IN UTERO COCAINE EXPOSURE AND PERSISTENT CHANGES IN COGNITION AND NEUROCHEMICAL MODULATION

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A growing body of evidence suggests that the cognitive changes produced by *in utero* cocaine exposure are subtle but functionally significant. The present studies were designed to investigate the influence of the timing and duration of exposure as well as the underlying neural mechanisms. In study one, rats were exposed to cocaine during either early or late in gestation or both ("full" exposure) and, in adulthood, tested on a series of extradimensional shift (EDS) tasks designed to tap attention and arousal. The pattern of changes during the early portion of the learning process indicated that for the cocaine-exposed animals, regardless of timing and duration of exposure, attention was captured by the most salient cues in the environment, which then affected attentional set formation and ease of shifting when task contingencies changed. In addition, both early- and "full"-exposed animals exhibited changes during the final learning phase indicative of impaired selective attention. In a second study, two doses of cocaine were explored: (1) 3.0 mg/kg cocaine once daily GD8-21 (1X COC) and (2) 3.0 mg/kg once/day GD8-16 and twice/day GD16-21 (2X COC). The pattern of findings suggested that the higher dose of cocaine significantly impaired transfer of learning involved in shifting attention, but only on EDS tasks in which the predictive stimuli were subtle relative to distractors. The lower cocaine dose impaired learning transfer both when distractors were salient and when they were subtle. Further, the lower dose of cocaine impaired selective attention. A final study examined correlations between density of α2 receptors in prefrontal cortex (PFC) for the controls and 1X COC group. This study revealed that although density of α2 receptors in PFC

did not differentiate the groups, nor did it predict performance in the control animals, this parameter did significantly predict performance of the COC rats. Specifically, those animals with low density of α2 receptors in PFC were significantly more impaired than those COC animals with high density of α2 receptors in PFC. These findings suggest that low α2 density in PFC may increase vulnerability to the lasting cognitive effects of prenatal cocaine exposure.

BIOGRAPHICAL SKETCH

Tara Lynn Benedetto was raised in Hammonton, New Jersey. Upon graduation from high school, she moved to Ithaca, New York, where she earned a B.A. in Biological Sciences in 2003 from the College of Arts and Sciences at Cornell University. Tara's academic interests center on neurotoxicology and behavior, neuropharmacology, and the developmental and psychosocial impacts of perinatal drug exposures.

This work is dedicated to my grandparents:

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CHAPTER ONE

EXPOSURE TO COCAINE *IN UTERO*: EXTENT OF THE PROBLEM

Historical Considerations

Cocaine is primarily derived from four *Erythroxylum* taxa: *E. coca* var. *coca*, *E. coca* var. *ipadu*, *E. novogranatense* var. *novogranatense*, and *E. novogranatense* var. *truxillense.* These taxa are indigenous to the Andean region of South America, including Colombia, Peru and Bolivia, but are also harvested in certain areas of Mexico and Hawaii (Johnson, Saunders, Mischke, Helling, & Emche, 2003; Johnson, Zhang, & Emche, 2005). Historically, the chewing of coca leaves was restricted to religious and ceremonial occasions among the Incans, where it was revered for its powerful stimulation properties (Meyer & Quenzer, 2005). The pharmacologically active alkaloid found in coca leaves began to be distributed for medical and recreational purposes after being isolated by a pharmaceutical company in 1859 (Julien, 1998). Use among Europeans and Americans grew throughout the late 19th century, when cocaine use was championed by many public figures, the most notable of which was Sigmund Freud (Meyer & Quenzer, 2005). The psychotropic effects of cocaine were widely marketed for recreational use, where products such as Vin Mariani (a combination of cocaine and ethanol) and Coca-Cola (a mixture of cocaine and caffeine) became popular. Medicinally, the local anesthetic properties of cocaine made the drug a popular relief to infant teething discomfort and surgical procedures (Meyer & Quenzer, 2005).

By the early 20th century, the negative effects of cocaine became widely recognized, and even its former proponents publicly distanced themselves from the drug (Meyer & Quenzer, 2005). Cocaine addiction was reported to cause psychosis, toxicity, and, ultimately, death (Steele, 1872). In response to the increasing concern

about the adverse effects of cocaine use, Congress passed the first law regulating this drug in 1914, the Harrison Narcotic Act, which banned the drug both medically and recreationally (Meyer & Quenzer, 2005). For the next 50 years, the popularity of cocaine remained relatively low, where it was restricted primarily among a select group of artists, musicians, and performers. However, a change in legislation in the mid-1960s induced a new surge in cocaine's popularity, when cocaine became less costly and more readily available than amphetamines (the "drug of choice" at the time) (Julien, 1998). Through the 1970s, the cost of cocaine limited its distribution primarily to the middle- and upper- classes, where it was considered the "champagne of drugs" (Meyer & Quenzer, 2005).

The advent of "crack" cocaine in the 1980s shifted the use of cocaine from the upper-class and avant-garde to the low-income, inner city poor. "Crack" was a free-base, smokeable form of cocaine that was much cheaper and more pure than the powdered drug. Pharmacologically, crack use (smoking) produced a remarkably different pharmacological profile than intranasal use (snorting). For the user, this translated into an immediate and extremely powerful "high" within five minutes of drug use, as opposed to the slower progression to a lower peak effect of the drug when snorted. (Meyer & Quenzer, 2005)

In 1985, according to the National Household Survey on Drug Abuse (NHSDA), approximately 7.1 million people admitted to using cocaine within the past year, and 1.7 million were classified as frequent users (this represents 0.8% of the population older than 12 years of age) (Substance Abuse and Mental Health Services Administration, 1997). By 1996, occasional users represented less than 0.3% of the population (Substance Abuse and Mental Health Services Administration, 1997). However, cocaine use again began to rise in 2000, and in 2006 there were 2.4 million

cocaine users, with approximately 682,000 specifically using crack (Substance Abuse and Mental Health Services Administration, 2007).

In a trend different from use of other drugs, crack has been found to be more prevalent among women of child-bearing age (Chavkin, 2001). In 1996, the rate of cocaine use was highest among those age 18-25 years old (2.0%) and age 26-34 years old (1.5%) (Substance Abuse and Mental Health Services Administration, 1997). At that time, the National Institute on Drug Abuse (NIDA) published results from the first nationally representative survey of drug use among pregnant women. The survey sampled 2,613 women who delivered babies in 52 urban and rural hospitals in 1992 to estimate the frequency of illicit drugs, alcohol and cigarettes used during pregnancy. These results estimated 1.1% of women in the United States, or approximately 45,000 individuals, used cocaine at some point during their pregnancy, with incidence of cocaine use higher among African-American women (4.5%) than Caucasian and Hispanic women (0.4% and 0.7%, respectively) (Mathias, 1995).

Between the mid-1980s and early-1990s, hundreds of articles appeared in popular media reporting a crack-baby "epidemic" (Glenn, 2006). The conclusions of these articles, which sensationalized both the incidence and severity of gestational cocaine exposure, were based only on limited anecdotal evidence and were not supported by any empirical, controlled findings (Slotkin, 1998). Despite a complete lack of any real proof, the articles classified so-called "crack babies" as a "biologic underclass" (Toufexis, 1991) who posed a societal dilemma "worse than smallpox" (Krauthammer, 1989). Rather than addressing the underlying social environment that contributed to the rising use of crack among pregnant women, these articles focused on the "negligence" of drug-using mothers. However, there are a number of underlying socioeconomic factors that may contribute to both the increased drug use

of these women and the increased incidence of developmental or cognitive abnormalities observed in their children (Litt & McNeil, 1997).

Indeed, cocaine-exposure is highly correlated with a number of social and economic confounders that may independently affect child development. Among these are maternal education and IQ, maternal employment status, access to and quality of prenatal care, stability of the postnatal environment, and use of multiple drugs (Chiriboga, 1998; Mayes, 1999). In consideration of these first two factors, the NHSDA assessed cocaine use based on education and employment status. In 1996, 1.3% of individuals 18 and older who had not completed high school reported using cocaine frequently, compared with 0.9% among those with just a high school education and 0.6% among those with some college education (Substance Abuse and Mental Health Services Administration, 1997). Similarly, the rate of current cocaine use was highest among the unemployed, as 2.4% of unemployed adults (age 18 and older) were cocaine users, compared with 1.1% and 0.9% of part-time and full-time employed adults, respectively (Substance Abuse and Mental Health Services Administration, 1997). These correlations between cocaine use and education or employment status have not changed appreciably since 1996 (Substance Abuse and Mental Health Services Administration, 2007).

Additionally, prenatal care is often lacking in the low socioeconomic areas in which cocaine use during pregnancy is prevalent. A women's health study conducted in New York City in 1990 estimated incidence of cocaine use to be 30-50% in urban women who lack prenatal care, as measured by cocaine metabolites in the urine (Chiriboga, 1998). Cocaine-using mothers report significantly lower weight gain during pregnancy, reflecting both maternal and fetal nutritional deficiencies (Bandstra et al., 2001). Prenatal care, including maternal nutrition and psychological care, is essential for appropriate development *in utero*, in order for the fetus to receive

adequate nutritional elements (e.g. folic acid) and to support the mother in abstaining from continued drug use. Further, substance-abusing pregnant women rarely use cocaine in isolation (Mayes, 1999). Cocaine-using mothers are more likely to use alcohol, cigarettes and/or marijuana (Bandstra, 2001a). In the NIDA survey, an estimated 9.5% of women used cigarettes, alcohol and cocaine during pregnancy; of those women who said they had not used cigarettes or alcohol, only 0.1% reported cocaine use (Mathias, 1995).

Comparability of Human and Animal Findings

In the mid-1990s, despite the increasing knowledge of prevalence of cocaine use, few controlled studies existed to elucidate the extent of physical and cognitive effects of *in utero* cocaine exposure. The initial reports of a "Crack Baby Syndrome," which suggested gross cognitive deficits and physical malformations associated with *in utero* cocaine exposure, had not be empirically substantiated (Chiriboga, 1998; Slotkin, 1998), and the long-term impacts of prenatal cocaine exposure are still unclear (Harvey, 2004). To provide greater insight into the long-lasting neurological, cognitive, and behavioral effects of gestational cocaine exposure, a number of studies in children exposed to cocaine have explored these outcomes over the past two decades. Although marked with a number of confounding factors and methodological issues, which will be discussed below, human research suggests a constellation of possible emotional, behavioral, and cognitive deficits (the putative "syndrome") related to cocaine exposure that is much more subtle and specific than originally suggested (Mayes, 1999; Slotkin, 1998).

Cocaine-exposed infants have shorter gestational ages, which may, in part, mediate the smaller growth of these newborns (Bandstra et al., 2001). It is of particular consequence that cocaine-exposed neonates are often smaller and delivered at an earlier gestational age than non-exposed infants, factors that may independently

be related to neural development and consequent behavioral deficit (Delaney-Black et al., 2000). For instance, premature birth in general is associated with cerebral palsy, learning disabilities, and developmental delays (Chiriboga, 1998; Morishima, Okutomi, Whittington, & Cooper, 2000).

Infants exposed to cocaine *in utero* demonstrate abnormal neurobehavior that represents disruption of several cognitive processes. A growing body of evidence suggests an association between prenatal cocaine exposure and altered arousal regulation (Mayes, Molfese, Key, & Hunter, 2005). These neonates show irritability, tachycardia, enhanced startle response, and diminished interactive behavior, as well as some motor deficits (e.g. tremors, hypertonia) (Alessandri, Sullivan, Imaizumi, & Lewis, 1993; Chiriboga, 1998; Gingras & O'Donnell, 1998). Early and prolonged cocaine exposure has also been associated with depressed cry characteristics and fewer facial expressions consistent with interest, joy, surprise, anger, and sadness, implying arousal dysregulation as early as 4 weeks old (Alessandri et al., 1993). The cocaine-associated effects on arousal seem to persist beyond infancy (Alessandri et al., 1993; Arendt, Angelopoulos, Salvator, & Singer, 1999; Azuma & Chasnoff, 1993; Bandstra, Morrow, Anthony, Accornero, & Fried, 2001), where older children experience rapid changes in heart rate associated with stimulation, increased arousal from sleep states and greater physiological lability (Mayes et al., 2005).

In general, global measures of IQ are not impaired in prenatally cocaine-exposed individuals. Some studies have found gender-specific impairments, with drug-exposed boys having lower IQ scores than unexposed boys but no differences seen in girls (Azuma & Chasnoff, 1993; Delaney-Black et al., 2004). IQ, however, is known to be profoundly affected by environmental variables, including quality of care-giving environment, so these inconsistencies may not be related to cocaine exposure at all (Frank et al., 2005). Instead, the association between cognitive

impairment and cocaine exposure implicates primarily attention and arousal regulatory processes, rather than more general deficits in learning ability.

Studies in young children exposed to cocaine *in utero* reveal deficits in both selective and sustained attention. Attentional disruption in cocaine-exposed children has most often been associated with conditions that are highly arousing and demand the greatest attentional control (Bendersky, Gambini, Lastella, Bennett, & Lewis, 2003; Savage, Brodsky, Malmud, Giannetta, & Hurt, 2005). Cocaine exposure has been associated with increased commission errors on the Continuous Performance Task as well as deficits on the Picture Deletion Task, the Object Assembly subtest and the Stroop test, all suggestive of attentional deficits in cocaine-exposed children (Bandstra, Morrow, Anthony, Accornero et al., 2001; Frank et al., 2005; Mayes et al., 2005; Noland et al., 2005). Teacher-assessed behavior scores also reveal a possible influence of cocaine on attention persisting into school age (Delaney-Black et al., 1998; Delaney-Black et al., 2000; Delaney-Black et al., 2004). These studies suggest that boys may be more sensitive to the long-lasting affects of prenatal cocaine exposure, as they were more likely to score in the "clinically significant range" on aggressive, hyperactive and attention deficit behaviors, although girls also demonstrate a greater tendency to inappropriately externalize behavior (Delaney-Black et al., 2000; Delaney-Black et al., 2004).

Even with reports from humans indicating an association between prenatal cocaine exposure and subtle disruptions in attention and arousal, the mechanism of impairment and the permanence of any deficit still remain to be elucidated.
Human studies fall short of providing causal mechanisms because of the number of uncontrollable factors inherent in evaluating a human population. As discussed above, cocaine-using mothers are more likely to fall victim to many other socioeconomic and physical conditions than their non-using counterparts. Many human studies employ

statistical techniques to control for these variables, but these do not entirely remove the impact of external factors on measured outcomes. For example, some research has shown that cocaine-using mothers withdraw from their infants, neglecting them or not providing sufficient emotional support, which may impact a number of behavioral outcomes (e.g. emotional regulation, internalizing vs. externalizing behaviors) (Slotkin, 1998), but this factor cannot be quantified, and thus cannot be experimentally controlled.

Additionally, determining cocaine use during pregnancy in a clinical sample is inherently unreliable; maternal self-report consistently identifies *less than half* of those individuals who have actually used the drug. Using biological markers (e.g. urine, meconium) in conjunction with self-report increases reliability of quantifying gestational drug exposure (Bandstra et al., 2001; Frank, Augustyn, & Zuckerman, 1998), but still provides limited information reflecting cocaine use only during later gestational stages. Further, these biological techniques do not provide information on the potency and purity of the drug used, which likely varies with each use within and between subjects (Frank et al., 1998).

Animal models provide an opportunity to control for these variables and thereby assess a direct cause/effect relationship between prenatal cocaine-exposure and long-term behavioral effects. Although the results of these animal studies have been somewhat inconsistent (as reviewed in Mayes 1999, 2005), several investigations have specifically implicated changes in attention (Gabriel & Taylor, 1998; Garavan et al., 2000; Gendle et al., 2003; Gendle et al., 2004; Romano & Harvey, 1998) and arousal (Gendle et al., 2003; Gendle et al., 2004; Morgan et al., 2002; Overstreet et al., 2000; Spear et al., 1989; Spear, Campbell, Snyder, Silveri, & Katovic, 1998) in cocaine-exposed animals. The inconsistencies observed in animal models stems, in part, from the route of administration used in early (1990s) studies of prenatal cocaine

exposure, which is critical to the pharmacokinetic profile produced and, ultimately, the relevance and interpretability of findings. These earlier models primarily employed a subcutaneous route of drug administration in pregnant animals. Because of cocaine's vasoconstrictive properties in the periphery, this commonly used procedure resulted in extremely painful necrotic lesions at the injection site (Scott, Morrell, & Vernotica, 1997). Subsequent maternal stress was measurable, with cocaine-exposed exposed dams showing higher levels of circulating cortisol (Lima, Spindola, Dias, Tomaz, & Barros, 2008). This maternal stress response is of particular consequence here, as studies in the rat have shown that chronic stress may lead to altered development of neurochemical pathways also thought to be disrupted by prenatal cocaine. For example, animals exposed to uncontrollable stressors have demonstrated an increase NE innervation in the PFC, as well as dendritic retraction and loss of dendritic spines from PFC pyramidal cells. The net effect of these stress-induced prefrontal changes is weakened PFC cognitive function. (Arnsten, Scahill, & Findling, 2007)

To circumvent these stress-related confounders, and to produce more reliable and interpretable results in animal models of cocaine exposure, Mactutus and colleagues (1994) developed an intravenous (IV) administration model in rats that produces a pharmacokinetic profile remarkably similar to that seen in human IV exposure. Briefly, an IV catheter was surgically implanted into the jugular vein of nulliparous female rats. After conception, the catheter served as a port for the painless injection of cocaine HCl (for details of surgery and drug administration procedure, see Mactutus, Herman, and Booze (1994)). In this model, the pharmacokinetic profile in IV exposed rodents is dose-dependent and characterized by a rapid but transient peak in arterial concentrations of cocaine (Booze, Lehner, Wallace, Welch, & Mactutus, 1997). Further, maternal stress in this IV exposure procedure is minimized, with no

evidence of skin necrosis and no overt signs of toxicity in either the dams or the pups (Mactutus, Herman, & Booze, 1994; Mactutus, Booze, & Dowell, 2000).

