group [Figure 14c; $t(26) = 1.15$, $P > 0.05$].

Figure 13: Basal DA

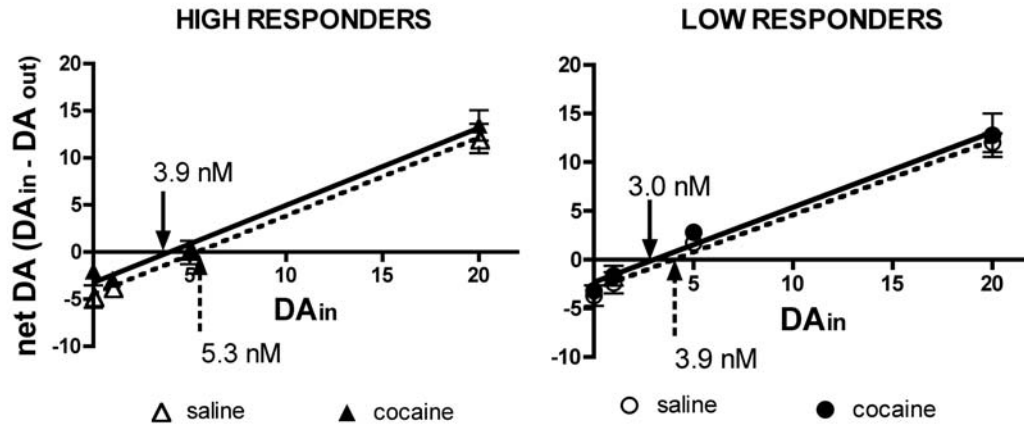


Figure 13: Quantitative microdialysis on a subset of rats showed no effect of Phenotype or Pretreatment on basal DA levels in the NAcc. The x-intercept of each regression line is equal to the extracellular value of DA while the slope of each regression line is equal to the extraction fraction (E_d)

Figure 14: Basal DA Comparisons

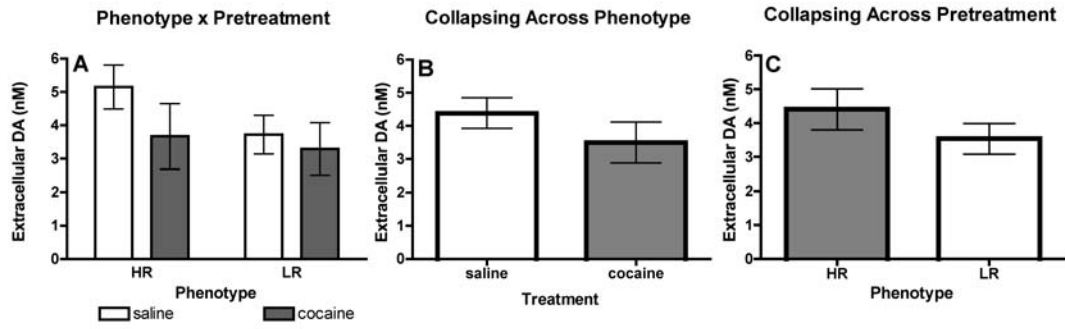


Figure 14: (A) There was no effect of Phenotype or Pretreatment on basal DA levels. Collapsing across (B) Phenotype or (C) Pretreatment did not reveal significant differences in basal DA levels.

There were no differences between any of the treatment groups nor was there an effect of Phenotype on E_d [Figure 11; Pretreatment x Phenotype: $F(1, 22) = 0.33, P > 0.05$]. The slope of each regression line in Figure 11 denotes E_d for each group. Mean levels of E_d for each group were 73% for saline pretreated high responders, 72% for saline pretreated low responders, 80% for cocaine pretreated high responders, and 68% for cocaine pretreated low responders.

The overall Group x Phenotype x Time ANOVA for DA levels in the NAcc was not significant [$F(24, 48) = 1.02, P > 0.05$]. This is to be expected given that analyzing high and low responders together in the same ANOVA would certainly produce a washed effect. Therefore, high and low responders were analyzed separately for time course effects of cocaine-induced DA levels in the NAcc.

In high responders, there was a significant interaction between Group and Time for DA levels in the NAcc [Figure 15a: $F(24, 40) = 2.57, P < 0.05$]. Further analyses of Time indicated there was a significant effect of Treatment for high responders at 20-50 minutes post cocaine injection [20 minutes: $F(3, 11) = 11.04, P < 0.05$; 30 minutes: $F(3, 9) = 11.59, P < 0.05$; 40 minutes: $F(3, 10) = 15.98, P < 0.05$; 50 minutes: $F(3, 9) = 5.63, P < 0.05$]. Post hoc analyses revealed the coc/CSF group had greater DA levels than sal/CSF, sal/sul and coc/sul groups from 20 to 40 minutes post cocaine-injection (indicated by #). Furthermore, the coc/sul group had greater DA levels than the sal/CSF group at 20 and 40 minutes post cocaine injection (indicated by *). There was also a significant difference 50 min post cocaine injection showing coc/CSF treated rats had greater DA levels than sal/CSF and sal/sul treated rats (indicated by **).

The pattern of DA increase in high responders was analyzed by area under the curve. When comparing time course data for pharmacological experiments, it is important to analyze for the pattern and shape of the data curve. Area under the curve is a statistical analysis commonly used in microdialysis experiments to compare patterns for DA increases between drug-treated and saline-treated groups. For high responders, there was a significant effect of Group [Figure 15b: $F(3, 12) = 7.01, P < 0.05$]. Post hoc analyses of Group for area under the curve revealed the coc/CSF group had greater DA levels than sal/CSF, sal/sul, and coc/sul groups (indicated by #).