The studies presented herein utilized this IV paradigm, which also produces a pattern of physiological responses similar to that observed in human users (e.g. increased heart rate and blood pressure) (Mactutus et al., 1994; Mactutus et al., 2000). While this administration procedure effectively models intravenous or intranasal intake in humans, we cannot necessarily extrapolate these findings to crack use. When crack cocaine is smoked, additional compounds are produced either as metabolites of the modified cocaine compound or as byproducts of the smoking procedure. These additional compounds include noranhydroecgonine methyl ester (a pyrolysis product) and anhydroecgonine ethyl ester (metabolite when crack is coadministered with ethanol) (Schindler, Tella, Erzouki, & Goldberg, 1995). Since these pyrolysis products are pharmacologically active and can cross the placenta, they may have significant developmental effects on the fetus which cannot be accounted for in our IV model.

Another caveat of the applicability of the current study to humans is that, here, cocaine was administered in isolation. This was done in order to elucidate specific effects of cocaine alone, which is an important investigative question. However, it is important to recognize that cocaine-using pregnant women rarely use the drug in isolation (Mayes, 1999), and most commonly report co-use of alcohol. Toxicologically, this represents a point of concern because co-administration of cocaine and alcohol produces a pharmacologically active metabolite, cocaethylene. While serum and liver microsomal carboxylesterases generally deactivate cocaine to non-pharmacologically active benzoylecogonine (BE), with co-adminstration of cocaine and ethanol they catalyze an ethyl transesterification to form cocaethylene, altering the metabolic profile (Bonate, Swann, & Silverman, 1996). That is, when

cocaine and ethanol are administered together, the amount of cocaine converted to BE is reduced by more than 50% than that produced when cocaine is administered alone; norcocaine (a normal metabolite five times as potent as the parent compound) formation is increased eight-fold with co-administration of alcohol and cocaine (Pan & Hedaya, 1999). Additionally, cocaethylene is more lipophilic than either cocaine or alcohol, and is thus able to quickly partition through the placenta, possibly exerting greater developmental effects on the fetus. Thus, the findings presented here are not necessarily applicable to considerations of human use that represent concurrent use of alcohol and cocaine, a caveat that must be noted when considering clinical relevance of findings from this rodent model.

Rationale and Goals of the Present Study

The present report details the results of two separate studies that examined persistent neurobehavioral changes associated with *in utero* cocaine exposure, using the IV rodent model described above. Previous work in this lab has revealed that animals exposed to low doses of cocaine during gestation exhibit long-lasting disruption in sustained and selective attention (Garavan et al., 2000; Gendle et al., 2003; Gendle et al., 2004; Morgan et al., 2002) and arousal dysregulation characterized by increased reactivity to errors (Gendle et al., 2003; Gendle et al., 2004; Morgan et al., 2002). Specifically, previous work has found that cocaine-exposed animals are particularly disrupted later in learning. On a sustained attention task, in which the duration, location and onset time of a brief visual cue was unpredictably varied, cocaine-exposed animals made more omission errors (failed to respond to the visual cue) at the end of the testing session and specifically on trials following an error. These treatment differences were attributed to failures in sustained attention and altered arousal regulation. (Gendle et al., 2003). Additionally, both Gendle (2004) and Garavan (2000) reported that cocaine-exposed animals are impaired in the ability to

filter out salient but irrelevant stimuli in the face of subtle, predictive cues, a deficit only observed in later phases of learning (Garavan et al., 2000; Gendle et al., 2004).

All animals evaluated here were tested in adulthood on an extensive battery of neurobehavioral tests, each designed to tap cognitive processes previously observed to be disrupted in cocaine-exposed subjects. The present report will focus on a series of extra-dimensional shift (EDS) tasks, in which animals were presented with stimuli from three dimensions (olfactory, spatial, and visual) of which only one cue-type was predictive of reward. The predictive dimension changed unpredictably after animals had achieved a high level of performance. Thus, this task series assessed attentional set formation (learning a bias towards a cue-dimension based on consistent response/reward parameters), attentional set-shifting (the ability to shift attention between dimensions upon an unexpected change in reward contingencies) and selective attention (filtering out irrelevant stimuli that is simultaneously presented with relevant cues).

The goals of the present study were three-fold. First, we aimed to assess whether there was a "sensitive period" of development in which cocaine exposure would exert its greatest effect on EDS performance. There is a small body of literature implicating changes in dopaminergic systems specific to later gestational exposure (Stanwood, Washington, & Levitt, 2001; Stanwood & Levitt, 2004) and morphological changes in noradrenergic systems specific to early gestational exposure (Snow et al., 2004). However, there is currently no research elucidating how the behavioral profile changes with these different periods of exposure. Therefore, we aimed to provide new information regarding alterations in neurobehavioral functioning by evaluating EDS performance in three treatment groups: early exposure (gestational days (GD) 8-15), late exposure (GD16-21) and early+late exposure ("full" GD8-21). Since there is a complete gap in the literature in regard to behavioral outcomes

associated with different periods of gestational exposure, all of our work regarding the early and late exposure groups was considered hypothesis-generating, rather than hypothesis-verifying; based on prior studies using the early+late exposure regimen, we expected a disruption in the "full" exposed animals to occur in the most attentionally-demanding tasks.

A second goal of these studies was to assess the cognitive profile associated with different doses of prenatal cocaine. We explored EDS behavioral outcomes in animals with a lower level of later-gestation exposure not previously investigated in this lab (i.e. animals were exposed only once per day from GD8-21). Additionally, we aimed to replicate prior findings in our "higher" exposure group, which represented the same exposure regimen as that used in previous studies, including the research presented in Chapter 2. This "higher" exposure group differed from the "lower" group only in drug administration frequency during the latter third of gestation (i.e.higher-exposed animals were exposed once per day GD8-15 and twice per day GD16-21). One of the most important contributions of this second investigation was the exploration of specific phases of the learning process in order to more thoroughly detail disrupted cognitive processes. General measures of learning rate, while useful, may obscure significant treatment effects that are only evident within one phase of learning (Garavan et al., 2000; Hilson & Strupp, 1997). Each learning phase (here, perseveration, chance, post-chance, and criterial phases) presumably taps different cognitive functions beyond that of general associative ability. That is, for the EDS tasks presented here, early learning phases likely employ associative ability as well as selective attention and attentional set shifting, while later learning phases more directly tap the ability to maintain focused attention in the face of distracting stimuli.

The final goal, and perhaps the most important, of the current report was to correlate behavioral outcomes and neural alterations <u>in the same animals.</u> While prior

studies have suggested that cocaine-associated deficits in executive functions are related to changes in the dopaminergic circuitry of the PFC and ACC (Mayes et al., 2005; Stanwood et al., 2001) and/or an upregulation of NE systems early in development (Arnsten et al., 2007; Mayes et al., 2005; Snow et al., 2004), there is very little information regarding direct correlations between altered behavior and underlying changes in neurochemical systems. Thus, the third chapter of this report will specifically address the relationship between prefrontal density of α2 receptors and EDS deficits associated with low-dose cocaine exposure.

The methods, results, and interpretation of cocaine-related EDS performance deficits and correlations with neural outcomes will be presented here in journal format.

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CHAPTER 2

IDENTIFICATION OF A SENSITIVE PERIOD OF *IN UTERO* COCAINE EXPOSURE: EVIDENCE FROM EXTRA-DIMENSIONAL SHIFT TASKS.

ABSTRACT

A growing body of evidence in both humans and animals implicates a constellation of effects associated with prenatal cocaine exposure that is both subtle and long-lasting, however little is currently known about the importance of timing and duration of prenatal cocaine exposure on cognitive outcomes. The present study was designed to evaluate the effects of *in utero* cocaine exposure in three exposure periods on performance in a series of extra-dimensional shift (EDS) tasks. Cocaine was administered intravenously to pregnant dams in three regimens: early (gestational day (ED) 8-15) or late (ED 16-21) in gestation, or both early and late (GD8-21, "full" exposure); a fourth group received saline injections (GD8-21). Progeny were tested in adulthood on a series of tasks in which stimuli from three dimensions (olfactory, visual, spatial) were simultaneously presented on each trial; only one dimension was predictive of reward. The predictive dimension was unpredictably shifted to one of the two previously irrelevant sets of cues after a high level of performance was achieved. Nine EDS tasks were administered; on each task the groups were evaluated in two learning phases. All exposure groups showed a pattern of performance in "early learning" across the series that suggested a specific disruption in attentional set shifting when the previously predictive stimuli were salient, and an impairment in attentional set formation to cues that were subtle, irrespective of the timing and duration of exposure. In addition, cocaine exposure during GD8-15 (regardless of total exposure duration) produced deficits in selective attention in later learning phases when the distracting stimuli were salient, a process that was spared in animals with

cocaine exposure limited to late gestation. Overall, these findings demonstrate that prenatal exposure to very low doses of cocaine results in an increased attentional focus to salient cues, which can facilitate or impair attentional set formation or shifting depending on the relative salience of relevant and irrelevant stimuli, and that exposure early in gestation produces a deficit in selective attention that persists into later learning.

INTRODUCTION

The advent of crack-cocaine in the 1970s expanded the popularity of cocaine to a wider subset of Americans. Compared with the more expensive powdered cocaine, which was popular in elite and avant-garde subsets of the population, crack's lower cost, higher purity, and widespread availability made it the "drug of choice" in urban populations (Meyer & Quenzer, 2005). The Reagan administration's "War on Drugs" campaign focused specifically on crack cocaine use in these low-socioeconomic areas and emphasized the long-lasting impacts of the drug on children. Subsequently, a number of case reports in the popular media (i.e. Time, Newsweek) emerged suggesting gross physical malformations and severe cognitive deficits in children exposed to cocaine *in utero*. Based primarily on these anecdotal accounts, the media claimed the emergence of a 'crack baby epidemic' in American society, although the actual prevalence of drug-exposed children had never been measured (Glenn, 2006). To explore the assertions that the US was steeped in an epidemic of cocaine-exposed children, in 1992 the National Institute on Drug Abuse (NIDA) initiated an extensive survey on drug use among American women. They estimated that approximately 45,000 women in the United States (1.1%) had used cocaine at some point during pregnancy; this figure represents a conservative estimate, as actual use was likely underreported (Mathias, 1995). While this report revealed a very real

problem of *in utero* cocaine exposure, the NIDA findings indicated that the incidence was far from epidemic.

As the "epidemic" proportion of the crack baby problem was clearly a media creation, so were the media's claims of the lasting effects of severe physical and cognitive impairments attributable to prenatal cocaine exposure, which had not been sufficiently explored at that time (Slotkin, 1998). That is, although there had been a number of anecdotal accounts at the time of the NIDA publication, there were very few empirical studies investigating the severity or permanency of *in utero* cocaine exposure. To characterize the extent of physical and behavioral impairment associated with prenatal cocaine exposure, the scientific community initiated a number of prospective studies in children exposed to the drug *in utero*. Over the past two decades, these studies have provided insight into the behavioral deficits associated with prenatal cocaine exposure. Although these studies fall short of reflecting a causal relationship between cocaine and behavioral outcomes because of confounding factors inherent in human studies, they are suggestive of a remarkably specific cognitive profile in children exposed *in utero* (Mayes, 1999). The deficits observed in cocaine-exposed children implicate disruption specifically in attention and arousal regulation, while learning and memory functions seem to be spared. That is, global measures of IQ are generally not impaired in prenatally cocaine-exposed individuals (Delaney-Black et al., 1998; Frank et al., 2005), while a number of reports suggest failures in sustained and selective attention, inhibitory control, task persistence and emotional regulation.

Prenatal cocaine exposure has been associated with altered arousal as early as 4 weeks of age. Alessandri (1993) reported that cocaine-exposed infants demonstrated depressed cry characteristics and facial expressions incompatible with environmental stimuli, suggesting arousal dysregulation (Alessandri, Sullivan, Imaizumi, & Lewis,

1993). More recently, Karmel and colleagues also suggested failures in arousal associated with *in utero* cocaine exposure, observing that cocaine-exposed infants failed to appropriately respond to auditory asynchrony (Karmel, Gardner, & Freedland, 1998). Studies in older children suggest that these alterations in arousal are persistent, as evidenced by increases in frustration (Bendersky, Gambini, Lastella, Bennett, & Lewis, 2003) and externalizing behavior scores on a teacher-assessed behavior scale (PROBS-14) (Delaney-Black et al., 1998; Delaney-Black et al., 2000).

Prenatal cocaine exposure has also been associated with attentional impairments in exposed children as young as six months old. Gaultney, et al. (2005) reported an increase in off-task distractibility in cocaine-exposed children up to nine months of age. They concluded that the distractibility of exposed children cannot be explained by differences in novelty preference but rather by impairments in the attentional domain (Gaultney, Gingras, Martin, & DeBrule, 2005). Deficits in selective and sustained attention have also been reported beyond infancy, in children age 2-10. Recently, Pulsifier, et al. (2008) reported an association between prenatal cocaine exposure and visual attention and sequencing at five years of age (Pulsifer, Butz, O'Reilly Foran, & Belcher, 2008). Further support for attentional impairments at school age comes from a survey of first-graders asked to self-rate their behavior; cocaine-exposed children reported a higher frequency of behaviors associated with Attention Deficit Hyperactivity Disorder (Linares et al., 2006). Mayes (2005) has observed failures in selective attention as characterized by performance on the Stroop Colour-Word subtest in 7-9 year olds (Mayes, Molfese, Key, & Hunter, 2005). In the oldest cohort studied to date, Savage and colleagues (2005) reported increased commission errors on a distractibility task under conditions of high arousal and subpar performance on the Trail Making Task (indicative of sustained and selective attention, respectively) in cocaine-exposed 10-year-olds (Savage, Brodsky, Malmud, Giannetta,

& Hurt, 2005). Taken together, these studies suggest persistent alterations in attentional functions associated with *in utero* cocaine exposure.

While these studies have provided insight into the long-lasting deficits associated with prenatal cocaine exposure, they fail to provide a causal relationship between attention and arousal dysfunctions and cocaine-exposure. That is, there are a number of factors inherent in human studies that confound interpretation of observed group differences. Maternal variables such as education, IQ, and nutritional status, quality of pre- and post-natal care, and polydrug use often coexist with cocaine use during pregnancy and have been shown to effect behavioral outcomes (Mayes, 1999). Even when children are matched for age and socioeconomic status, the exposed and control groups often differ in these other ways, which have been shown to independently influence emotional and cognitive development. Animal models provide an opportunity to control for these confounding variables in order to establish a causal relationship between *in utero* cocaine exposure and behavioral outcomes.

Because animal models allow inference of causal relationships between drug exposure and behavior, a number of such studies have been conducted in the past two decades. While most have reported some level of cocaine effect on behavior, the specificity of deficits is largely inconsistent. Much of this inconsistency can be attributed to the route of drug administration employed in earlier investigations. In the 1990s, the most common route of administration in these prenatal cocaine exposure models was subcutaneous (SC) administration. SC administration, though widely used, poses a number of issues that complicate interpretability of results. SC administration produces necrotic skin lesions at the injection site, a result of the vasoconstrictive effects of cocaine, which are extremely painful and induce stress responses in the mother (Mactutus, Herman, & Booze, 1994). Recent work from Arnsten and colleagues revealed that maternal stress, on its own, can produce lasting

changes in the same neural systems thought to be affected by prenatal cocaine and may thereby influence observed behavioral deficits (Arnsten, Scahill, & Findling, 2007). Additionally, in order to replicate the peripheral and cognitive effects observed in human recreational use with SC animal models, extremely high doses of cocaine need to be administered (as high as 100 mg/kg). Even with these high doses, the resulting pharmacokinetic profile of circulating cocaine in the SC-exposed pregnant dams does not consistently produce the concentration:time profile (in brain and plasma) observed in humans upon injection or inhalation (the two most common methods of intake) (Mactutus et al., 1994; Mactutus, Booze, & Dowell, 2000). Because of these problems with SC administration, more recent animal studies have turned to an intravenous exposure protocol that accurately models the pharmacokinetic profile observed in human recreational users while minimizing maternal stress.

Studies using IV exposure in several species suggest an impairment of selective attention and reversal learning in prenatally exposed animals. A study of infant monkeys exposed to cocaine *in utero* revealed dysfunction in toy manipulation and orientation, suggesting altered sustained attention as early as 1 week of age (He, Bai, Champoux, Suomi, & Lidow, 2004). In one of the most comprehensive primate studies to date, Chelonis and colleagues (2003) investigated visual attention and reversal learning in rhesus monkeys from infancy (6 months) to nine years of age. They reported that cocaine-exposed monkeys were persistently impaired in reversal learning; the "escalating dose" group continued to show performance deficits for more than two years on the same task. Additionally, cocaine-exposure was significantly related to longer latency on a visual discrimination task, which Chelonis interpreted as impaired stimulus encoding and attention (Chelonis, Gillam, & Paule, 2003). Sustained and selective attention have also been found to be disrupted in cocaine-exposed rabbits. Romano and Harvey (1998) observed that cocaine-exposed animals

learned faster than controls when a salient cue (tone) was predictive and the simultaneously presented subtle stimulus (visual) was irrelevant. However, when the visual cue served as predictive and the tone was distracting, cocaine-exposed rabbits were impaired in learning. These findings suggest altered attentional processing in animals exposed to cocaine *in utero,* where attention is captured by the most salient environmental stimulus (Romano & Harvey, 1998), a conclusion supported by the work of Gabriel and Taylor (1998).

Studies in rodents have also contributed to evidence of cocaine exposure and attentional deficits. In a review by Mayes (2002), prenatal cocaine exposure was associated with increased perseveration, impaired performance on serial reversal tasks, and altered selective attention specifically when subtle predictive cues were presented with salient but irrelevant stimuli (Mayes, 2002). More recent findings in rats suggested altered sustained attention and reactivity to errors (Garavan et al., 2000; Gendle et al., 2003; Gendle et al., 2004; Morgan et al., 2002), as well as deficits in a visual discrimination task with olfactory distractors (Gendle et al., 2004).

Although these studies have demonstrated a causal relationship between impaired arousal/attention and *in utero* cocaine exposure, they focus on animals that were exposed to the drug throughout gestation. Relatively few studies have explored whether exposure during different periods of development produces a different constellation of effects and how the duration of exposure influences behavioral outcomes. In the rat, the development of dopaminergic and noradrenergic receptors occurs between gestational days 11-15 (depending on brain area), in a timeline that corresponds with the development of cocaine binding sites in the fetal rat brain (Ferris et al., 2007; Snow et al., 2004). Cocaine exposure during this critical period of neural maturation may produce permanent alterations in the distribution of receptor sites and monoaminergic neurotransmission (Mayes, 1999). Since monoamines have been

shown to act as trophic factors early in development, cocaine may interfere with developmental processes beyond those directly associated with cocaine-binding sites (Snow et al., 2004). Understanding the neural changes and associated behavioral alterations associated with exposure during different developmental periods can, therefore, allow better characterization of the nature of the deficits caused by *in utero* cocaine exposure.

Measures of cocaine use by trimester, as determined by maternal self-report, indicate that usage patterns are not constant across the duration of pregnancy. That is, approximately 22-24% of women reported cocaine (crack) use during the first trimester, but only 3-5% reported use into the second and third trimesters (Richardson, Conroy, & Day, 1996). Dose of cocaine used, too, has been reported to decrease over pregnancy, with an average use of 3.3 grams per month in the first trimester but only 0.1 grams and 0.2 grams in the second and third trimester, respectively (Snow et al., 2004). Based on these statistics, the societal implication of prenatal cocaine is greatest in regard to early (first trimester) exposure. When considered in conjunction with the developmental time line of neural systems, cocaine-exposure early in gestation may primarily effect the noradrenergic system, in which primary neurogenesis occurs in humans during the 5^{th}-6^{th} week of pregnancy (corresponding to approximately GD11-13 in the rat) (Snow et al., 2004). Dopaminergic development occurs slightly later, with first evidence of DA in frontal systems at 5.5 weeks and exponential increases through week 8 (Almqvist et al., 1996), corresponding to GD13-16 in the rat (Snow et al., 2004). Thus, the pattern of maternal cocaine use may differentially affect the developing child. Understanding how cocaine affects brain development and behavior when used at varying times or for differing durations of gestation is of critical importance both in developing appropriate preclinical models and when designing effective pharmacological interventions.

In humans, the assessment of maternal cocaine use (timing, dose, duration) is inherently unreliable, based either on maternal self-report and/or biological assays that insufficiently estimate exposure level across all three trimesters (meconium and urine analysis). Because quantifying these variables is impossible, there are presently no studies in humans that specifically assess a sensitive period in development and subsequent effects on behavioral or neural outcomes. Therefore, animal models are necessary to target specific developmental periods and ultimately may aid in developing effective pharmacological interventions.

The majority of available literature relating critical periods of development and *in utero* cocaine exposure comes from a rabbit model of IV cocaine exposure developed by Stanwood and colleagues. This research group recently identified a sensitive period of development in a rabbit model of IV cocaine exposure. In several replications, they exposed pregnant dams to cocaine either early (GD8-15), late (GD16-25), or early and late in gestation (GD8-25 or GD8-29) and examined subsequent morphological changes in the anterior cingulate cortex. These researchers reported no changes in animals exposed only early in gestation but significant alterations in corticogenesis in animals exposed on GD16-25, regardless of the total duration of exposure. That is, offspring of late-exposed and early+late-exposed animals had alterations in the development of pyramidal neurons and interneurons in the anterior cingulate cortex, suggesting that this later period of development (equivalent to late first trimester/early second trimester in humans) is particularly sensitive to cocaine's effects (Stanwood, Washington, & Levitt, 2001; Stanwood & Levitt, 2004). The anterior cingulate cortex is thought to underlie the attention and arousal regulatory functions altered in cocaine-exposed animals, but studies from Stanwood and Levitt did not directly investigate correlations between observed neural changes and behavioral deficits (Stanwood et al., 2001).

More recently, a rodent model assessing sensitive periods of development was developed (Snow et al., 2004). Snow and colleagues reported that animals exposed only early in gestation had shorter neurite length per cell and total neurite length in the LC immediately after birth. Animals exposed only in the later gestational period also showed a decrease in total neurite length relative to non-exposed animals, but animals exposed in both early and late gestation showed no developmental differences from controls. Thus, prenatal cocaine exposure interfered with LC neurite outgrowth when restricted to a short period of development; longer duration of exposure produced neural characteristics in the LC similar to controls (Snow et al., 2004).

While these previous studies have provided insight into neural changes related to prenatal cocaine exposure in specific developmental periods, the literature lacks any significant investigation of associated behavioral responses. The present study was designed to fill this gap in the literature by examining the behavioral effects caused by cocaine exposure during different developmental periods. We used a series of tasks designed to primarily tap attention and arousal regulation, functions found to be most sensitive to prenatal cocaine exposure in prior studies (from this lab and others). The present report will present the results of a series of extra-dimensional shift (EDS) tasks, which provided a measure of attentional control specifically in an animal's ability to switch attention between stimulus dimensions (visual, olfactory, spatial). For each task in the EDS series, one domain was predictive (e.g. visual) while stimuli from the other dimensions served as distractors. These EDS tasks tap a number of cognitive processes; of particular interest here are selective attention (filtering out irrelevant cues) and attentional set shifting (learning the new rule upon an unpredictable change in task contingencies). These tasks were expected to be sensitive to effects of prenatal cocaine exposure because prior work in rodents on a 2-choice EDS paradigm revealed specific deficits in distinct aspects of attention in cocaine-exposed animals.

Prior work in this lab demonstrated a cocaine-induced impairment in selective attention, specifically when salient distracting stimuli were presented prior to or simultaneously with relatively subtle relevant cues. Gendle and colleagues reported that animals exposed *in utero* to low doses of cocaine were significantly impaired on a visual attention task in which unpredictable olfactory distractors were presented while rats waited for a visual cue (Gendle et al., 2004). Garavan and colleagues also reported specific deficits in selective attention when olfactory stimuli served as distractors. The study by Garavan, et al. (2000) utilized a 2-choice EDS task in which the predictive cues were switched between olfactory and spatial dimensions. These investigators found that cocaine-exposed animals took significantly longer than controls to learn the spatial-predictive task when previously predictive olfactory stimuli were simultaneously presented; these animals were not impaired when the olfactory cues were predictive of reward despite the presence of previously predictive spatial cues. These investigators also looked at animals' performance within different phases of learning in order to elucidate the specific cognitive processes disrupted in cocaine-exposed animals. In-depth analysis revealed that performance on the spatial-predictive tasks was impaired for cocaine-exposed animals particularly in the later phases of learning, a deficit attributed to altered selective attention processes (Garavan et al., 2000).

In the current task series, we aimed to build upon the observation that cocaine-exposed animals' attention was "captured" by the most salient stimuli and to further investigate where in the learning process this attentional alteration occurred. To this end, the EDS procedure used in Garavan et al. was modified to increase the difficulty of the tasks and further challenge selective attention ability. We used a three-choice EDS paradigm, in which the predictive cues switched between olfactory, spatial and visual dimensions. The visual dimension was expected to be the most challenging for

all animals, as the visual domain is subdominant to olfaction in rodents. Because subtle visual cues were presented as the predictive stimuli with simultaneous olfactory distractors, we expected this task type to be particularly sensitive to selective attention deficits in cocaine-exposed animals.

METHODS

Subjects

Nulliparous Long–Evans rats were obtained from a commercial supplier (Harlan Sprague–Dawley, Indianapolis, IN) at approximately 11 weeks of age. These rats were maintained according to National Institutes of Health guidelines in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care. All breeding and surgical procedures were conducted at the University of Kentucky (Lexington). The animal facility at the University of Kentucky was maintained at 21± 2 °C, 50% ± 10% relative humidity, and had a 12-hr light/dark cycle, with lights on at 7:00AM. Food (Prolab RMH 1000, PMI Nutrition International, Brentwood, MO) and water were available ad libitum.

Prior to mating, a sterile intravenous catheter (22 gauge; Becton/Dickson, General Medical Corporation, Grand Prairie, TX) with a Luer-lock injection cap (Medex, Kensington, MD) was surgically implanted into the jugular vein to provide an IV port for drug administration after conception. Details of the catheters and surgical procedures used can be found in Mactutus, Herman, and Booze (1994).

Mating

After recovery from surgery (4-8 days), the females were group-housed (n=3) with a male rat. Conception (Gestational Day 0; GD0) was confirmed with a sperm-positive lavage. The impregnated females were then split into four treatment groups corresponding to the timing and duration of cocaine exposure.

Drug Administration

Drug injection procedures were conducted as described in Mactutus, Herman and Booze. (1994). Briefly, the dams were divided into four treatment groups: control (saline), early (GD8-14) exposure, late (GD15-21) exposure, or exposure spanning both of these gestational periods (GD8-21). (The early+late exposure group is referred to as "full" exposure in this report.) Cocaine hydrochloride (Research Triangle Institute, NC) or saline was administered as a bolus injection once per day from ED 8-14 and twice per day from ED 15-21, at a volume of 1 ml/kg (15s), followed by flushing (15s) the catheter with 0.2 mL of heparinized (2.5%) saline. All catheterized dams received daily IV saline injections from ED 1-7. See dosing regimen chart (Table 2.1). Cocaine treated animals (COC) were given the same dose of cocaine at each injection (3.0 mg/kg). The drug was dissolved daily immediately prior to injection. The decision to not administer cocaine from GD1-7 and to administer cocaine only once daily from GD8-14 was based on evidence that two injections per day may be fetotoxic, by compromising implantation or producing spontaneous abortion.

Table 2.1 Dosing regimen for pregnant dams

	GD8-14 (1x/day)	GD15-21 (2x/day)
Control	Saline	Saline
Early Exposure	Cocaine	Saline
Late Exposure	Saline	Cocaine
Full Exposure	Cocaine	Cocaine

The 3.0 mg/kg dose was selected to model human recreational use. In experimental conditions, humans will self-administer this dose multiple times in a 2.5-hour session, reflecting the appropriate cognitive and behavioral outcomes expected with "recreational" use (Fischman & Schuster, 1982). This dose yields peak arterial plasma levels similar to those reported for humans administered 32 mg of cocaine

intravenously (Booze, Lehner, Wallace, Welch, & Mactutus, 1997; Evans, Cone, & Henningfield, 1996). Additionally, the peripheral responses (e.g. acute increases in heart rate and blood pressure) in the pregnant rat administered 3.0 mg/kg are similar to those observed in other species (Mactutus et al., 2000) and the pharmacokinetic profile in the periphery is similar to that observed in human recreational users. In previous studies, this drug injection protocol (route, dose, and rate) produced no evidence of overt maternal or fetal toxicity, no maternal seizure activity, no effect on maternal weight, and no effect on offspring growth or mortality (Mactutus et al., 1994; Mactutus et al., 2000).

Offspring Care

After birth, litters were culled to four males and four females. After weaning on postnatal day (PND) 21, one male and one female offspring from each litter (86 litters total) were transported under environmentally controlled conditions to Cornell University, where behavioral testing was conducted. All animals were housed in same- sex pairs and placed on a reversed day/night schedule (lights off at 6:30AM, lights on at 9:30PM EST) to allow testing during the animals' active cycle. They were allowed to acclimate to the testing room and housing conditions for approximately three weeks prior to behavioral testing.

After the first week of acclimation, animals were placed on a food restriction schedule to accustom them to the feeding regimen used during behavioral testing. All females were initially restricted to 18 grams of rat chow (Pro-Lab Rat/Mouse/Hamster Chow) per day, and the males to 21 grams per day. Animals were monitored daily during testing for changes in body weight and motivation. Those whose response patterns indicated low motivation (evidenced by a high number of non-responses during testing) had their daily allotment reduced 1-3 grams as needed to increase motivation and still maintain a healthy body weight. Additionally, those animals

whose body weight consistently declined after acclimation had their daily allotment increased by 1-3 grams of chow per day. All modifications in food allotment were made by individuals blind to treatment conditions of the individual animals. Animals were tested six days a week (Sunday-Friday) for the duration of the study. On these days, the amount of food received via the food rewards in the task was subtracted from the animals' daily allotment of chow described above. Animals were allowed three hours immediately after testing to consume the remainder of their food individually before being returned to their home cage with their cage mate. On non-testing days (Saturdays), animals were given five hours to eat their food allowance. Tap water was provided ad libitum throughout the study.

This study was conducted in two successive replications (cohorts), resulting in the behavioral testing of 144 total offspring (72 animals/cohort). Within each cohort, the animals were balanced across sex and treatment condition, such that there were a total of nine animals in each of the eight treatment by sex sub-groups.

Apparatus

For each cohort, behavioral testing was conducted in 12 custom-built Plexiglas automated operant chambers, each housed in a wooden enclosure lined with sound-attenuating material, and controlled by a PC. Each testing chamber consisted of a rectangular waiting area (26.5 cm x 25 cm x 30 cm) with a smaller testing alcove extending from one wall. A motorized guillotine-type door controlled entrance into the alcove and prevented responses during the intertrial interval. Each of the three walls of the alcove contained a funnel-shaped port. The left and right ports were at an approximate 45-degree angle to the center port. In the behavioral tasks, a one second nosepoke into one of these ports constituted a 'choice'. A green light-emitting diode (LED) was located above each of the three ports in the alcove; illumination of one of these LEDs served as either the predictive cue or the visual distractor in the present

report. Additionally, the narrow end of each port was connected by tubing to three bottles containing liquid odorants, attached to a board placed outside of the box (9 bottles total). Compressed air was forced through the liquid odorants, allowing strawberry, rose, and lilac scents to be emitted into the testing chamber during the task. These odors served as either the predictive cue or the olfactory distractor in the current study. A set of infrared phototransistors and a light source monitored the entrance to the alcove and to each port. Correct responses were rewarded with a 45-mg Noyes food pellet delivered directly onto the alcove floor from a pellet dispenser.

Behavioral Testing Procedure

Within each cohort, each rat was assigned to one of the 12 testing chambers so that assignments were balanced across treatment groups. Each chamber was designated for rats of one sex.

Animals began training on PND 53. Prior to the onset of testing, the animals were trained to make a one second nose poke into the response ports to receive a food pellet (for details of training procedure, see Hilson & Strupp, 1997). As mentioned above, a 1-second nose poke constituted a 'choice' for all tasks presented. After completing the training tasks, behavioral testing began. Before being tested on the task series described in the present report, all animals had previously been tested on a series of visual attention tasks, a visual attention task with olfactory distractors, and an olfactory serial reversal task (for details of these prior tasks see (Gendle et al., 2003; Gendle et al., 2004). Animals began the EDS series described below on approximately PND 170.

Extra-Dimensional Shift (EDS) Tasks

The EDS series consisted of an initial olfactory-predictive task with irrelevant visual distractors followed by nine subsequent "shifts" in which the predictive dimension (olfactory, visual, spatial) was switched with each successive task. For each

task, entry of the rat into the testing alcove at trial onset produced the immediate illumination of one of the three LEDs and the emission of three odors (one from each port). The odor triad was always strawberry-rose-lilac and the port from which each was emitted varied pseudo-randomly. In the olfactory-predictive tasks (Tasks 1, 4, 7, 9), a correct response was a 1-second nosepoke to the port from which strawberry scent was emitted; the visual and spatial dimensions were irrelevant. Task 1 was considered the baseline olfactory task. This task was preceded by an olfactory serial reversal task; because olfactory cues were predictive in this prior task series, Task 1 does not constitute an extradimensional "shift." In the visual-predictive tasks (Tasks 2, 6, 10), the animal was rewarded for a response to the port above which the LED was illuminated; olfactory and spatial information were irrelevant. In the spatial-predictive tasks (Tasks 3, 5, 8), a correct response was a 1-second nose poke to the center funnel, irrespective of the locations of the visual and olfactory stimuli.

The olfactory and visual cues were presented continually for 60 seconds or until the animal made a response. A correct response was rewarded with delivery of a food pellet; there was no consequence of an incorrect response. If an animal failed to make a response after 60 seconds, a nontrial was scored. After the rat left the testing alcove following a response, the alcove door was lowered, followed by a 10 second intertrial interval. For all tasks, a daily testing session consisted of 200 response trials (trials on which the animal entered the alcove within 60 seconds after the door was raised) or two hours, whichever came first.

All animals completed the ten tasks of the EDS series in the same order, as illustrated below (Table 2.2). Animals were tested on a given task in the series until the learning criterion was reached – one session of ≥88% correct. Reward contingencies for each task type are summarized in Table 2.3.

Table 2.2 Order of tasks for all animals on EDS series

Task Number	Predictive Dimension
Task 1	Olfactory
Task 2	Visual
Task 3	Spatial
Task 4	Olfactory
Task 5	Spatial
Task 6	Visual
Task 7	Olfactory
Task 8	Spatial
Task 9	Olfactory
Task 10	Visual

Table 2.3 Reward contingencies for extra-dimensional shift tasks

	LED ILLUMINATED	ODOR EMITTED	SPATIAL LOCATION	Correct Response is:
Visual-predictive	Relevant	Irrelevant	Irrelevant	illuminated LED
Olfactory-predictive	Irrelevant	Relevant	Irrelevant	strawberry odor
Spatial-predictive	Irrelevant	Irrelevant	Relevant	center port

Dependent Measures

For each task in the EDS series, overall learning rate was assessed with errors to criterion, defined as the total number of errors (summed across sessions) each animal committed prior to reaching ≥88% correct in a single testing session. In-depth analyses of learning phases on an EDS series in another dataset (Chapter 3) suggested the importance of distinguishing between "early" versus "later" learning in characterizing the lasting effects of prenatal cocaine exposure on this task series. For that reason, we also analyzed the duration of early versus late learning phases. For this analysis, total errors were further divided into two blocks of learning. For each task, the trial-by-trial data was examined for each animal to determine the point at which an individual achieved eight consecutive correct responses. Errors made prior to this demarcation point were deemed "Block 1" errors; errors committed after this

point were classified as "Block 2" errors. To ensure that any observed differences were not solely due to this particular demarcation point, we also analyzed the duration of these two blocks using as the demarcation point strings of 5, 10, and 12 correct responses; these analyses confirmed that a string of 8 responses accurately captured the learning pattern in all animals.