Figure 15: Effects of Intra-NAcc Sulpiride on DA in High Responders

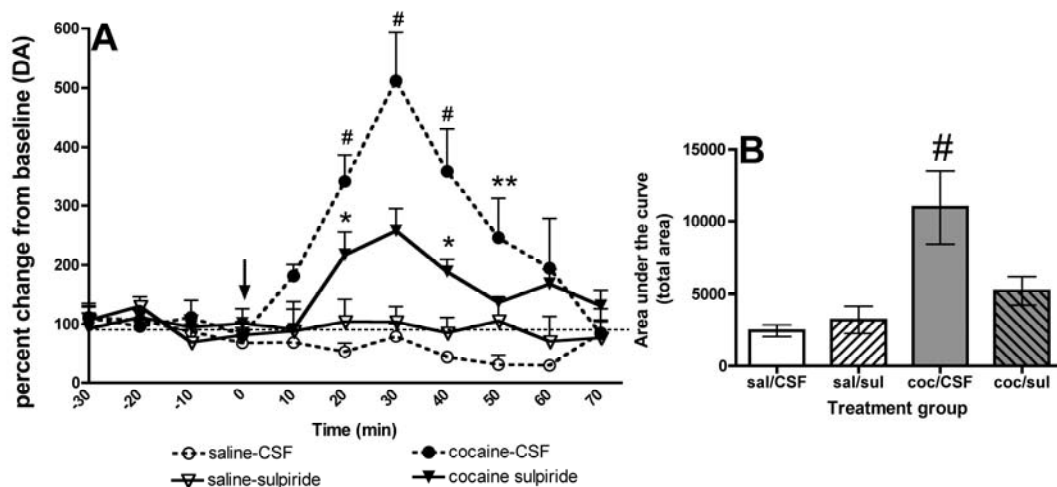


Figure 15: (A) Cocaine increased NAcc DA levels in high responders for cocaine reward (dotted lines and filled circles; indicated by # and **). Intra-NAcc sulpiride attenuated cocaine-induced increase in NAcc DA (solid lines and filled diamonds; indicated by *). (B) Results of area under the curve show cocaine induced increases in NAcc DA (grey bars; indicated by #) and blockade by intra-NAcc sulpiride (grey striped bars).

There was not a significant interaction between Group and Time in low responders for DA levels in the NAcc [Figure 16a; $F(24, 40) = 0.33, P > 0.05$]. Furthermore, there was no effect of Group in low responders for area under the curve [Figure 16b; $F(3, 12) = 0.87, P > 0.05$]. However, because of the pattern of cocaine-induced DA, a post-hoc test was conducted to see if coc/CSF was significantly different from sal/CSF. Post hoc analyses did reveal greater DA levels in coc/CSF treated rats in comparison to sal/CSF controls [Figure 16b; $t(6) = 2.44, P = 0.05$; indicated by *].

Figure 16: Effects of Intra-NAcc Sulpiride on DA in Low Responders

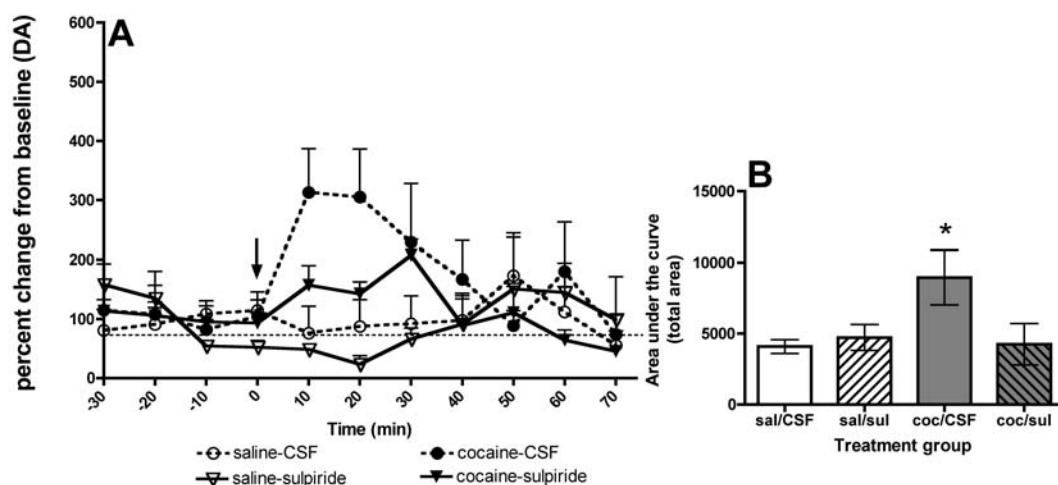


Figure 16: (A) Cocaine increased NAcc DA levels in high responders for cocaine reward (dotted lines and filled circles). (B) Results of area under the curve show cocaine induced increases in NAcc DA (grey bars; indicated by *).

Similar to what was found in the intra-NAcc perfused groups, the overall Group x Phenotype x Time ANOVA for DA levels in the NAcc was not significant [$F(24, 96) = 1.45, P > 0.05$]. Therefore, high and low responders were analyzed separately for time course effects of cocaine-induced DA levels in the NAcc.

There was a significant interaction between Treatment and Time for DA levels in the NAcc [Figure 17a; $F(24, 56) = 2.51, P < 0.05$]. Further analyses of Time indicated there was a significant effect of Treatment for high responders at 30, 40 and 60 minutes post cocaine injection [30 minutes: $F(3, 12) = 7.99, P < 0.05$; 40 minutes: $F(3, 12) = 6.88, P < 0.05$; 60 minutes: $F(3, 11) = 4.11, P < 0.05$]. Post hoc analyses revealed the coc/CSF group had greater DA levels than sal/CSF, sal/sul, and coc/sul groups from 30 to 40 minutes post cocaine injection (indicated by #). There was also a significant difference 60 min post saline injection showing the sal/sul group had greater DA levels than sal/CSF and coc/sul groups (indicated by \$).