Finally, we analyzed the duration of the "perseverative phase", which refers to a relative short period, immediately following the shifting of predictive dimensions, during which the rat consistently responded to the previously correct cue. Perseverative responding yields chance levels of performance which cannot be distinguished, based on percentage correct, from hypothesis-testing, random responding, or side biases. Therefore, in order to determine the duration of this perseverative responding to the previously predictive dimension, we calculated a moving average of responses (bin size 20) and identified the bin at which the average responses to the previously predictive cue fell below 55.8% (upperbound of "chance" level responding for bin size 20). The development of a side bias, or repeated responding to one port regardless of the location of olfactory and visual stimuli, was a common strategy observed in all animals upon a shift in task contingencies. Therefore, on tasks preceded by the spatial-predictive dimension, we could not differentiate between a response strategy that characterized "perseveration" and that representative of "side bias." To avoid erroneous classification of these errors committed early in learning, we did not evaluate a perseverative phase for tasks 4, 6, and 9, which were all preceded by a spatial-predictive task. An in-depth discussion of perseveration and side bias is presented in Appendix A.

Statistical Procedures

All statistical analyses were conducted with SAS v8.2 (SAS Institute, Cary, NC) for Windows 2000 Professional. A repeated measures analysis of variance

(ANOVA) was used to assess statistical significance, a procedure which considered within litter correlations (multiple rats from same dam) and within animal correlations (multiple observations for each rat).

For each dependent measure, we analyzed tasks within each predictive dimension independently from tasks in other predictive domains, such that visual-predictive tasks were evaluated separately from those in which olfactory or spatial were the predictive dimensions, etc. Task 1 was considered separately from the other olfactory-predictive tasks because it did not involve shifting between different relevant dimensions. All three cocaine-exposed groups and controls were analyzed in the same model. Pairwise comparisons of LSMeans between each cocaine group and controls was then evaluated for significant effects on each dependent measure.

Separate analyses were conducted for each of the three cocaine-exposure regimens versus the control group, such that higher order interactions involving treatment (if observed) would be more readily interpretable. The independent variables in each model included treatment condition, sex, cohort (1 or 2), task number (first, second, etc. for a given predictive dimension), and relevant interactions. The distributions of residuals and random effects were evaluated for normality to ensure model assumptions were satisfied. In cases where the assumptions of the parametric model could not be verified, a nonparametric test was used to minimize the influence of very high or very low data points. The significance level was set at 0.05.

It was determined *a priori* that each of the three cocaine-exposure groups would be compared to the controls for each task, regardless of whether a significant treatment effect or treatment by task interaction was detected. Because very little is known about the behavioral effects of cocaine during different periods of development, this study served as hypothesis-generating rather than hypothesis-verifying. In order to avoid overlooking functionally relevant impairments produced

by cocaine-exposure during a specific developmental period, we evaluated such comparisons within and between tasks even when fixed effects involving treatment did not meet our set significance level. For this reason borderline effects should be viewed as tentative and need to be replicated in future studies.

RESULTS

Body Weight

A main effect of sex was found for body weight across the duration of the EDS series [$F(1, 126) = 2583.11$, $p<.0001$], with the male animals weighing significantly more than the females (means: 460 vs. 283 grams). There was also an effect of treatment on mean body weight [$F(3,124)=2.84$, $p=0.04$]. None of the COC groups were significantly different from controls (all $p>0.2$ for COC vs controls), but animals exposed throughout gestation were of slightly lower weight than those animals in the early exposure group [$t(126)=2.15$, $p=0.03$] and the late exposure group [$t(126)=2.30$, $p=0.02$].

Effects of Cohort and Sex

For all dependent measures, we examined whether there was an interaction between treatment group and cohort (1 or 2). The treatment by cohort interaction was not significant for errors to criterion or errors in each block across the EDS series [all $p >0.1$]. We also evaluated whether there was a treatment by sex interaction; this term was not statistically significant for any of the outcomes measured in any task type [all $p>0.4$]. The three-way interaction between treatment, sex, and cohort was non-significant for all dependent outcomes measured, across the EDS series [all $p>.2$]. Thus, males and females from both cohorts were combined (n=144).

Baseline Olfactory-Predictive Task (Task 1)

For Task 1, there were no significant treatment differences in errors to criterion [$F(3,120)=1.03$, $p=0.4$] or Block 1 errors [$F(3,121)=0.11$, $p=0.9$]. There was,

however, a trend towards a treatment effect for Block 2 errors [F(3,122)=1.89, p=0.13]. Contrasts comparing each of the three cocaine exposed groups to the controls revealed that full-exposed animals committed more Block 2 errors than controls on Task 1 [p=0.03]; there were no differences in Block 2 performance between controls and the other two COC groups [all p>0.6] (Figure 2.1).

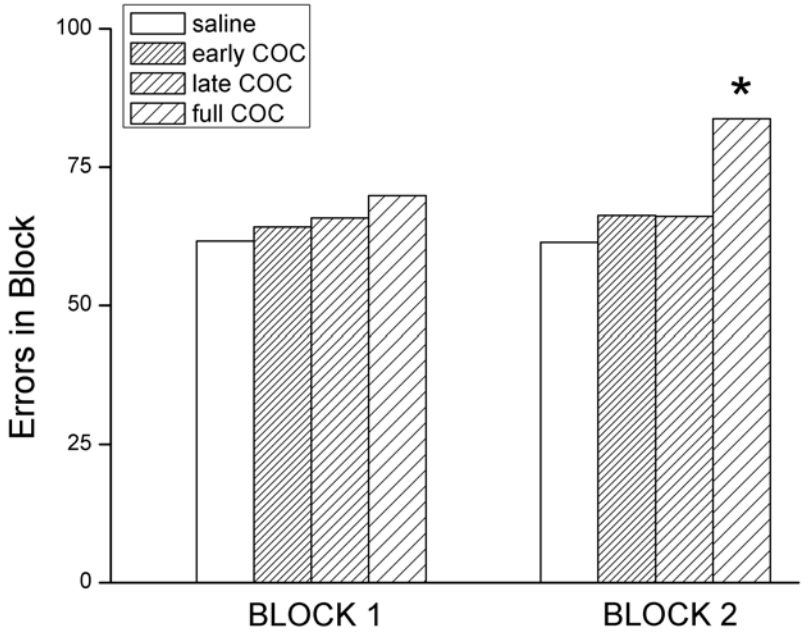

Figure 2.1 Errors committed within each phase of learning for the first olfactory-predictive task (Task 1). There were no differences between groups in errors within Block 1, but full-exposed COC animals committed significantly more later learning errors than saline controls (p=0.03).

Olfactory-Predictive EDS Tasks (Tasks 4, 7, 9)

Errors to Criterion

The analysis of errors to criterion comparing the early-exposed and control animals did not reveal a main effect of treatment [F(1,58.3)=0.57, p=0.4] or a significant interaction of treatment and task [F(2, 115)=1.20, p=0.3]. The analysis comparing learning rate of the late-exposed and control animals for the olfactory-predictive tasks also did not reveal a main effect of treatment [F(1, 62.7)=0.11, p=0.7],

but a significant interaction between treatment and task was found [F(2,120)=3.84, p=0.02] (Figure 2.2). Comparison of least square means revealed that the controls committed significantly more errors than late-exposed animals within Task 7 [t(141)=1.96, p=0.05]; performance of these two groups was similar on Tasks 4 and 9.

The analysis comparing learning rate of the full-exposed animals and the controls did not reveal a main effect of treatment [F(1,61.8)=0.56, p=0.4] but the treatment X task interaction approached significance [F(2,123)=2.33, p=0.10]. Pairwise comparisons revealed that the full-exposed animals tended to commit more errors to criterion than controls on Task 4 [t(140)=-1.90, p=0.06], but did not differ from controls in Tasks 7 (p =0.7) or 9 (p =0.8).

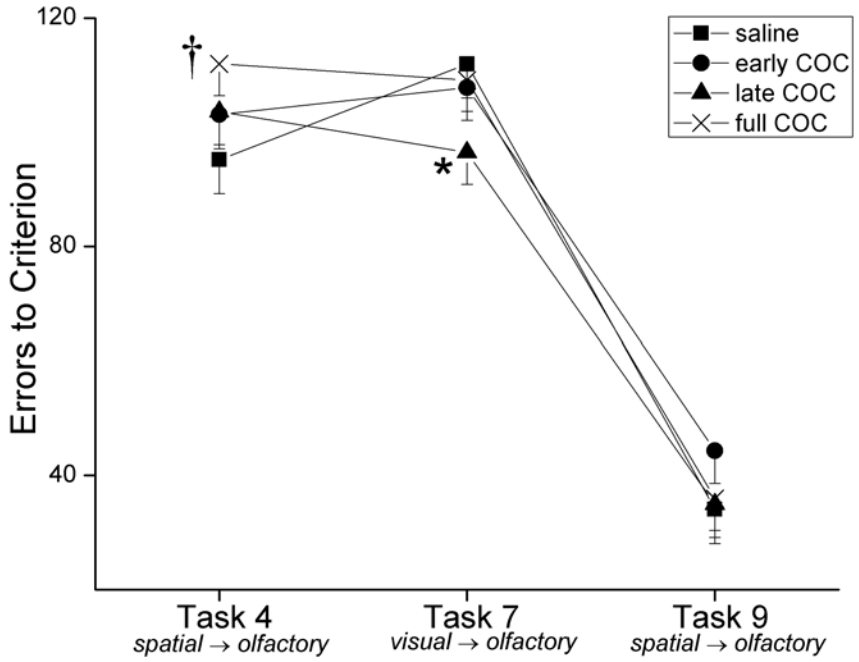

Figure 2.2 Total errors committed across olfactory-predictive tasks. Full-COC animals demonstrated a trend toward higher errors to criterion relative to controls on Task 4; late-COC animals were superior to controls within Task 7 (†p<0.10, *p<0.05).

Block 1 Errors

The analysis comparing the controls and early-exposed animals for Block 1 errors in the three olfactory predictive tasks did not reveal a main effect of treatment [F(1, 58.8=1.85), p=0.17;) or an interaction of treatment and task [F(2, 117)=0.28, p=0.8]. The similar analysis comparing the controls and late COC groups did not reveal a main effect of treatment but did reveal a significant treatment X task effect [F(2,118)=5.55, p=0.005]. The analysis comparing the controls and full-exposed animals revealed a similar interaction [F(2,124)=4.23, p=0.02], as shown in Figure 2.3. Pairwise comparisons revealed that for Task 4, a shift from spatial-predictive to olfactory-predictive, both the late-COC [t(168)=-2.31, p=0.02] and full-COC [t(171)=-2.84, p=0.005] groups committed significantly more Block 1 errors than controls. No significant group differences were seen for Block 1 errors in tasks 7 and 9.

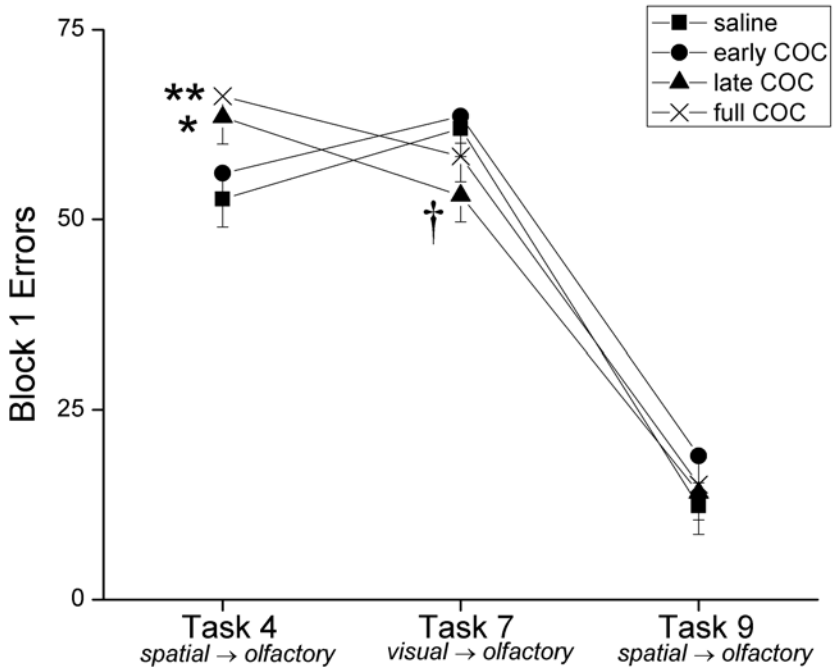

Figure 2.3 Early learning errors across olfactory-predictive tasks. Both late- and full-COC animals committed more errors in Block 1 than controls on Task 4. Late-COC were superior in Block 1 performance on Task 7 (†p<0.10, *p=0.02, **p=0.005).

Perseverative errors

On Task 7, late-exposed animals tended to have fewer perseverative errors than controls [Wilcoxon Rank Sum p=0.07] (Figure 2.4). There were no differences in perseverative errors between controls and early COC or full COC groups [p=0.6, p=0.7, respectively]. As discussed above, because the other olfactory-predictive EDS tasks (Tasks 4 and 9) were preceded by spatial-predictive tasks, we could not evaluate true perseveration at these timepoints.

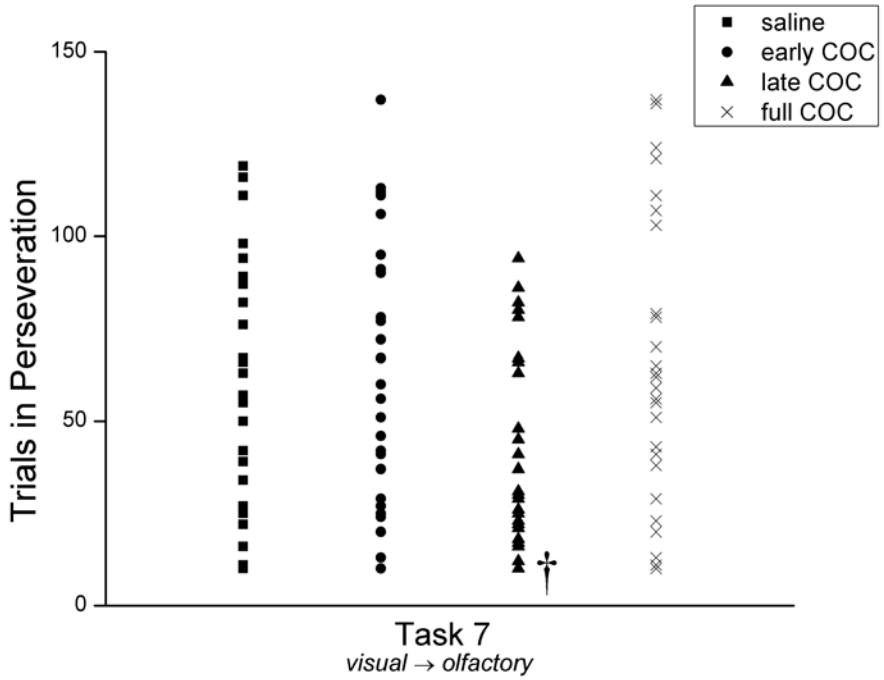

Figure 2.4 Responses to previously predictive visual cue upon shifting in predictive dimension to olfactory-predictive task. Late-COC committed fewer perseverative errors than saline controls on this visual → olfactory shift (†p<0.10).

Block 2 Errors

There were no significant group differences in Block 2 errors for any of the three olfactory-predictive EDS tasks (all p>.2).

Visual Predictive Tasks (Tasks 2, 6, 10)

Errors to Criterion

The three tasks in which the predictive dimension was visual tended to be the most difficult type of EDS task for all groups, as reflected in the high average number of errors committed before achieving criterion. The analysis comparing errors to criterion for the early-COC animals and controls for the visual-predictive tasks did not reveal a significant effect of treatment [$F(1,71.5)=1.5$, $p=0.2$] or a significant treatment X task interaction [$F(2,91.2)=1.67$, $p=0.2$]. However, pairwise comparisons revealed that the early exposure group tended to master the first two visual tasks (Tasks 2 and 6) more slowly than controls; this difference approached significance only on task 6 [$t(88.8)=-1.76$, $p=0.08$] (Figure 2.5). Late-COC and full-COC animals did not differ from controls for errors to criterion for any of these three tasks [all $p>0.2$].

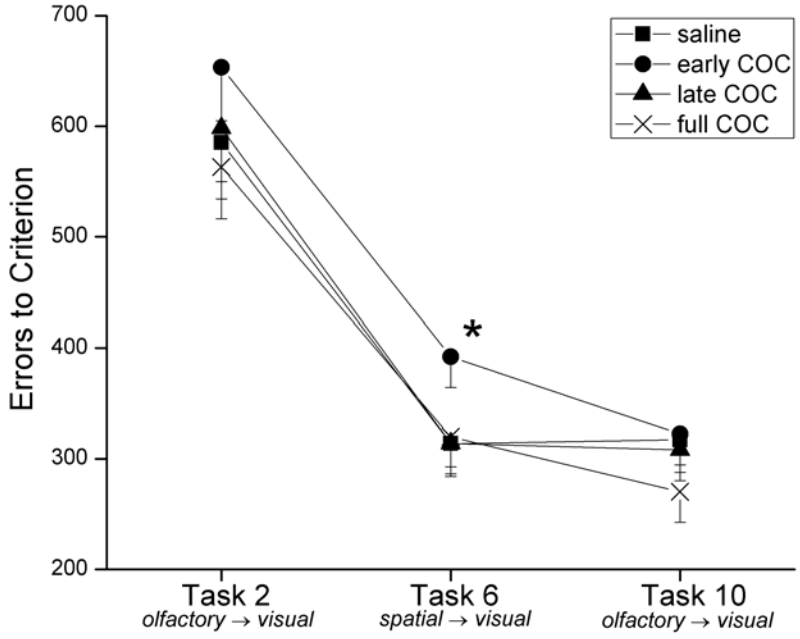

Figure 2.5 Total errors committed on visual-predictive tasks. Early-exposed animals committed more errors than controls on Task 6 (* p=0.08).

Block 1 Errors

The analysis comparing the controls and early COC groups for Block 1 errors in the visual-predictive EDS tasks did not reveal a main effect of treatment [F(1,179)=1.72, p=0.2] or treatment X task interaction [F(2,179)=1.39, p=0.3] (Figure 2.6). Pairwise comparisons revealed that the early-COC group committed more errors during Block 1 than controls on Task 2 [t(174)=-1.98, p=0.05]; there were no differences between these groups within Task 6 or Task 10. Late-exposed animals showed no significant main effect of treatment [F(1,59.6)=0.15, p=0.7], but the treatment by task interaction was significant [F(2,118)=5.55, p=0.005]. Contrasts revealed that this interaction was driven by the higher number of Block 1 errors committed by the late-COC group specifically in Task 2 [t(180)=-1.76, p=0.08]. There were no treatment related differences in mean Block 1 errors within Task 6 or Task 10.. The comparison of full-COC animals and controls revealed no significant differences in this outcome across visual-predictive tasks.

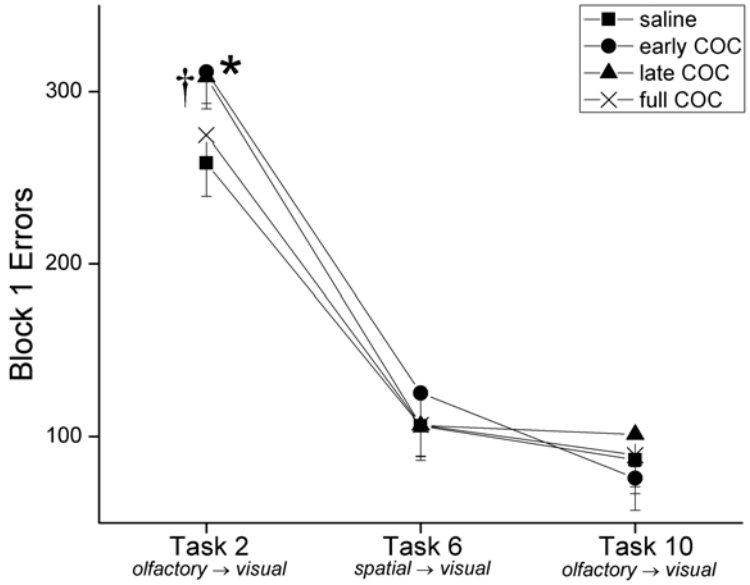

Figure 2.6 Block 1 errors on visual-predictive tasks. Both early- and late-COC animals tended to commit more errors early in learning relative to controls (†p<0.10, *p<0.05).

Perseverative responses

For Tasks 2 and 10, the number of perseverative responses to the previously correct olfactory cue was analyzed with the nonparametric Wilcoxon Rank Sum procedure (Figure 2.7). On Task 2, the duration of perseverative responding for early-exposed animals and late-exposed animals was not different from controls [$p>0.3$]. Full-exposed animals showed a trend towards greater perseverative errors than controls on Task 2 only ($p=0.07$). There were no treatment differences in duration of perseveration for Task 10, a task learned very rapidly by all groups. Perseverative errors could not be accurately quantified for Task 6, which was preceded by a spatial-predictive task.

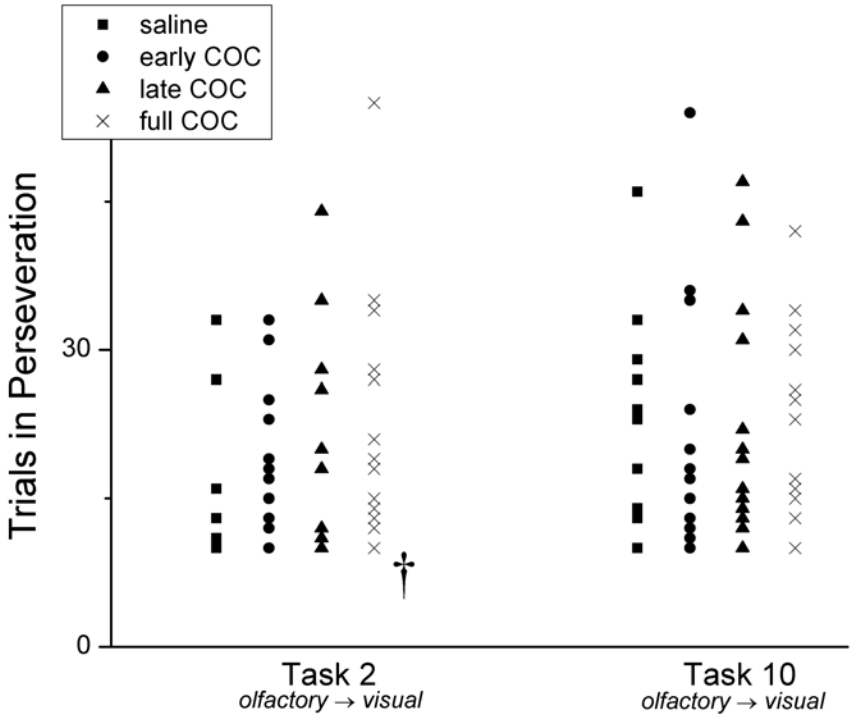

Figure 2.7 Perseverative errors on visual-predictive tasks. Full-COC animals persistently responded to the olfactory cue on the first shift to visual-predictive dimension, a relationship that suggested a trend (†$p<0.10$).

Block 2 Errors

For the comparison of each cocaine group versus controls, the treatment main effect and the treatment X task interaction for Block 2 errors failed to reach significance ($p>0.2$). Within task comparisons between COC and controls uncovered no significant differences in mean Block 2 errors.

Spatial Predictive EDS Tasks (Tasks 3, 5, 8)

Errors to Criterion

The spatial-predictive tasks were the easiest for animals to acquire, as evidenced by the low number of mean errors committed for all groups; most animals achieved criterion in 1 or 2 testing sessions. For the comparison of each cocaine group versus controls, there were no treatment or treatment X task effects [all $p>0.14$] in the errors to criterion outcome (Figure 2.8).

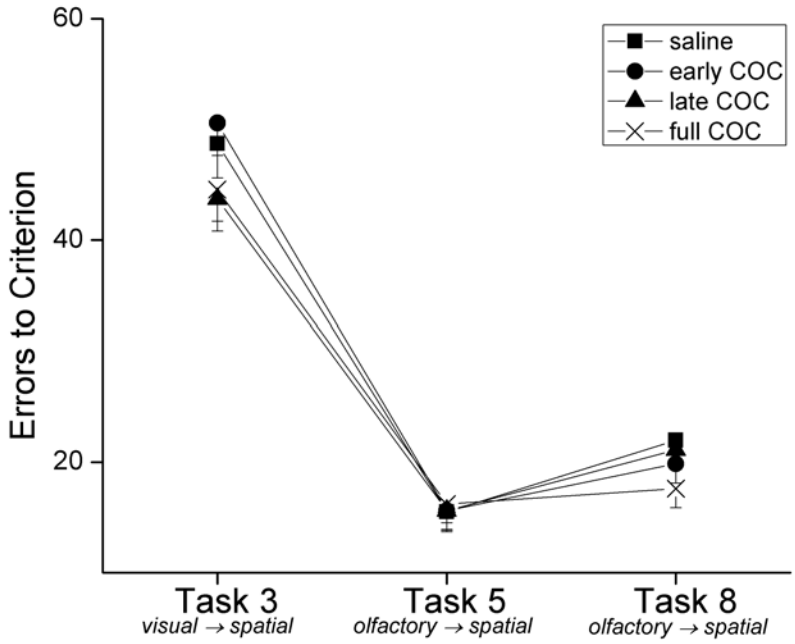

Figure 2.8 Total errors committed on spatial-predictive tasks. There were no significant differences between treatments within any spatial-predictive task.

Block 1 Errors

The analysis comparing the controls and early COC group for Block 1 errors in the spatial-predictive tasks revealed a main effect of treatment [$F(1,98.9)=4.82$, $p=0.03$], but no treatment X task interaction [$F(2,82.7)=1.46$, $p=0.2$]. Pairwise comparisons showed fewer Block 1 errors for the early COC group relative to controls on Task 3 [$t(61.2)=2.06$, $p=0.04$], a shift from visual-predictive to spatial predictive (Figure 2.9), which represented a difference in LSMeans of 6 errors; there were no differences between these groups for Tasks 5 or 8.

The analysis of late-exposed animals vers' s controls also showed a significant main effect of treatment [$F(1,85.5)=5.10$, $p=0.03$] and a significant treatment by task interaction [$F(2,104)=5.20$, $p=0.007$]. Contrasts revealed that this interaction was driven by the lower number of Block 1 errors committed by late-COC in Task 3 [$t(64.5)=2.92$, $p=0.005$]. There were no treatment related differences in mean Block 1 errors within Task 5 or Task 8.

The comparison of full-exposed animals and controls revealed a significant main effect of treatment [$F(1,96.9) =6.56$, $p=0.01$] and a treatment X task interaction [$F(2,87.7)=3.37$, $p=0.04$]. Pairwise comparisons revealed that full-exposed COC animals performance on Task 5, the first olfactory to spatial shift, was not different from controls. However, full-exposed animals committed significantly fewer Block 1 errors than controls on both Task 3 (a visual to spatial shift) [$t(65.3)=2.45$, $p=0.02$] and Task 8 (a second olfactory to spatial shift) [$t(114)=2.02$, $p=0.05$].

Block 2 Errors

In the analysis comparing controls and early-exposed animals for Block 2 errors, the main effect of treatment was not significant [$F(1,59.3)=1.22$, $p=0.3$], although the treatment by task interaction approached significance [$F(2, 119)=2.56$, $p=0.08$]. Contrasts suggested that this treatment by task interaction was driven

primarily by differences with Task 3, in which early-exposed COC animals committed significantly more Block 2 errors than controls [t(169)=-2.35, p=0.02] (Figure 2.9).

In the analyses evaluating late-exposed or full-exposed animals versus controls on Block 2 errors, there were no significant main effects of treatment [F(1,62.7)=0.21, p=0.6; F(1,59.4)=0.02, p=0.9] and no interaction between treatment and task [all F<1]. Pairwise comparisons within each task indicated no differences in Block 2 performance between controls and either late COC or full COC animals (all p>0.4).

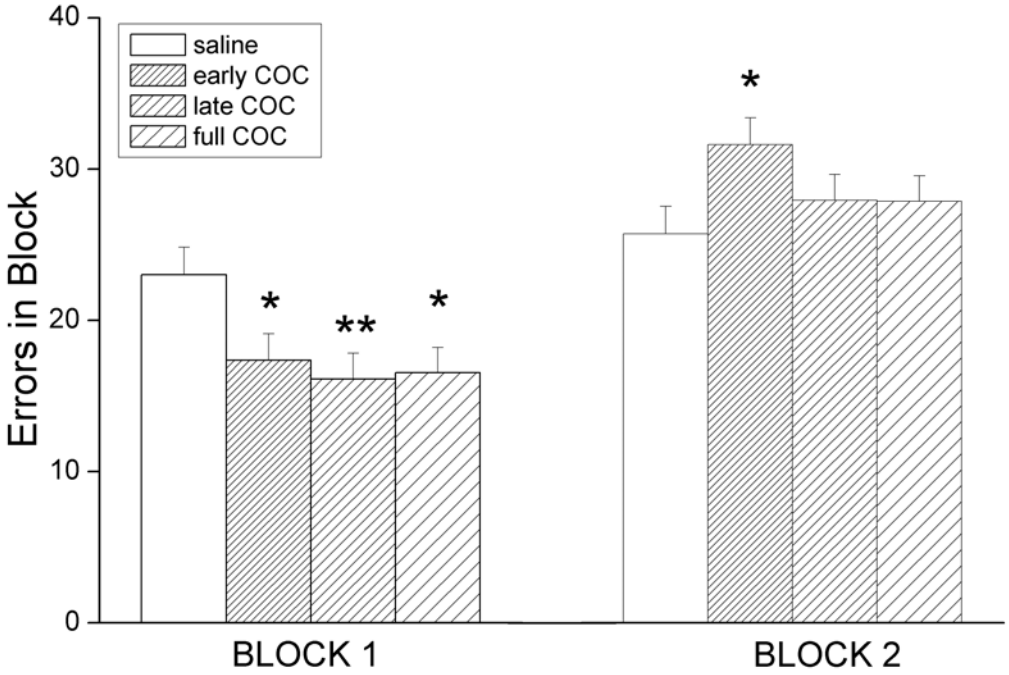

Figure 2.9 Errors committed in each block on Task 3, the first shift to the spatial-predictive dimension. All cocaine-exposed animals committed significantly fewer errors than controls in Block 1 (* p<0.5, ** p<0.01). Only early exposed animals were impaired relative to controls in Block 2 (p=0.04).

DISCUSSION

The results of the present study contribute to a growing body of evidence that the cognitive effects of prenatal cocaine exposure are both subtle and specific, primarily disrupting various aspects of attention and arousal. These findings indicate that prenatal cocaine exposure, at doses that model maternal recreational use, leads to lasting impairments in attentional set formation and shifting and selective attention on extra-dimensional shift tasks, in a pattern dependent on the timing and duration of exposure. Although the dependent measures used here are, in point of fact, measures of learning rate, the constellation of effects, observed in the present study and in prior reports, suggests that the observed group differences do not reflect effects of cocaine on learning ability (associative processes) per se. In EDS tasks, different phases of learning tap distinct cognitive processes. Specifically, the "early learning phase" encompasses a number of cognitive processes in addition to basic associative ability, including (1) shifting attention from the previously predictive cues (which can be affected by both the strength of the attentional set that was formed, as well as flexibility in reallocating attention), (2) hypothesis testing to ascertain which cues are currently predictive, and (3) selective attention to filter out the irrelevant cues. In contrast, the duration of the "later learning phase" reflects primarily selective attention. It is the *pattern* of differences that provides the greatest insight into the disrupted processes underlying observed differences. The behavioral changes associated with varying timing and duration of *in utero* cocaine exposure is delineated below.

Effects of early cocaine-exposure on EDS performance

Animals with cocaine exposure limited to early gestation differed in performance from controls under very specific circumstances, which provides clues to the lasting effects of this particular timing and dose of *in utero* cocaine exposure. The

number of errors in the "early learning" phase was significantly greater for the early COC animals than for controls in Task 2, the first shift from an olfactory-predictive task to one in which visual cues were predictive. Interestingly, these animals also committed significantly fewer Block 1 errors in Task 3, the first shift from a visual predictive task to one in which spatial cues were predictive. The latter finding, along with the lack of Block 1 differences in the olfactory-predictive tasks, clearly indicates that these animals do not have a more general associative deficit. Rather, this pattern of results suggests that these COC animals are drawn to very salient cues, which then affects both the strength of the attentional set that is formed and the ease with which the attentional set can be shifted when contingencies change. Thus, these animals progress more slowly on EDS tasks in which the previously predictive cue was highly salient and the current predictive cues were subtle (e.g. olfactory to visual shift), but progress more rapidly than controls when shifting from a less potent set of cues (e.g. visual) because they form a weak attentional set under these conditions. Early-exposed animals are thereby able to learn the new task contingencies very quickly. A similar pattern of behaviors has been reported for primates with DA depletion of the prefrontal cortex (Crofts et al., 2001; Robbins & Roberts, 2007; Roberts et al., 1994; Rogers, Andrews, Grasby, Brooks, & Robbins, 2000). These lesioned animals showed no improvement across a series of IDS tasks but were superior to controls on EDS tasks – both effects apparently due to a failure to form an attentional set.

In addition, early-exposed animals also committed significantly more Block 2 errors on visual-predictive and spatial-predictive EDS tasks but not on the olfactory-predictive tasks. The absence of group differences in Block 2 of the olfactory predictive tasks again rules out a more general associative deficit. Rather, this pattern of effects is suggestive of a deficit in selective attention, the ability to focus selectively on the newly predictive cues and filter out the previously predictive cues. This deficit

in selective attention, not surprisingly, was seen under conditions in which the irrelevant cues were the most salient (i.e., olfactory).

It is notable that the performance differences in both the early and late learning phases were not seen by the final task in each EDS series. This pattern indicates that the alterations in attentional set formation and selective attention experienced by the early-exposed animals may be overcome with extensive experience on this type of task and/or habituation to the potent olfactory stimuli.

Effects of late cocaine exposure on EDS performance

The pattern of effects was slightly different for the animals with cocaine exposure limited to late gestation. Similar to the early-exposed group, late-exposed animals committed more errors than controls in Block 1 of Task 2 (when the predictive stimuli shifted from olfactory to visual) and a shorter early learning phase on the first visual to spatial shift. Additionally, late-COC animals exhibited significantly less perseveration than controls on Task 7, a visual to olfactory shift. Late-COC animals also showed an elongated early learning phase in Task 4, the first shift from spatial- to olfactory- predictive cues. This pattern suggests that the late-exposed rats, like the early-exposed animals, are impaired in forming an attentional set to visual cues, presumably because they are not salient cues for these animals. That is, on tasks in which the previously predictive dimension was subtle (e.g. visual), COC animals were able to quickly learn the new rule because an attentional set to the subtle visual stimulus was only weakly acquired. This deficit can explain the faster early learning of tasks which were preceded by a visual-predictive task (Tasks 3 and 7). In contrast, when the previously predictive dimension was more salient (e.g. olfactory, spatial), an appropriate attentional bias to that dimension was formed and deficits on the subsequent task represent failures in "attentional set shifting." The lack of

differences in Block 2 on all tasks indicates that selective attention is not impaired when cocaine exposure is limited to later gestation.

As with the early-exposed animals, differences between the late exposed animals and controls were no longer evident by the final task of each type in the series. Again, it seems that the deficit can be overcome with repeated training and habituation to the more salient cues.