There was a significant effect of Group for area under the curve in high responders [Figure 17b; $F(3, 12) = 3.88, P < 0.05$]. Post hoc analyses of Group for area under the curve revealed the coc/CSF group had greater DA levels than sal/CSF, sal/sul, and coc/sul groups (indicated by #).

Figure 17: Effects of Intra-VTA Sulpiride on DA in High Responders

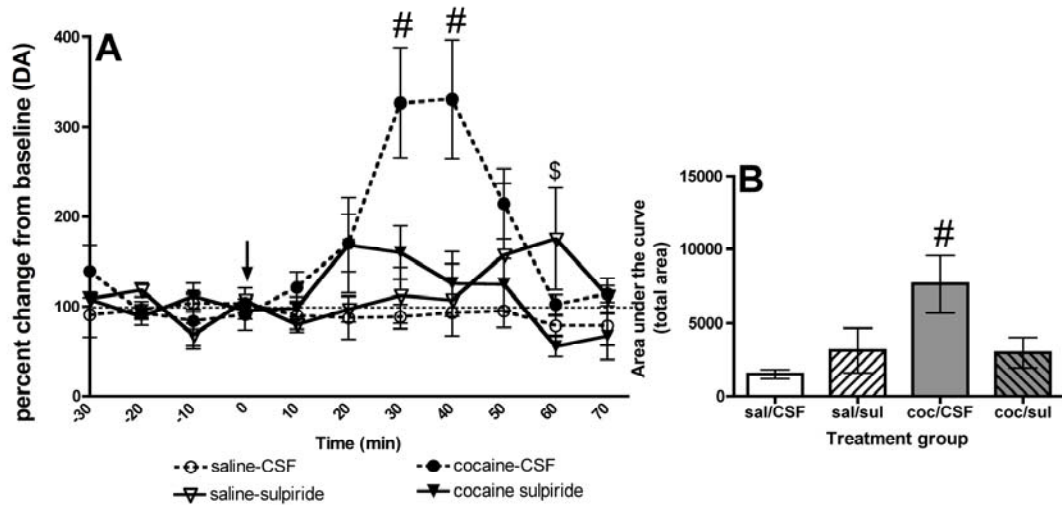


Figure 17: (A) Cocaine increased NAcc DA levels in high responders for cocaine reward (dotted lines and filled circles; indicated by #). Intra-VTA sulpiride attenuated cocaine-induced increase in NAcc DA (solid lines and filled diamonds). (B) Results of area under the curve show cocaine induced increases in NAcc DA (grey bars; indicated by #) and blockade by intra-VTA sulpiride (grey striped bars).

There was not a significant interaction between Group and Time in low responders for DA levels in the NAcc [Figure 18a; $F(24, 40) = 1.52, P > 0.05$]. Furthermore, there was no effect of Treatment in low responders for area under the curve [Figure 18b; $F(3, 11) = 2.04, P > 0.05$]. However, because of the pattern of cocaine-induced DA, a post-hoc test was conducted to see if the coc/CSF was significantly different from the sal/CSF. Post hoc analyses did not reveal an effect of Group in the coc/CSF group in comparison to the sal/CSF group [Figure 18b; $t(6) = 1.96, P > 0.05$].

Figure 18: Effects of Intra-VTA Sulpiride on DA in Low Responders

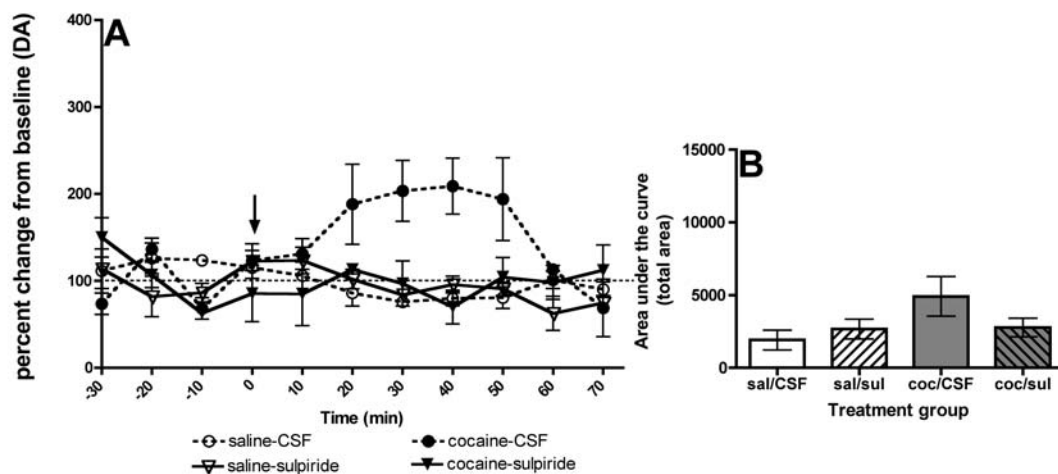


Figure 18: There were no significant differences of Group for low responders even though a pattern towards cocaine induced DA was seen (A) across the sampling time and (B) by area under the curve.

Discussion

The present series of experiments revealed individual differences in cocaine place conditioning for male adolescent rats. Following a median split of rats into high and low responders based on expression score, only high responders were found to have a cocaine place preference (Figure 4a). High responders were specifically vulnerable to cocaine and cocaine-associated cues given that only high responders showed cocaine-induced reinstatement of cocaine place preferences (coc/sal group in Figures 7a and Figure 8a). Cocaine-induced reinstatement of cocaine place preferences in high responders was blocked by intra-VTA infusions of the D2 antagonist sulpiride (coc/sul group in Figure 8a); however, intra-NAcc sulpiride in high responders did not alter cocaine-induced reinstatement (coc/sul group in Figure 7a). Interestingly, cocaine-induced DA levels in the NAcc were found to be effected by sulpiride. Local infusions of sulpiride into either the VTA or NAcc attenuated cocaine-induced increases in accumbal DA levels (Figures 15-18). The attenuating effect of intra-NAcc sulpiride on cocaine-induced DA was evident in both high and low responders while intra-VTA sulpiride significantly attenuated cocaine-induced DA in high responders and only approached significance in low responders. Given that intra-VTA infusions of a D2 antagonist blocked both reinstatement and cocaine-induced increases in accumbal DA levels, it can be suggested D2 receptors in the VTA are one major factor mediating cocaine-seeking behaviors in male rats previously classified as high responders for cocaine during adolescence.

The present report is the first to show individual differences in cocaine place conditioning in adolescent rats. There were two divergent responses to cocaine place conditioning with some rats expressing a cocaine preference (Figure 4a) and others showing a lack of a cocaine effect (Figure 4b). Many have shown adolescent rats are able to acquire and express a place preference for cocaine (Badanich et al, 2006; Laviola et al, 1992; Pruitt et al, 1995; Schramm-Sapyta et al, 2004) as well as other drugs of abuse (Vastola et al, 2002; Belluzi et al, 2004; Philpot et al, 2003; Torella et al, 2004); however no one has investigated whether individual differences exist in cocaine reward for adolescents. Furthermore, the majority of adult rodent data investigating individual differences in response to cocaine have not used place conditioning as the measure for splitting rodents into high and low responders. The majority of data on individual differences in response to drug sensitivity has used locomotor activity and reactivity to novelty as predictors of drug sensitivity (Klebaur and Bardo, 1999; Piazza et al, 2000; Hooks et al, 1991).

Given the majority of investigations on individual differences in drug vulnerability has used locomotor activity in a novel environment to split subjects into high and low responders, it is very likely reactivity to stressful situations played a role in drug use liability. Plasma levels of corticosterone were higher in Fischer rats when compared to their addiction prone Lewis comparisons (Ortiz et al, 1995). Furthermore, Fischer rats have greater increases in locomotor activity following exposure to corticosterone suggesting Fischer rats and addiction resistant phenotypes may be more reactive to stressful situations (Ortiz et al, 1995). Levels of corticosterone were suggested to regulate tyrosine hydroxylase and GFAP levels in the VTA of only Fischer rats (Ortiz et al, 1995). Furthermore, stress of environment or stress of injection may make low

responders less likely to develop place preference because the negative effects of the chamber or injection outweigh the rewarding effects of cocaine. (van de Kam et al, 2006).

Since most reports of individual differences in adolescent and adult rats classify high and low responders based on their locomotor response in a novel environment, more research is needed on low and high responders based on drug reward such via use of place conditioning scores as in the present experiment. One report divided adult rats into high and low responders based on their locomotor activity in the place conditioning chamber during a preconditioning trial (Shimosato and Watanabe, 2003) yet is still classified as individual differences in novel-environment-induced activity. A recent report from our lab revealed adolescents that were low responders for free choice novelty showed more robust cocaine place preferences than adolescent high responders suggesting low responders for novelty have greater sensitivity to the rewarding properties of cocaine (Stansfield and Kirstein, 2007). Furthermore, adult high responders for novel-environment induced activity show more robust psychostimulant induced place preferences (Klebaur and Bardo, 1999). Phenotypic differences in novelty that predict sensitivity to cocaine reward could be associated with phenotypic differences in cocaine reward. It would seem likely neurochemical factors mediating high responsivity to novelty are also present in those vulnerable to drug-seeking behaviors as well as in adolescents exhibiting high responses for cocaine reward.

Underlying physiological mechanisms mediating differences in low and high responders for cocaine reward in developing rats from the present study could be one of several potential factors. Differences in dopaminergic response to drugs are likely given Stansfield and Kirstein (2005) showed adolescent high responders for novelty-induced exploration had higher cocaine-induced DA levels than low responders, an effect that

differed from adult comparisons regardless of phenotype. High responders for cocaine reward in the present study are likely to have a greater dopaminergic response to drugs of abuse than low responders given adult Lewis rats have greater cocaine-induced DA levels in the NAcc (Cadoni and Di Chiara, 2007). High responders for novel-environment-induced locomotor activity have greater maximal peak levels of cocaine-induced DA in the NAcc (Chefer et al, 2003). Interestingly, high responders for cocaine self-administration had moderate accumbens DA levels while low responders either had lower or higher accumbal DA levels suggesting non-monotonic or moderate levels of DA were optimal for producing maximal operant responding for cocaine (Glick et al, 1994). Although the present microdialysis data for cocaine-induced DA did not reach statistical significance for an effect of Phenotype, it does not rule out the fact cocaine-induced DA could be mediated by phenotype in a dose dependent manner. Furthermore, the aforementioned studies split subjects into high and low responders based on either genotype (Cadoni and Di Chiara, 2007) or reactivity to novelty (Chefer et al, 2003) while the present experiments split subjects according to place preference scores.