"Full"-exposure group: effects on EDS task performance

Animals exposed to cocaine both early and late in gestation (the "full" exposure group) exhibited a pattern that was different from either of the two other exposed groups. First, contrary to either of these two other groups, the full exposure group exhibited impaired performance during Block 2 of Task 1, the first olfactory-predictive task in the series. Although this task does not constitute a true "shift," as it was preceded by a long series of olfactory serial reversal tasks, this was the first task in which irrelevant visual cues were presented. Although one might not expect irrelevant visual cues to be very distracting when presented with potent predictive olfactory cues, it is notable that (a) the olfactory stimuli (strawberry, rose, and lilac) used in this task were the same as those that had been used for a long series of serial reversal (SR) tasks administered immediately prior to the EDS series, and thus may be less salient due to habituation; and (b) these rats had previously been trained on a long series of visual attention tasks (prior to the SR series), rendering LED illumination as a potentially important stimulus. Thus, we suggest that this extensive prior experience with this odor triad may have reduced the relative salience of olfactory stimuli for all animals, leaving "attentional capacity" available to be captured by the more subtle visual cues which had previously been associated with reward. The impaired performance of the full-exposure group later in learning suggests that they were more vulnerable to this influence than controls, due to an impaired ability to focus

selectively on the predictive cues. Evidence for this type of change comes from two previous studies that were designed to tap selective attention. Garavan and colleagues (2000) reported that this same *in utero* cocaine exposure regimen led to impaired performance specifically during the final learning phase of an EDS task (Garavan et al., 2000). Further, in a visual attention task in which olfactory distractors were presented immediately prior to the predictive stimulus, COC-exposed animals demonstrated impaired performance indicative of deficits in the domain of selective attention (Gendle et al., 2004).

The full exposure group was also impaired in learning the first visual-predictive task (Task 2), as evidenced by a borderline increase in errors to criterion. Phase analysis revealed that this trend towards slower attainment of the learning criterion was due to increased errors in Block 1, including a longer period of perseverative responding to the previously predictive olfactory cues; Block 2 errors did not differ from controls. Interpretation of these findings is informed by the additional finding that these full-exposed animals exhibited superior Block 1 performance, relative to controls, in shifts from visual cues to spatial cues (Task 3). This pattern suggests that, for the COC animals, the strength of the attentional set that is formed is very much affected by the relative salience of the cues. When the cues are highly salient (e.g., the olfactory cues), a strong attentional set is formed, and attentional set shifting is difficult for them, whereas when the cues are subtle (e.g., visual), a weak attentional set is formed, and shifting is in fact faster than for controls.

One additional, perhaps unexpected finding (in light of the pattern of effects overall) is that the full-exposed animals also performed better than controls on Task 8, a shift from olfactory to spatial cues. We did not expect to find treatment differences on this task because the pattern of performance on previous tasks suggested that animals would form a strong attentional set to the prior olfactory-predictive cues,

which would inhibit (rather than facilitate) acquiring the rule on the subsequent spatial-predictive task. Although unexpected, the finding that full-exposed animals were superior to controls on Task 8 may be reconciled with the pattern of effects on other tasks when considering that visual cues, while "distracting" in the sense that they have been previously associated with reward, were still subtle relative to the other two dimensions (i.e. the sensory characteristics of visual stimuli are not as inherently salient to these animals as olfactory stimuli). If this effectively eliminates "visual" as a potent distractor (on this task), we speculate that when spatial cues are pitted against olfactory cues (to which animals may have become habituated to), the spatial cues may be more novel and therefore more salient at this point in the series. Since cue salience accommodates the pattern of findings on all task types for the full-exposed animals, it is reasonable to assume that it may be a relevant factor in the final task. It is also important to note here that the magnitude of the superior performance of the full-exposed animals on Task 8 was small, representing an average difference of fewer than 4 errors between treatments; the clinical relevance for a difference of such small magnitude is unclear.

Synthesis of findings

The specificity of deficits observed here reveals the importance of timing and duration of *in utero* cocaine exposure, and suggests that interrupting the normal developmental timeline with toxic exposure disrupts subsequent cognitive processes in a way dependent on the specific developmental events occurring at the time of insult (Snow et al., 2004). In the present study, prenatal cocaine exposure produced a specific disruption in attentional set shifting when the previously predictive stimuli were salient, and disrupted attentional set formation to cues that were subtle, irrespective of the timing and duration of exposure. In general, attention in COC animals was captured by the most salient cues in the environment (salience being

determined by relative novelty, prior association with reward and sensory characteristics), which then affected attentional set formation and ease of shifting when task contingencies changed. In some instances, this cognitive change resulted in inferior performance relative to controls, and in some instances, superior performance. In addition to this cognitive disruption (which was seen in all groups), cocaine exposure that included the period from GD8-15 (regardless of total exposure duration) produced deficits in selective attention in later learning phases when the distracting stimuli were salient (again, where cue salience was determined not only by the potency of the stimulus but also by prior experiences with the distracting cues). This cognitive process was spared in animals with cocaine exposure limited to late gestation.

The present findings support prior studies in this lab, which have observed that animals with our "full" exposure regimen demonstrated impairments in selective attention when the distracting stimulus was more salient than the predictive cue (Garavan et al., 2000; Gendle et al., 2004). In both of these previous studies, COC animals showed an increased attention to salient olfactory stimuli, which disrupted the animals' ability to attend to less potent predictive dimensions. The current study complements these previous findings by showing a similar pattern of deficits and suggests that the salience of cues is not only dependent on the stimulus dimension but is also influenced by prior experiences. Cue salience in the context of prior experiences will thereby influence the extent of attentional impairment experienced by these COC animals both early and later in learning.

Proposed Neural Mechanism for Observed Behavioral Deficits

Our current understanding of a "sensitive period" in development is restricted primarily to information regarding neural development in cortical areas, with little prior investigation of subsequent behavioral changes associated with specific timing

and/or durations of exposure. Stanwood and colleagues reported that exposure to cocaine during the later gestational period (GD16-25 in rabbits) was both necessary and sufficient to produce permanent effects in anterior cingulate cortex structure and function; they reported no morphological changes in the ACC in animals exposed to cocaine prior to GD16. Most notably, late-exposed animals had a loss of dopamine receptor coupling with its G-protein, which downregulated dopamine release. Other animal studies have suggested that prenatal D1 receptor signaling changes may contribute to persistent deficits in arousal regulation (Gabriel & Taylor, 1998; Stanwood et al., 2001; Stanwood & Levitt, 2004). However, the present results suggest no behavioral deficits specific to prenatal cocaine exposure limited to later gestation. The only impairments observed in our late-COC group were also observed in the early- and full-exposure groups, namely altered acquisition of attentional set and subsequent attentional set shifting based on salience of previously predictive stimuli. Thus, it appears that the neural systems (ACC) investigated by Stanwood do not underlie the behavioral dysfunction observed in the present study.

We suggest that the attentional impairments observed in the present investigation may be mediated by changes in the noradrenergic system. Littermates of the early-exposed animals tested here were found to have decreased neurite outgrowth from the locus coeruleus, which reduced noradrenergic innervation of the prefrontal cortex (Snow et al., 2004). Behaviorally, a growing body of evidence suggests that NE plays a specific role in selective attention and responses to salient stimuli (Arnsten & Li, 2005; Aston-Jones, Rajkowski, & Cohen, 1999; Aston-Jones & Cohen, 2005). Recently, Eichenbaum and colleagues (2008) reported that NE in medial prefrontal cortex was necessary for extradimensional set shifting; these researchers proposed that EDS disruption in lesioned animals was due not to failures in set shifting per se, but rather to "over-attention" to previously predictive, irrelevant stimuli (McGaughy,

Ross, & Eichenbaum, 2008), a mechanism similar to what is suggested by the findings presented here. Taken together, the noradrenergic changes observed in COC animals may be, in part, responsible for the behavioral deficits observed here.

Conclusions

Overall, these findings demonstrate that prenatal exposure to very low doses of cocaine results in an increased attentional focus to salient cues, which can facilitate or impair attentional set formation or shifting depending on the relative salience of relevant and irrelevant stimuli. Further, the findings presented in this report provide the first evidence that selective attention deficits observed in rodents prenatally exposed to cocaine are specific to the timing and duration of drug exposure, perhaps due to the disruption of specific critical periods in development. It seems reasonable to predict that this area of dysfunction is related, in part, to alterations in noradrenergic projections to the prefrontal cortex, a system that plays an important role in attentional control (e.g., (Arnsten & Li, 2005; Ramos & Arnsten, 2007)) and that show lasting alterations as a result of prenatal cocaine exposure (see Booze et al., 2006; Dey, Mactutus, Booze, & Snow, 2006; Snow, Smith, Booze, Welch, & Mactutus, 2001; Snow et al., 2004).

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CHAPTER THREE

DOSE-DEPENDENT DISRUPTION IN LEARNING TRANSFER AND SELECTIVE ATTENTION IN A RODENT MODEL OF *IN UTERO* COCAINE EXPOSURE

ABSTRACT

The present study was designed to evaluate the effects of *in utero* cocaine exposure at different doses on performance in a series of extra-dimensional shift (EDS) tasks. Cocaine was administered intravenously with a regimen that accurately models the pharmacokinetic profile and physiological effects observed with human recreational use. Two doses of cocaine were assessed: one group of pregnant dams (1X COC) received cocaine once a day from gestational day (GD) 8-21; another group (2X COC) was administered cocaine once a day during GD8-15 and twice daily GD16-21. A third group, the controls, received saline injections from GD8-21. Offspring of these dams were tested in adulthood on a series of tasks in which stimuli from three dimensions (olfactory, visual, spatial) were simultaneously presented on each trial; only one dimension was predictive of reward, the other dimensions were irrelevant and randomly associated with reward. After a high level of performance was achieved on each task, the predictive dimension was shifted to one of the two previously irrelevant sets of cues. Nine EDS tasks were administered, each of which were evaluated in two learning phases. The 1X COC animals were impaired in both "early" and "later" learning phases, an effect observed on both olfactory- and visual-predictive tasks. The 2X COC animals also showed lack of improvement in rate of "early learning" across the three visual-predictive tasks, but showed no early-learning deficits in the other task types. Furthermore, unlike the 1X COC group, the 2X COC animals, did not commit more "later learning" errors than controls for any task type.

This pattern of findings suggests that the higher dose of cocaine significantly impaired transfer of the learning involved in shifting attention when the predictive domain changed, but only on EDS tasks in which the predictive stimuli were subtle relative to distractors. In contrast, the lower cocaine dose impaired learning transfer both when distractors were salient and when they were subtle. Further, the lower dose of cocaine impaired selective attention, irrespective of the relative salience of predictive and non-predictive cues.

INTRODUCTION

The late 1980s and early 1990s brought a surge of reports in the popular media suggesting a rising "crack baby epidemic," in which prenatal cocaine exposure produced severe physical malformations and neurological impairments (Glenn, 2006). These sensationalized media reports were backed by little empirical research, instead relying primarily on anecdotal evidence and case reports (Slotkin, 1998). In 1992, the National Institute of Drug Abuse (NIDA) conducted the first large-scale survey assessing the prevalence of illicit drug use during pregnancy. NIDA reported that approximately 1.1% of women in the United States used cocaine at some point during their pregnancy, a figure which likely underestimates actual incidence due to underreporting (Mathias, 1995).

It has been estimated that the annual cost of acute and long-term medical and social care for cocaine-exposed infants exceeds $1.3 billion (National Institute on Drug Abuse, 2001), with more than $350 million spent annually in special education services alone (National Institute on Drug Abuse, 1998). The financial and societal costs of prenatal cocaine exposure are, therefore, non-trivial, and demand the development of clinical and preclinical models to accurately gauge the cognitive, neurochemical and behavioral effects of *in utero* cocaine exposure. For the past two decades there have been numerous studies attempting to characterize the immediate

and long-lasting effects of prenatal cocaine exposure on physical and cognitive development. Although complicated by numerous external factors and methodological problems, research with cocaine-exposed humans suggests a subtle, but specific, constellation of deficits associated with *in utero* cocaine exposure (Mayes, 1999; Slotkin, 1998).

Physically, prenatal cocaine exposure is associated with lower birth weight and smaller head circumference, although cranial ultrasounds in the immediate postnatal period do not indicate gross malformations or infarctions of the central nervous system in cocaine-exposed infants (Behnke & Eyler, 1993; Frank, Augustyn, & Zuckerman, 1998; Frank, Augustyn, Knight, Pell, & Zuckerman, 2001). Cognitively, general measures of intelligence have not revealed a relationship between IQ and prenatal cocaine exposure in school-aged children (Azuma & Chasnoff, 1993; Delaney-Black et al., 1998; Delaney-Black et al., 2000; Frank et al., 1998; Frank et al., 2005; Loebstein & Koren, 1997). While conflicting results have been reported, IQ is known to be profoundly affected by a number of social and environmental variables often co-existing with prenatal cocaine exposure, complicating the interpretation of these studies (Frank et al., 2005). While some deficits in motor maturity and language development have also been observed in cocaine-exposed children, the majority of human prospective studies are suggestive of a relationship between prenatal cocaine and impairments in the domains of attention and arousal regulation (Mayes, Grillon, Granger, & Schottenfeld, 1998; Mayes, 2002; Pulsifer, Butz, O'Reilly Foran, & Belcher, 2008).

The effects of *in utero* cocaine exposure on executive functions are evident within the first month of life and persist into pre-adolescence; there is no available literature on effects in children beyond age 10 (Mayes, 1999). Infants exposed prenatally to cocaine exhibit dose-related deficits in stress and startle responses,

indicative of impaired arousal regulation (Karmel & Gardner, 1996; Karmel, Gardner, & Freedland, 1996; Karmel, Gardner, & Freedland, 1998; Williams & Ross, 2007), as well as increased off-task distractibility (an index of sustained and selective attention) (Gaultney, Gingras, Martin, & DeBrule, 2005). Evidence from school-aged children suggests that these alterations in attention and arousal persist into later childhood. Richardson (1996) was among the first to suggest a cocaine-related attention deficit in school-aged children. In a study evaluating a wide range of behaviors, performance on a continuous performance task in 6-year olds was the only behavior correlated with cocaine-exposure, where exposed children committed more errors reflecting lapses in sustained attention (Richardson, Conroy, & Day, 1996), a finding recently replicated by Accornero (Accornero et al., 2007). Cocaine-exposure has also been associated with deficits in selective attention, as evidenced by an increased rate of commission errors on a Continuous Performance Task (Noland et al., 2005) and impaired learning in the Stroop Color Word subtest (Mayes, Molfese, Key, & Hunter, 2005). Cocaine-exposed children also demonstrate impaired arousal regulation, as evidenced by decreased inhibitory control (Savage, Brodsky, Malmud, Giannetta, & Hurt, 2005; Bendersky & Lewis, 1998; Bendersky, Gambini, Lastella, Bennett, & Lewis, 2003; Mayes, 2002; Pulsifer et al., 2008), increased reaction to frustrating problem-solving tasks (Delaney-Black et al., 2000; Pulsifer et al., 2008), failures in task persistence (Bandstra, Morrow, Anthony, Accornero, & Fried, 2001) and more frequent externalizing behaviors (Delaney-Black et al., 2000; Delaney-Black et al., 2004).

While such human studies have helped to characterize the cognitive deficits associated with prenatal cocaine exposure, there are significant factors that confound their interpretation. Maternal cocaine use is associated with other variables that are also risk factors for developmental delay, including nutritional status, quality of pre- and post-natal care, household instability, socioeconomic status and low maternal

education and IQ (Mayes, 1999). Maternal depression is also a significant variable, with new evidence suggesting that gestational cocaine exposure may actually buffer the impact of maternal depression on the fetus, further complicating the development of an accurate cognitive profile in exposed children (Salisbury et al., 2007). Additionally, the majority of women who report cocaine use during pregnancy also report the use of other substances, most frequently indicating concomitant use of nicotine, alcohol and/or marijuana with cocaine (Mathias, 1995; Mayes, 1999). Exposure to these drugs during pregnancy may have physical and cognitive effects both independent from and synergistic with cocaine, further confounding interpretation of findings in the human literature.

Animal models allow researchers to control for the extraneous influential factors that confound interpretation of human studies, which provides an opportunity to make causal inferences in the relationships between cocaine exposure and cognitive and behavioral outcomes. Early animal models (e.g. 1990s) utilized subcutaneous (SC) administration of cocaine, which, due to the vasoconstrictive properties of the drug, produced necrotic lesions at the site of injection and thereby increased maternal stress (Mactutus, Herman, & Booze, 1994). Maternal stress during pregnancy has been reported to independently effect fetal development and long-term cognitive functioning in offspring (Arnsten, 2007). Thus, the SC exposure protocol complicated the interpretation of results in these early studies. Further, SC administration required extremely high doses of cocaine (as high as 100 mg/kg) to create the expected behavioral impairments in exposed offspring (Church & Overbeck, 1990), doses that did not adequately model human recreational use and failed to produce the appropriate pharmacokinetic profile of cocaine in the periphery (Mactutus, Booze, & Dowell, 2000).

Due to the problems associated with the SC injection route, many recent studies have turned to the intravenous (IV) route of administration. In the rodent, Mactutus and colleagues have developed an IV administration technique that, at a dose of 3.0 mg/kg, produces a pharmacokinetic profile in the periphery comparable to that observed with recreational human use. This IV-exposure regimen also minimizes maternal stress, producing no skin lesions, no evidence of maternal seizure activity, no effect on maternal weight, and no reduction in maternal food intake. Additionally, pups from IV-exposed mothers have body weights comparable to that of saline controls, indicating no effect of the IV drug on offspring growth or mortality (Mactutus et al., 1994).

Previous research from this lab using Mactutus's IV protocol has suggested that rodents prenatally exposed to low doses of cocaine exhibit lasting deficits in executive functions, specifically in the domains of sustained and selective attention and arousal (Gendle et al., 2003; Gendle et al., 2004; Morgan et al., 2002). Of particular relevance to the present study, these previous findings indicate that cocaine-exposed animals are specifically impaired in tasks that required attention to a less salient modality in the presence of highly salient stimuli (Garavan et al., 2000; Gendle et al., 2004). Selective attention deficits caused by prenatal cocaine exposure have also been observed in other species, including rabbits and non-human primates (Chelonis, Gillam, & Paule, 2003; He, Bai, Champoux, Suomi, & Lidow, 2004; Gabriel & Taylor, 1998; Romano & Harvey, 1998).