Another plausible factor mediating individual differences in cocaine reward for developing rats could be the DAT. High and low responders for cocaine-induced locomotor activity showed differences in the rate of DA clearance (Sabeti et al, 2003). Whether affinity or density of the DAT are likely factors mediating differences between high and low responders for cocaine reward is difficult to determine given mixed results in the kinetics of the DAT have been found (Briegleb et al, 2004; Chefer et al, 2003; Dietz et al, 2005; Flores et al, 1998). No differences in K_i or B_{max} were found between high and low responders for cocaine-induced locomotor activity (Briegleb et al, 2004) while basal DA uptake and affinity for the DAT was reduced in high responders for novel environment-induced locomotor activity (Chefer et al, 2003). Another report showed

high responders for novel environment-induced locomotor activity had a greater density of DAT in the VTA and substantia nigra (Dietz et al, 2005) but Lewis rats, had lower levels of D2 receptors and DATs in the mesolimbic pathway (Flores et al, 1998). Other brain regions, such as the PFC, show decreased cell surface expression of DATs in high responders for novel-environment induced locomotor activity (Zhu et al, 2007)

Individual differences in cocaine reward for developing rats may also be attributed to levels of tyrosine hydroxylase. Lewis rats had greater amounts of tyrosine hydroxylase in the VTA but less in the NAcc than their 'addiction resistant' Fischer comparisons (Bietner-Johnson et al, 1991; Haile et al, 2001). However, high and low responders for novel-environment-induced locomotor activity had similar levels of tyrosine hydroxylase in the VTA and substantia nigra (Dietz et al, 2005). High responders interestingly had a larger storage pool for DA accompanied by more VMAT (Verheij et al, 2008).

Whether individual differences in cocaine reward in developing rat were due to pharmacokinetics are not clearly understood due to the lack of data in adolescence and mixed results in adults (Caster, et al, 2005; Piazza et al, 2000). Brain cocaine levels did not differ in adolescents (Caster et al, 2005) nor between drug vulnerable and drug resistant phenotypes suggesting individual differences were not due to differences in the pharmacokinetics of cocaine (Piazza et al, 2000; Kosten et al, 1997; Gulley et al, 2003). Others showed Lewis and Fischer rats had different plasma levels of cocaine (Camp et al, 1994).

Due to the paucity of data investigating individual differences in cocaine reward based on cocaine place preference scores, it is not currently understood what physiological mechanisms mediate individual differences in adults or developing rodents. Future research is needed to explore neurophysiological, neurochemical and

neuroanatomical differences in high and low responders for cocaine reward. These measures can be used as predictors of future vulnerability to drug dependency.

Cocaine-induced reinstatement of place conditioning has been previously shown in both adolescent and adult rodents (Balda et al, 2006; Brenhouse and Andersen, 2008). Cocaine induced reinstatement of place conditioning was more robust in adolescent rats following pretreatment with a lower cocaine dose (Brenhouse and Andersen, 2008). Even if adolescents were followed into adulthood, cocaine priming injections were still able to reinstate place preferences (Balda et al, 2006). However no one has examined the involvement of mesolimbic D2 receptors in the reinstatement of cocaine place preferences in developing rats or whether these effects manifest differently in high and low responders. The present study revealed intra-VTA, but not intra-NAcc, infusions of the D2 antagonist sulpiride blocked cocaine-induced reinstatement of cocaine place preferences only in rats classified as high responders for cocaine reward during adolescence. Individual differences in psychostimulant-induced reinstatement were previously shown in adult rodents suggesting high responders exhibit a more robust reinstatement (Homberg et al, 2004). Strain differences in the ability of either intra-NAcc AMPA or systemic cocaine to induce reinstatement of cocaine self-administration was found with Lewis rats showing more robust reinstatement at lower doses of AMPA while Fischer rats showed more robust reinstatement at higher AMPA doses (Kruzich and Xi, 2006).

Cue-induced locomotor activity during the place preference expression test had a similar but non-significant pattern to cocaine place preferences only in high responders (Figure 9a). Unlike what was seen for place conditioning, cue-induced locomotor activity was similar between high and low responders with both phenotypes showing main effects of Session and Pretreatment but not significant interactions. The present data

are unable to determine whether cue-induced locomotor activity could be contributing to place preferences seen in high responders; however it is unlikely given low responders showed an effect of cocaine only on locomotor activity (Figure 9b) and not on place conditioning (Figure 4b). Furthermore, cocaine-induced reinstatement of cocaine place conditioning was not associated with cocaine-induced locomotor activity during the reinstatement test, regardless of phenotype (Figures 11-12). Similar effects have been shown in adult rodents suggesting a dissociation between conditioned place preference and locomotor activity as well as cue-induced locomotor activity (Kosten and Miserendino, 1998; van de Kam et al, 2006). In adult rodents, DA agonists/antagonists alter reinstatement of cocaine seeking. These data suggest DA receptors are involved in mediating reinstatement but not cocaine-induced locomotor activity in developing rats.

Since D2 receptors were found to mediate cocaine-induced reinstatement of cocaine place conditioning in developing rats, it is likely high and low responders for cocaine reward differ in DA receptor density, functionality or intracellular signaling mechanisms. D1 and D2 receptors differentially mediate sensitivity to cocaine in Lewis and Fischer rats (Haile and Kosten, 2001) and could also demonstrate phenotypic differences in cocaine-induced reinstatement in developing rats from the present study. Phenotypic differences in developing rats are likely potentiated by altered levels of D2 receptors or Gi-alpha proteins in the VTA that typically couple to D2 receptors and inhibit adenylyl cyclase. Given the present data showed D2 antagonist induced blockade of reinstatement via the VTA but not NAcc, atypical functionality or density of D2 receptors may be even more pronounced in the VTA than in the NAcc of high responders for cocaine reward. Furthermore, high responders for novel-environment-induced locomotor activity needed higher doses of the D2 agonist quinpirole in the somatodendritic field to inhibit firing of midbrain DA cells (Marinelli and White, 2000).

These data suggest D2 receptors specifically in the VTA of rodents previously exposed to cocaine during adolescence may mediate phenotypic differences in cocaine sensitivity.