In the only study to date to use a rodent model of cocaine-exposure on attentional set shifting, Garavan and colleagues (2000) explored the competition between subtle, predictive cues and simultaneously presented salient, irrelevant stimuli. Garavan utilized a two-choice EDS paradigm, and observed that animals exposed to low-dose cocaine daily during gestation were slower to learn the task

parameters when the predictive cues were subtle (spatial) and distracting stimuli were salient (olfactory), but not when salient cues were predictive of reward (Garavan et al., 2000). This pattern of responses indicates that impairments in performance are not due to task difficulty, per se, as evidenced by disruption in the relatively "easy" spatial predictive tasks (Garavan et al., 2000; Hilson & Strupp, 1997). Further investigation of discrete learning phases revealed that disruption on spatial-predictive EDS tasks was restricted to later learning phases, after the animals had mastered the task contingency, suggesting that cocaine-associated differences were due to dysfunction in selective attention. A similar mechanism was thought to govern impaired performance in the later stages of learning of a serial olfactory discrimination tasks observed in these same animals (Garavan et al., 2000).

This prior study was valuable in forming the conceptual framework for evaluating discrete learning phases in the present report. The basis of our phase analysis methodology was specifically informed by the work of Hilson and Strupp (1997), Garavan, et al. (2000), and others. Each of these studies suggested a specific progression in learning and subsequent response rates that we expected to be relevant in our extradimensional shifting paradigm. Specifically, evidence from reversal tasks suggested that a shift in task contingencies would first produce a brief period of consistent (but non-rewarded) responses to the previously predictive dimension (perseveration), followed by an extended period of hypothesis testing (possibly including the adoption of a side bias). As the new task rule is learned, response rate to the correct dimension (i.e. percent accuracy) would gradually but consistently increase until criterion is achieved (Garavan et al., 2000; Hilson & Strupp, 1997). Such in-depth analysis has provided, in previous studies, information on the specific nature of cognitive deficits in monkeys with discrete brain lesions (Jones & Mishkin, 1972) and in humans and primates with immature neural systems (Overman, Bachevalier,

Schuhmann, & Ryan, 1996). Phase analysis on olfactory serial reversal tasks from our lab has also provided insight into the cognitive dysfunction of rodents with perinatal lead exposure (Hilson & Strupp, 1997) and prenatal cocaine exposure (Garavan et al., 2000). While the rationale of demarcating learning phases was based on reversal tasks, the conceptual framework behind this response pattern was expected to hold true for the 3-choice extradimensional shift task used in the present study. The in-depth phase analysis conducted here was intended, therefore, to illuminate the specificity of cocaine-associated deficits in the learning process, perhaps providing insight regarding the locus of underlying neural changes that may produce dysfunction in certain phases of learning.

The current report describes a series of three-choice extradimensional shift (EDS) tasks designed to tap a range of cognitive processes. Mastery of the EDS tasks used here required associative learning (establishing the reinforcement contingency), inhibitory control to overcome perseveration upon a change in task rule, selective attention to focus on the predictive dimension and effectively learn the task, and transfer of learning over time. Perhaps most importantly, these EDS tasks require shifting attention between sensory modalities; we hypothesized that such cognitive flexibility may be dependent on the salience of the predictive dimension relative to that of the distracting stimuli. To further challenge such cognitive flexibility, we included a visual dimension into the EDS series that prior work in this lab had not evaluated. Since the visual domain is known to be subdominant to olfaction in these animals, we expected that the visual-predictive tasks with salient olfactory distractors would place a higher demand on attentional set shifting and selective attention for all animals (across exposure level). Because the literature in primates, rabbits and rodents suggests that cocaine-exposed animals' attention is "captured" by the most salient environmental stimuli (relative to saline controls), we expected dose-dependent

significant performance deficits in the cocaine-exposed animals on these visual-predictive tasks, with the higher dose exhibiting the greatest behavioral disruption. In addition, the present study aimed to further characterize the dose-specific effects of IV *in utero* cocaine exposure on specific phases of learning, as detailed above. To this end, we investigated discrete phases of learning in addition to overall learning rate by incorporating an analysis of response patterns across individual trials. Previous research suggests that such in-depth phase analyses may uncover effects not seen in assessments of overall learning rate (Garavan et al., 2000; Hilson & Strupp, 1997). Therefore, we expected that in-depth examination of specific learning phases would provide insight into the nature of any disruption of specific cognitive processes related to *in utero* cocaine exposure, and may further inform the underlying neural mechanism of such behavioral dysfunction.

METHODS

Subjects

Male and female Long–Evans rats were obtained from a commercial supplier (Harlan Sprague–Dawley, Indianapolis, IN) at approximately 11 weeks of age. The health of this animal colony and their housing conditions were monitored according to guidelines set forth by the National Institutes of Health and the American Association for the Accreditation of Laboratory Animal Care. All breeding and surgical procedures were conducted at the University of South Carolina (Columbia). The animal facility at the University of South Carolina was maintained at 21 ± 2 °C, 50% ± 10% relative humidity, and a 12-hr light/dark cycle, with lights on at 7:00AM. Food (Prolab RMH 1000, PMI Nutrition International, Brentwood, MO) and water were available *ad libitum*.

Catherization and Mating

A sterile intravenous catheter (22 gauge; Becton/Dickson, General Medical Corporation, Grand Prairie, TX) with a Luer-lock injection cap (Medex, Kensington, MD) was implanted into the jugular vein of nulliparous female Long-Evans rats; this catheter served as the port for IV injection of cocaine or saline after conception. Details of the catheters and surgical procedures used can be found in Mactutus, Herman, and Booze (1994).

After recovery from surgery (4-8 days), the females were group-housed (n=3) with a male rat. Conception (Gestational Day 0; GD0) was confirmed with a sperm-positive lavage. Pregnant females were then split into three treatment groups: saline control, lower-dose cocaine (1X COC), and higher-dose cocaine (2X COC).

Drug Administration

Drug injection procedures were conducted as described in Mactutus, Herman & Booze (1994). Briefly, all catheterized dams received once daily IV saline injections from GD 1-7. Cocaine hydrochloride (Research Triangle Institute, NC) or saline was then administered as a bolus injection of 3.0 mg/kg once per day from GD 8-14 and once or twice per day (depending on treatment group) from GD 15-21, at a volume of 1 ml/kg (15s), followed by flushing (15s) of the catheter with 0.2 mL of heparinized (2.5%) saline (Table 3.1). The drug was dissolved daily immediately prior to injection. Food and water were provided *ad libitum* for the duration of drug administration.

Table 3.1 Dosing regimen for pregnant dams

	GD8-14	GD15-21
Control	Saline 1x/day	Saline 2x/day
Lower-dose COC	Cocaine HCl 1x/day	Cocaine HCl 1x/day
Higher-dose COC	Cocaine HCl 1x/day	Cocaine HCl 2x/day

The dose per injection (3.0 mg/kg) has been shown to appropriately model recreational cocaine use in humans, as this dose yields peak arterial plasma levels similar to those reported for humans administered 32 mg of cocaine intravenously (Booze, Lehner, Wallace, Welch, & Mactutus, 1997; Evans, Cone, & Henningfield, 1996) The 3.0 mg/kg dose also produces an appropriate pattern of self-administration observed in humans in experimental conditions, suggesting that this dose produces the expected psychological response in the adult user (Fischman & Schuster, 1982). High levels of cocaine administered early in gestation may result in spontaneous abortion of the fetus; to reduce the risk of such fetotoxicity, higher levels of cocaine were restricted to later gestational stages.

Offspring Care

After birth, litters were culled to four males and four females. After weaning on postnatal day (PND) 21, one male and one female offspring from each of 36 litters were transported under environmentally controlled conditions from the University of South Carolina to Cornell University, where behavioral testing was conducted. Behavioral testing was conducted on 72 animals, twelve in each treatment by sex condition.

All animals were housed in same sex pairs and placed on a reversed day/night schedule (lights off at 5:30AM, lights on at 8:30PM EST); animals were acclimated to the testing room and housing conditions for approximately three weeks before behavioral testing began. All behavioral testing occurred during the animals' active (dark) cycle.

Food Restriction

Animals were placed on a food restriction schedule on approximately PND 28 to accustom them to the feeding regimen used during behavioral testing. Females were initially restricted to a daily allotment of 18 grams of rat chow (Pro-Lab

Rat/Mouse/Hamster Chow); males were allowed 21 grams of chow per day. During this acclimation period, animals were allowed five hours in individual feeding cages to consume their food allocation. Behavioral testing occurred six days a week (Sunday-Friday) for two hours a day for the duration of the study. On testing days, the amount of food reward received during testing was subtracted from the animals' daily allotment of chow (described above) to normalize caloric intake. Animals were allowed three hours immediately after testing to consume the remainder of their food individually before being returned to their home cage with their cage-mate, for a total of five hours to consume allowed food. On non-testing days (Saturdays), animals were given five hours in their individual cages to eat their food allowance. Tap water was provided *ad libitum* throughout the study.

Upon the commencement of behavioral testing, changes in each animal's body weight and activity level were monitored on a daily basis, and food allotment was adjusted accordingly. That is, individuals whose response patterns indicated low motivation (evidenced by a high number of non-responses during testing) had their daily intake reduced 1-3 grams as needed to increase motivation and still maintain a healthy body weight. Chow allotment was increased by 1-3 grams/day for animals whose body weight consistently declined after acclimation. All modifications in food allotment were made by researchers blind to the treatment conditions of the individual animals.

Apparatus

Behavioral testing was conducted in 12 custom-built Plexiglas automated operant chambers, each housed in a sound-attenuating wooden enclosure. The chambers consisted of a rectangular waiting area (26.5 cm x 25 cm x 30 cm) with a smaller testing alcove extending from one wall. A motorized guillotine-type door controlled entrance into the alcove and prevented responses between trials. Recessed

into each of the three walls of the alcove was a funnel-shaped port. The left and right ports were at an approximate 45° angle to the center port. A set of infrared phototransistors was located at the alcove entrance and at the opening of each of the three ports; breaking the infrared beam signaled trial initiation or a nosepoke, respectively.

A green light-emitting diode (LED) was located above each of the three ports in the alcove; illumination of one of these LEDs served as either the predictive cue or the visual distractor in the present report. Additionally, the narrow end of each port was connected by tubing to three bottles containing liquid odorants (anise, almond, and maple scents), attached to a board placed outside of the box (9 bottles total). Compressed air was forced through the scented liquids, allowing scented air to be emitted into the testing chamber during a testing session.

Behavioral Testing Procedure

Each animal was assigned to one of the 12 testing chambers such that each chamber was balanced across treatment groups; each chamber was designated for rats of one sex only and each rat used the assigned apparatus for the duration of the study. Animals began nose-poke training on PND 53 (for details of training procedure, see Hilson & Strupp, 1997). During this training, all animals learned to make a one-second nose-poke into the response ports. For subsequent all tasks, this 1-second nose-poke constituted a 'choice'. Correct responses were rewarded with a 45mg Noyes food pellet delivered directly onto the alcove floor from a pellet dispenser. Following successful completion of these training phases, behavioral testing began. For all tasks, a daily testing session consisted of 200 response trials (trials on which the animal entered the alcove within 60 seconds after the door was raised) or two hours, whichever came first. Before being tested on the task series described in the present report, all animals mastered a visual sustained attention task, a visual

predictive/olfactory distraction task, and an olfactory serial reversal task. The odor triad utilized in the preceding serial reversal task was different from the three odors used in the EDS series discussed here. Animals began the EDS series on approximately PND 180.

Extra-Dimensional Set (EDS) Shifting Tasks

The EDS series consisted of an initial olfactory-predictive task with visual distractors and nine subsequent "shifts" in which the predictive dimension (olfactory, visual, spatial) was switched with each successive task. For each task, the animal's entry into the testing alcove at trial onset produced the immediate illumination of one of the three LEDs and the emission of three odors (one from each port). The odor triad was always anise-almond-maple, and the port from which each was emitted varied pseudo-randomly. The presentation of the stimuli on each trial also included a spatial dimension (i.e., corresponding to left, center, or right ports). The olfactory and visual cues were presented continuously for 60 seconds or until the animal made a response. A correct response was rewarded with delivery of a food pellet; there was no consequence of an incorrect response. If an animal failed to make a response after 60 seconds, a nontrial was scored. The alcove door was lowered immediately following a response or a nontrial, and was followed by a 10 second intertrial interval. All animals completed the ten tasks of the EDS series in the same order, as described below (Table 3.2).

In tasks 1, 4, 7, and 9, the olfactory cues were predictive, and a correct response was a 1-second nose-poke to the port from which maple scent was emitted; the visual and spatial dimensions were irrelevant for these tasks. Task 1 was considered the baseline olfactory task, which did not constitute an "extradimensional shift" as it was preceded by an olfactory serial reversal task (in which olfactory cues were also predictive). However, this was the first task in which a visual cue was

presented to serve as a distractor. In Tasks 2, 6, and 10, a correct response was a 1-second nose-poke into the port below the illuminated LED; the spatial location of the ports as well as the olfactory cues were randomly associated with reward. In Tasks 3, 5, and 8, a correct response was a 1-second nose-poke to the center funnel, irrespective of the locations of the visual and olfactory stimuli. Reward contingencies for each task type are summarized in Table 3.3.

Table 3.2 Order of tasks in Extra-Dimensional Shift Series

Task Number	Predictive Dimension
Task 1	Olfactory
Task 2	Visual
Task 3	Spatial
Task 4	Olfactory
Task 5	Spatial
Task 6	Visual
Task 7	Olfactory
Task 8	Spatial
Task 9	Olfactory
Task 10	Visual

Table 3.3 Reward contingencies for Extra-Dimensional Shift tasks

	LED ILLUMINATED	ODOR EMITTED	SPATIAL LOCATION	Correct Response is:
Visual-predictive	Relevant	Irrelevant	Irrelevant	illuminated LED
Olfactory-predictive	Irrelevant	Relevant	Irrelevant	maple odor
Spatial-predictive	Irrelevant	Irrelevant	Relevant	center port

Animals were tested on a given task in the series until the learning criterion was reached. Based on prior evidence that asymptotic performance on the visual-predictive task was only 80-85% correct (relative to 90-95% on the spatial- and olfactory-predictive tasks), it was deemed optimal to have the learning criterion on this

task type be 80% correct for a single session, whereas the criterion for the other two task types was designated as 88% correct for a single session.

Summary of Behavioral Outcomes

Learning rate:

The measure of overall learning rate for each task was errors to criterion – the total number of errors (summed across sessions) each animal committed prior to reaching the learning criterion.

In-depth analyses (assessment of different phases in the learning process):

Two different types of in-depth analyses were examined in order to elucidate the specific nature of cognitive dysfunction. First, performance on each EDS task was divided into four distinct phases of learning: chance, early post-chance, late post-chance, and criterial (Phase Analysis). Each of these four phases of learning was thought to tap different cognitive processes. We also evaluated the data using a broader demarcation point, such that performance on each task was divided into two phases (Blocks Analysis). The specific methodology for each type of in-depth analysis is detailed below. In this study, Phase Analysis (i.e. four learning phases) was investigated only for the olfactory-predictive (4, 7, 9) and visual-predictive (2, 6, 10) tasks, as progression through each phase of the spatial tasks was so rapid for all animals that the measures were not sensitive to group differences. However, because of the broader demarcations of learning phases in the Blocks Analysis (and thus greater number of trials within each "phase"), spatial-predictive tasks were assessed for those outcomes.

Phase Analysis

Determination of Learning Phases

Performance on each task in the EDS series was divided into chance, early post-chance, late post-chance, and criterial phases; these phases were demarcated by

the point at which responses to the correct dimension for a given task consistently exceeded predetermined levels. The cutoff for chance phase, 44.6%, was the upper bound of the 90% Confidence Interval around chance performance (33.3%), based on 60 trials. That is, there was a 5% probability of observing a response rate greater than 44.6% out of 60 trials when the true underlying probability of a correct response was 33.3%. Post-chance phases were classified as all responses between the end of chance phase until the animal performed at a level consistently greater than criterion. The division of this phase into "early post-chance" and "late post-chance" was based on a performance level midway between the end of chance and criterial level (approximately 66%). Criterial phase was quantified as the end of post-chance until an animal achieved a full session (200 response trials) at greater than or equal to 88% for olfactory-predictive tasks (80% for visual-predictive tasks). These learning phases cutoffs are graphically depicted in Figure 3.1.

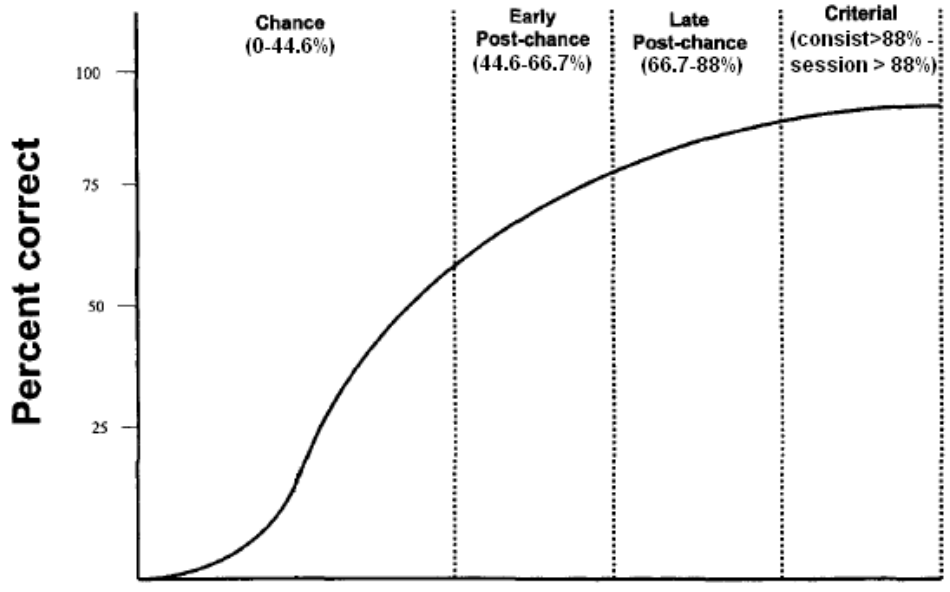

Figure 3.1 Learning phases for visual and olfactory extra-dimensional shift tasks (modified from Garavan, et al., 2000)

To determine the duration of learning phases, we evaluated the trial-by-trial data to quantify responses to each dimension (e.g. light, center, maple); a smoothed curve describing the frequency of responses to each dimension was generated using a moving average with a bin width of 60 trials (bins were calculated in one-trial increments e.g. 1-60, 2-61, 3-62, etc). Separate graphs were created for the full duration (total trials to criterion) of each task for each rat. Each graph showed three curves, one each for responses to light, center, and maple; Figure 3.2 depicts a representative graph of one animal's performance on Task 2. The moving average bin width of 60 response trials (excluding nontrials) was selected to maximize accuracy of phase demarcation by reducing the likelihood that calculated averages were based on a short period of unusually high or unusually low performance.