Repeated cocaine exposure during adolescence did not alter basal DA levels in the NAcc later on in adulthood and seems plausible given only 4 cocaine injections were administered during adolescence (Figure 13-14). Local infusions of sulpiride into either the VTA or NAcc attenuated cocaine-induced increases in accumbal DA levels in rats previously exposed to cocaine during adolescence (Figures 15-18). The attenuating effect of intra-NAcc sulpiride on cocaine-induced DA was evident in both high and low responders for cocaine reward while intra-VTA sulpiride significantly attenuated cocaine-induced DA in high responders and only approached significance in low responders.

To our knowledge, no one has investigated the effects of the present pharmacological manipulations on cocaine-induced DA in developing rodents. However, much research has been conducted on the involvement of DA receptors/transporters in both basal and cocaine-induced DA levels for adult rodents (Beyer and Steketee, 2000; Chen and Pan, 2000). Intra-VTA infusions of D1 antagonists or systemic D2 antagonists blocked psychostimulant-induced increases in NAcc DA (Veznia, 1996; Parsons et al, 1993) while others have shown intra-VTA or intra-NAcc D2 antagonist infusions increased cocaine-induced DA levels (Yan, 2003; Tanabe et al, 2004). Co-infusion of D2 antagonists and another DAT blocker, GBR12909, blocked of accumbal DA levels (Rahman et al, 2001) suggesting effects of D2 antagonists on DA levels are brain region and drug specific.

One mechanism mediating decreased DA following cocaine and sulpiride may be that DA cells were overexcited and developed depolarization block, a well know neurophysiological result from too much excitation. In cells exhibiting depolarization

block, the membrane potential becomes so depolarized the cell stops or slows firing in order to compensate for overexcitation (Grace and Bunney, 1986). This is a likely factor given D2 antagonists into the VTA should block autoinhibition of DA cells via D2 autoreceptors and decrease negative feedback mechanisms (Kohl, et al, 1998). Electrophysiological data would be ideal for corroborating this theory. Furthermore, it is not known whether depolarization block could occur following intra-NAcc infusion of D2 antagonist by way of blocking terminal release of accumbal DA. It should also be noted local infusion of D2 antagonist into the VTA or NAcc only partially reduced cocaine-induced DA and was irrespective of phenotype. Further data are needed to determine whether other DAergic mechanisms or other neurotransmitters (glutamate, GABA) mediate cocaine-induced reinstatement of cocaine place conditioning in rats previously showing a place preference during adolescence.

Reinstatement and microdialysis data in the present studies nicely support current theories of cocaine-induced reinstatement related neurocircuitry. The VTA, NAcc core, and PFC have been shown to mediate cocaine-induced reinstatement in adult rodents by pharmacologically manipulating or reversibly inactivating target brain regions (McFarland et al, 2003; Di Ciano and Everitt, 2004; See, 2005). Hypothesized involvement of these key brain regions in cocaine-induced reinstatement is illustrated on the left side of Figures 19 and 20. Drugs of abuse enhance DA cell firing in the VTA and release DA into both the PFC and NAcc. DA has an inhibitory effect on glutamatergic projection cells from the PFC to the NAcc core thereby removing tonic excitation of NAcc core cells. GABAergic cells in the NAcc core are inhibited thus removing inhibition of downstream motor circuitry and enhancing approach behavior towards a salient stimulus. The right side of Figure 19 illustrates VTA data from the present paper in combination with hypothesized reinstatement related circuitry. Intra-VTA infusion of D2

antagonist may have overexcited DA cells and produced a depolarization block. This effect reduced DA levels in the PFC as well as in the NAcc, as revealed in the present paper. Decreased DA release in the PFC provided less inhibition and therefore allowed cortical glutamate projection cells to excite NAcc core cells. Excitation of NAcc core GABAergic cells send inhibitory signals to downstream motor circuitry and block approach behavior towards a salient stimulus. Less approach behavior was manifested as blocked cocaine-induced reinstatement. The right side of Figure 20 illustrates NAcc data from the present paper in combination with hypothesized reinstatement related circuitry. Intra-NAcc infusion of D2 antagonist overexcited DA terminals and produced a depolarization block. Since The VTA was not effected, the VTA-PFC pathway should respond with an optimal amount of inhibition on the PFC. Given PFC glutamatergic innervation of the NAcc core is optimal and VTA DA innervation of the NAcc core is dampened (i.e., less inhibition), then the overall net effect on the NAcc core is moderate excitation. However, this would lead to a blockade of approach behavior and reinstatement, an effect not supported by the present data. Therefore, an alternative mechanism must occur to inhibit GABAergic cell firing in the NAcc core. Plausible alternative mechanisms inhibiting the NAcc could be altered sensitivity of intracellular signaling mechanisms, ion channels or the functionality of D1, NMDA, and AMPA receptors. Changes in a third mechanism must be needed in order to see unaltered cocaine-induced reinstatement following intra-NAcc infusions of D2 antagonist.

Figure 19: Relating VTA Data to Reinstatement Circuitry

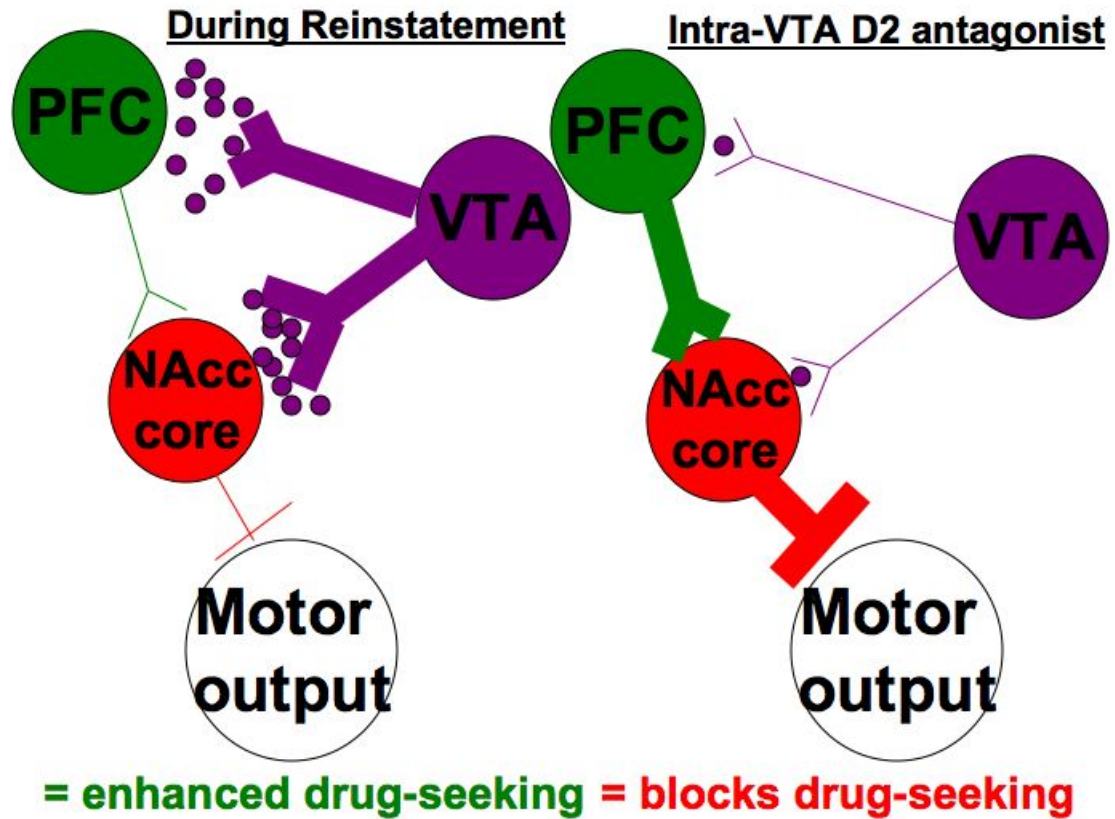


Figure 19: Hypothesized involvement of key brain regions in cocaine-induced reinstatement is illustrated on the left side. Drugs of abuse enhance DA cell firing in the VTA and release DA into both the PFC and NAcc. DA has an inhibitory effect on glutamatergic projection cells from the PFC to the NAcc core thereby removing tonic excitation of NAcc core cells. GABAergic cells in the NAcc core are inhibited thus removing inhibition of downstream motor circuitry and enhancing approach behavior towards a salient stimulus. The right side of the Figure illustrates VTA data from the present paper in combination with hypothesized reinstatement related circuitry. Intra-VTA infusion of D2 antagonist overexcited DA cells and produced a depolarization block. This effect reduced DA levels in the PFC as well as in the NAcc, as revealed in the present paper. Decreased DA release in the PFC provided less inhibition and therefore allowed cortical glutamate projection cells to excite NAcc core cells. Excitation of NAcc core GABAergic cells send inhibitory signals to downstream motor circuitry and block approach behavior towards a salient stimulus. Less approach behavior was manifested as blocked cocaine-induced reinstatement.

Figure 20: Relating NAcc Data to Reinstatement Circuitry

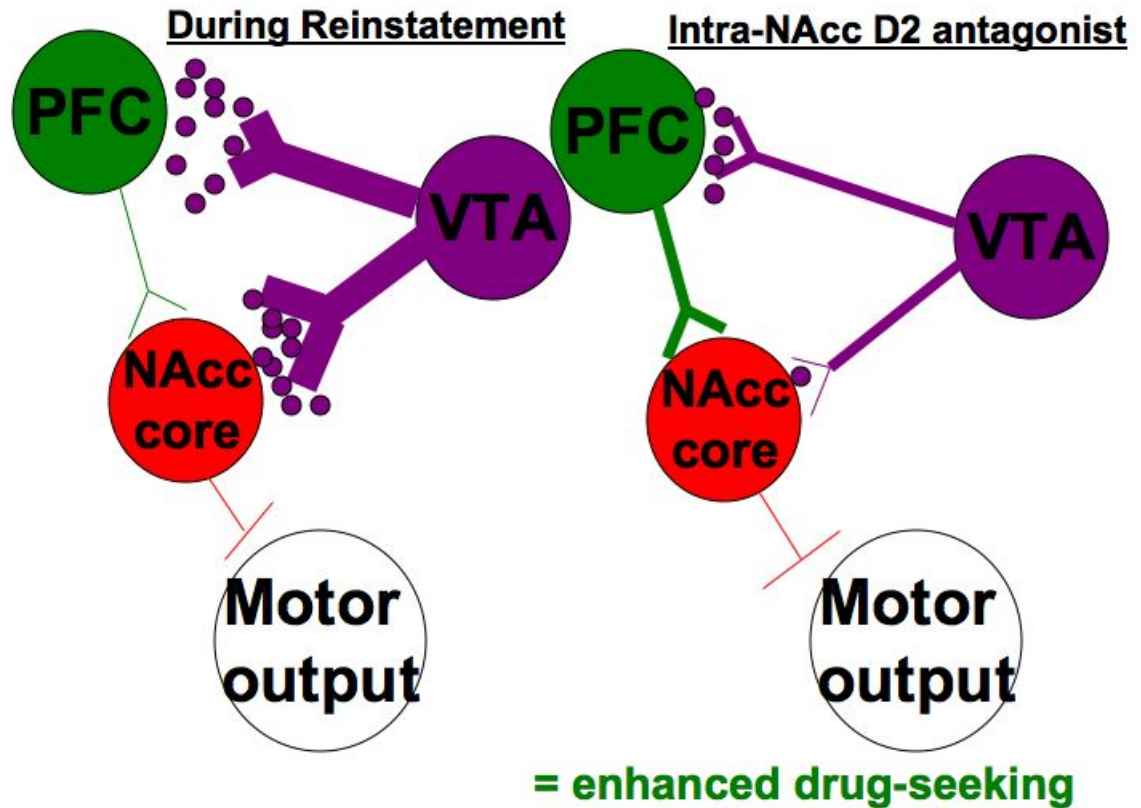


Figure 20: Hypothesized involvement of key brain regions in cocaine-induced reinstatement is illustrated on the left side. Drugs of abuse enhance DA cell firing in the VTA and release DA into both the PFC and NAcc. DA has an inhibitory effect on glutamatergic projection cells from the PFC to the NAcc core thereby removing tonic excitation of NAcc core cells. GABAergic cells in the NAcc core are inhibited thus removing inhibition of downstream motor circuitry and enhancing approach behavior towards a salient stimulus. The right side of the Figure illustrates NAcc data from the present paper in combination with hypothesized reinstatement related circuitry. Intra-NAcc infusion of D2 antagonist overexcited DA terminals and produced a depolarization block. Since The VTA was not effected, the VTA-PFC pathway should respond with an optimal amount of inhibition on the PFC. Given PFC glutamatergic innervation of the NAcc core is optimal and VTA DA innervation of the NAcc core is dampened (i.e., less inhibition), then the overall net effect on the NAcc core is moderate excitation. However, this would lead to a blockade of approach behavior and reinstatement, an effect not supported by the present data. Therefore, an alternative mechanism must occur to inhibit GABAergic cell firing in the NAcc core.

Human adolescent brains undergo a physiological shift in primary brain activity from the limbic system during adolescence to more involvement of cortical areas in adulthood (Lewis et al, 1997). Together, the limbic mediated adolescent brain and fluctuating DA levels in the NAcc (Badanich et al, 2006) would support the hypothesis adolescence is a time of transitional neuronal activity in the mesolimbic system. These data support studies comparing DA activity in the NAcc and PFC of adolescent rats. Spear (2000) discusses the inverse relationship between DA activity in the NAcc and PFC with early adolescent rats (PND 30) showing greater PFC and less NAcc DA activity than older adolescent rats (PND 40). Similarly, rats expressing high DA levels also have heightened basal firing patterns in the mesolimbic system (Grace et al., 1995). These heightened basal concentrations, or tonic DA release, are regulated by glutamatergic afferents from the PFC, hippocampus and amygdala (Grace, 2000; O'Donnell et al, 1999). Brenhouse and colleagues (2008) reported the density of glutamatergic projection cells from the PFC to the NAcc core progressively increased across age with adults having greater cortical innervation of the NAcc than younger rats. Additionally, D1 receptor expression on these glutamatergic projection cells peaked during adolescence as compared to younger and older rats. Given the VTA, dPFC and NAcc core mediate cocaine-induced reinstatement (Kalivas and McFarland, 2003; Cornish and Kalivas, 1999; De Vries et al, 1999; Capriles et al, 2002; McLaughlin and See, 2003; Everitt and Wolf, 2002) and it is these same brain regions undergoing developmental transitions during adolescence (Brenhouse et al, 2008), investigating the involvement of these brain regions on reinstatement in the developing rodent was warranted.

Taken together, the present series of experiments suggest individual differences in cocaine place conditioning are present during adolescence. The ability of the place conditioning paradigm to reveal individual differences is key given not all humans respond to drugs in the same manner. Rats classified as high responders for cocaine reward were more likely to show cocaine-induced reinstatement of cocaine place conditioning. Reinstatement in high responders was blocked by intra-VTA, but not intra-NAcc, D2 antagonist infusions. Interestingly, intra-VTA and intra-NAcc infusions of the D2 antagonist attenuated cocaine-induced increases in accumbal DA levels, an effect that was irrespective of phenotype. Investigating the interaction between phenotype and environment is an important step towards modeling human drug use patterns during adolescence and understanding the long-term development of drug dependency. Understanding individual differences in behavioral and neurochemical responses to factors inducing drug relapse will aid in the development of more effective treatment strategies.

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Appendices

Appendix A: Abbreviation List

5-HT = 5-hydroxy tryptamine
6-OHDA = 6-hydroxydopamine
aCSF = artificial cerebrospinal fluid
ANOVA = analyses of variance
BLA = basolateral amygdala
CPP = conditioned place preference
DA = dopamine
DA_{in} = amount of DA perfused
DA_{out} = amount of DA in dialysate
DAT = dopamine transporter
Ed = extraction fraction
FI = fixed interval
FR = fixed ratio
GABA = gamma-amino-butyric acid
HPLC = high performance liquid chromatography
ICSS = intracranial self stimulation
min = minute
NAcc = nucleus accumbens septi
NE = norepinephrine
NMDA = N-methyl-D-aspartate
PND = postnatal day
PR = pre response (for electrophysiological data)
PR = progressive ratio (for operant conditioning data)
PR+RF = pre response + reinforcement
RFe = reinforcement excitation
RFi reinforcement inhibition
VTA = ventral tegmental area
nA nanoamp
μL = microliter
nM = nanomole
μM = micromole
mM = millimole

About the Author

Kimberly A. Badanich was born in Pittsburgh PA, received a B.S. in Psychology at the College of Charleston in Charleston SC, and will receive her Ph.D. in Neuroscience from the University of South Florida, Tampa FL. She can't wait to start her post-docotoral position at the Medical University of South Carolina in Charleston SC were she accepted a fellowship on a NIAAA T32 training grant. She is way too exhausted to write anymore details about herself...