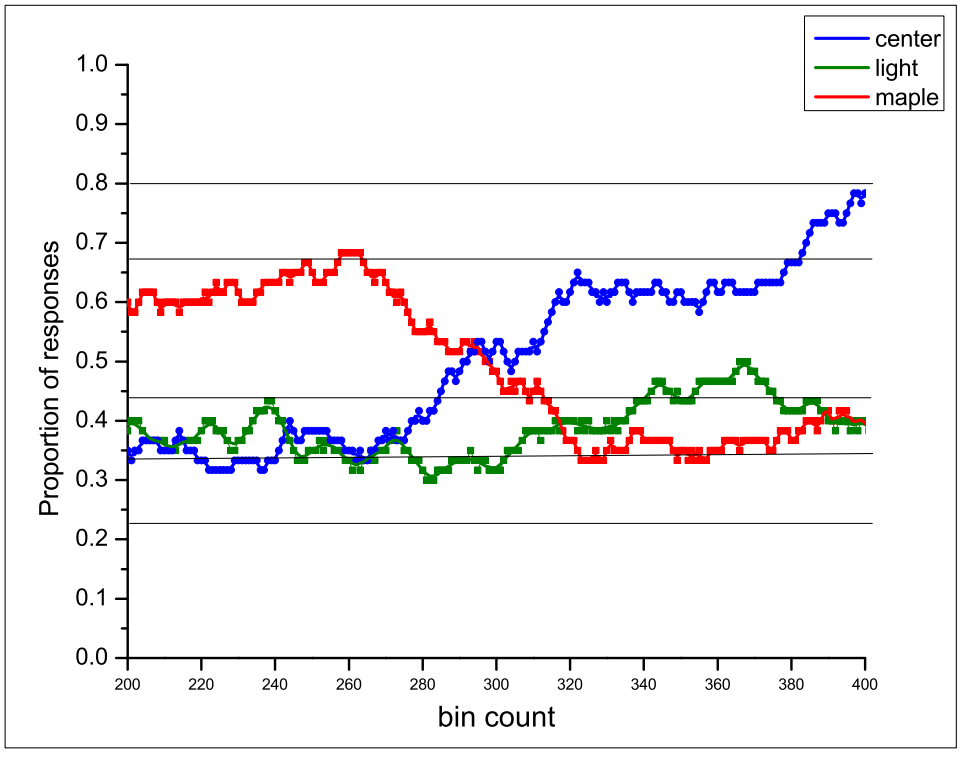

Figure 3.2 Representative graph of individual animal's (B32) performance on Task 2 (visual-predictive) bins 200-400. Each colored line represents the moving average of percent response to each relevant dimension (center, light, maple). The horizontal lines served as reference points for reviewers.

The point at which animals shifted from one phase of learning to another was determined by two reviewers blind to treatment conditions. Each reviewer independently evaluated individual graphs for the 72 animals across the 7 tasks (olfactory and visual) in the EDS series. As shown in Figure 3.2, each graph had three curves; when the line representing rate of responding to the correct dimension was *consistently* above the predetermined levels (mentioned above), coders recorded a shift to the next phase. The rate of responding to the two non-predictive dimensions in each task was important information for the coders, as it often revealed a change in strategy that reflected the progression of learning over the task. After independently determining phases for each animal, the reviewers then compared values for the phase changes in order to ensure consistency in the decision process. The scores identified by the two coders were averaged if they differed by no more than ten bins. In cases of discrepant demarcations greater than 10 bins, another reviewer provided a third independent assessment and, if necessary, examined the trial-by-trial data at the point of inconsistency. Specific rules followed by these reviewers can be found in Appendix A.

Chance Phase

The first phase, the period of chance-level performance, started at the beginning of each task (trial 1) and continued until the center of the bin at which responses to the correct dimension were consistently significantly greater than chance (44.6% for 3-choice task). Chance phase encompassed two components of the learning process: perseveration (responding to the previously predictive dimension) and hypothesis testing (determining new stimulus-reward associations). For some tasks, separate analyses were conducted on the perseveration phase, as discussed below.

Post-Chance Phases

Two post-chance phases were demarcated: early post-chance and late-post chance. Early post-chance was defined as performance between 44.6% and 66.7% correct, whereas late post-chance referred to performance consistently greater than the 66.7% cutoff and yet consistently below criterial performance (88% for olfactory tasks, 80% for visual tasks). Learning within both post-chance phases required learning of the new contingencies as well as selective attention to permit filtering out previously predictive cues.

Criterial Phase

The final phase for each task, the criterial phase, spanned the period from when the animal was consistently performing greater than criterion until the animal achieved criterial performance in a single testing session (88% correct in a single session for olfactory-predictive tasks, 80% for visual-predictive tasks). Criterial phase did not represent a "learning" phase per se, as the animals had already learned the new task contingencies in order to achieve this high rate of correct responding. Rather, criterial phase was indicative of the animal's ability to maintain an attentional set in the face of salient irrelevant cues.

Perseveration

The perseverative phase was a relatively short period of inaccurate responding early in the task in which an animal persistently responded to the previously correct cue; this is the period where the animals were learning that the previously predictive cues were no longer predictive. The duration of this phase provides an index of flexibility and also reflects the strength of the attentional set to the previously predictive dimension. In EDS tasks, perseverative responding yielded chance levels of performance which could not be distinguished (based on percentage correct) from hypothesis testing, random responding, or side biases. Therefore, in order to quantify

the duration of perseveration it was necessary to examine the trial by trial data, which provided information on which stimuli were associated with the port to which the rat responded on each trial.

For Tasks 2 and 10 (visual-predictive) and Task 7 (olfactory-predictive), we quantified the perseverative phase by again graphing a moving average of responses to each of the three dimensions. However, because the perseverative phase was expected to be of short duration relative to the other learning phases described previously, a smaller bin size was used for graphing. A bin size of 20 trials was selected because the perseverative phase was expected to be sufficiently short such that a larger bin width would be an insensitive measure, possibly masking actual levels of perseverative responding; additionally, this bin size had previously been shown to appropriately capture perseveration in EDS tasks (Garavan et al., 2000). These graphs were again evaluated by two independent reviewers blind to treatment, as above. The end of perseveration was demarcated by the center of the bin at which average responding to the previously predictive dimension fell below the upperbound of chance (55.8% for bin width 20). Note that designation of other phases was based on response rate to the currently predictive dimension; here, responding to the previous cue was the relevant curve on the graph. Again, the presence of responding rates to the other dimensions served to clarify shifting of response strategy. An example of a perseveration graph can be found in Appendix A.

Limitations in determining duration of perseveration

A side bias, or responding consistently to one port regardless of the olfactory or visual stimuli paired with it, was a common response pattern adopted when rats were confronted with a new task and had not yet learned the contingencies. In all previous tasks, the center port was never solely predictive of reward, yet early in the EDS series (Tasks 1 and 2) animals quickly adopted a center-port bias rather than a

response bias to left or right ports. This pattern of responses on these first two tasks was unexpected, as center had never previously been predictive in all 100 prior days of behavioral testing, and suggested to the coders that development of a side bias specifically to the center port does occur. This observation proved to be important when differentiating hypothesis testing from perseverative responding. A graph representing coders observations of side bias is presented in Appendix A.

Tasks 4, 6, and 9 were each preceded by a spatial-predictive task. Interpreting a pattern of consistent responses to the center port early in learning when the previously predictive task was spatial-predictive (with the center port being correct) was, based on the above logic and observations, ambiguous. Such a pattern could arise from either perseveration or development of a side bias. To avoid erroneous classification of such responses, duration of a "perseverative phase" was not calculated in tasks immediately preceded by a spatial-predictive tasks.

Outcome of Phase Analyses

The analyses of specific learning phases were designed to provide insight into the changes in learning and strategy over time on each task. However, the results revealed that group differences were better captured by analyses that demarcated "early" learning and "late" learning blocks, rather than the finer demarcations of phases. For this reason, the present chapter includes only the results of this latter analysis. The detailed results of the phase analyses can be found in Appendix A.

Blocks Analysis

As noted above, the phase analyses indicated that differences between the cocaine exposed and control groups was best captured by demarcations into "early" versus "later" learning, rather than by specifying four distinct learning phases. Therefore, learning on each task was divided into two phases demarcated by the point at which an animal achieved eight consecutive correct responses; these two phases

were designated “Block 1” and “Block 2”. Varying strings of correct responses (strings of five, 10, and 12 correct responses) were compared for the demarcation point to ensure that any observed differences were not solely due to the point of demarcation. Results of these additional analyses confirmed that the string of 8 consecutive correct responses provided the most sensitive demarcation for revealing group differences, although the basic patterns were the same for the other demarcation points.

Statistical Procedures

All statistical analyses were conducted with SAS v9.1 (SAS Institute, Cary, NC) for Windows XP Professional. For all dependent measures (errors to criterion, duration of each phase, errors in block), a repeated measures analysis of variance (ANOVA) was used to assess statistical significance, accounting for both the correlation induced by using littermates and multiple testing on each rat. The variables in the different ANOVA models for the EDS tasks included treatment condition (saline, lX COC, 2X COC), sex, task, and relevant interactions. Graphical methods were used to confirm that model residuals were approximately normally distributed and to identify outliers in both the fixed and random effects. In instances where the distribution of the raw data and did not satisfy the assumptions of normality necessary for a parametric test, a nonparametric procedure was used to minimize the effects of very high or very low data points.

Separate analyses were conducted for each type of EDS task (e.g., olfactory-predictive, visual-predictive, spatial-predictive). No direct comparisons were made between tasks with different predictive dimensions. For each type of task, separate analyses were conducted to compare each cocaine-exposed group to the controls. This procedure was followed so that the treatment by task interaction term would be most readily interpretable (as it specifically tested whether one COC group differed from

controls across the three tasks included in each analysis). In addition, we decided *a priori* that we would test for treatment differences within each task, regardless of whether the main effect of treatment or the treatment X task interaction was significant. This procedure was followed to avoid missing functionally important effects. In addition to comparing group differences for each task, we were also interested in treatment-related differences in the rate of improvement across the task series (i.e., "learning to learn").

Because relatively little is known about the effects of prenatal cocaine exposure on EDS tasks, we viewed these analyses as hypothesis-generating rather than hypothesis-testing. Due to this approach, observed group differences should be viewed as tentative, and in need of replication by future studies.

RESULTS

Maternal and Pup Characteristics

Cocaine-exposed dams did not differ from saline controls in maternal weight gain, length of gestation and litter size [all $F<1$]. Offspring from the controls and both cocaine-exposed groups were not significantly different in pup birthweight or other characteristics (data not presented).

Body Weight and Food Restriction

On Day 1 of EDS testing, there were no significant differences in body weights between treatments [$F(2,58)=0.06$, $p=0.9$]. The average body weight across the duration of EDS tasks was also not significantly different between treatment groups [$F(2,58)=0.19$, $p=0.8$]. Additionally, there was no treatment effect in average food intake [$F(2,58)=0.03$, $p=1$]. As expected, there was a significant main effect of sex for these three outcomes, with males weighing significantly more than females on Day 1 of EDS testing [$F(1,58)=791.03$, $p<0.0001$; 429g vs. 265g] and on average over the duration of the task series [$F(1,58)=806.18$, $p<0.0001$; 443g vs. 270g]. For all of these

outcomes, there was no statistical evidence of a treatment X sex interaction [all p>0.7].

Effect of Sex

For all dependent measures, we examined whether the treatment effect varied by sex. The treatment by sex interaction was not statistically significant for all of the outcomes measured in all task types [all p>.3]. Since both sexes within each treatment performed similarly on the evaluated outcomes across the series, males and females were combined into a single group for analyses of overall learning rate and duration of each learning phase (n=72).

Nontrials

The incidence of nontrials committed across sessions within a given task was usually very low, and the distribution of these values did not meet the criteria for parametric evaluation of the outcome. Nontrials were summed for each rat and task type; these scores were analyzed with a one-way Wilcoxon Rank Sum nonparametric test. There were no treatment related differences in number of nontrials for all task types [all p>0.3].

Olfactory-Predictive EDS Tasks

Task 1

Because Task 1 was not a true extradimensional shift, it was analyzed separately from the other olfactory-predictive EDS tasks. Thus, there was only one observation per animal for each dependent measure pertaining to this task. A General Linear Model including all three treatment groups was used to evaluate treatment differences within this task. For errors to criterion, the overall effect of treatment was not significant [$F(2,63)=1.90$, $p=0.16$] . Contrasts revealed that controls tended to commit more errors on Task 1 than the 1X COC group [p=0.08]; controls and 2X COC animals did not differ on this task (Figure 3.3). The analysis of Block 1 errors

revealed a trend towards a treatment effect [F(2,63)=2.43, p=0.10]. Pairwise comparisons within Task 1 revealed that controls tended to commit more errors in Block 1 than both 1X and 2X cocaine-exposed groups [p=0.04, p=0.09, respectively] (Figure 3.4). The treatment groups did not differ for Block 2 errors [F(2,63)=0.71, p=0.5].

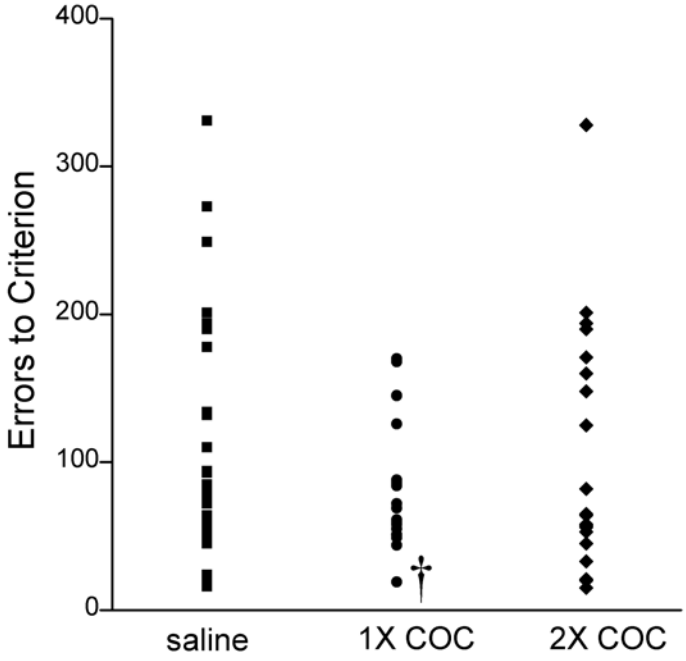

Figure 3.3 Total errors committed on Task 1. The lower dose cocaine-exposed animals committed more errors than control, a difference that approached significance (p=0.08).

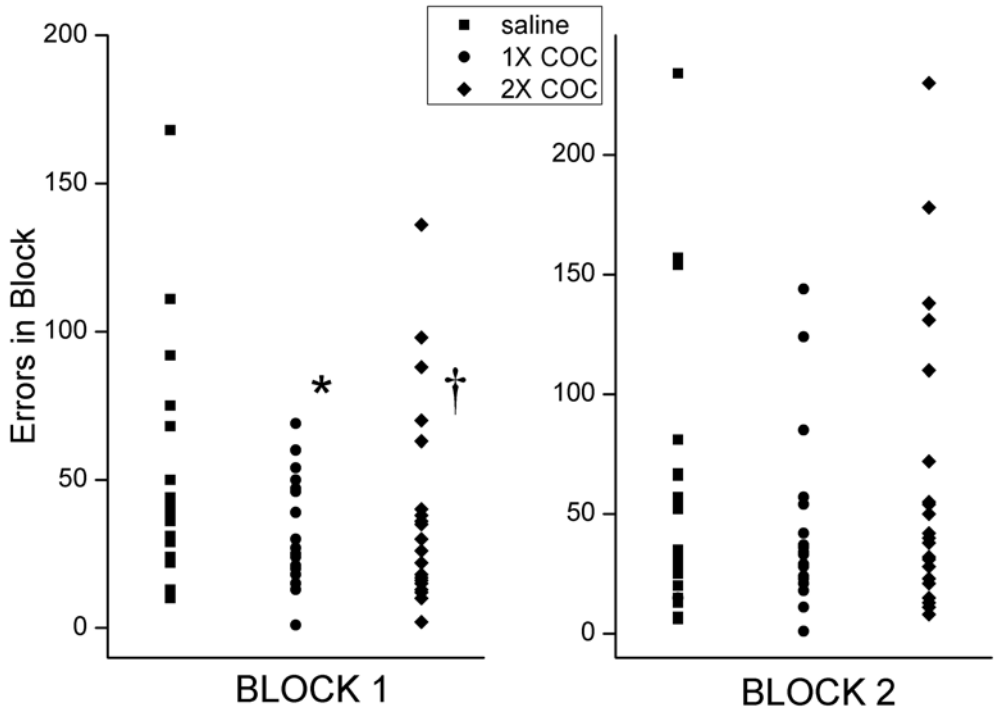

Figure 3.4 Errors committed within each block of learning on Task 1 [F(2,63)=2.43, p=0.10]. COC-exposed animals at both doses committed fewer errors than controls early in learning [*p<0.05, †<0.10]. There were no treatment differences in errors committed later in learning.

Tasks 4, 7, 9

Errors to Criterion

In the analysis comparing the 1X COC group and controls, the main effect of treatment on errors to criterion was not significant [F(1,34)=2.61; p=.12] (Figure 3.5). The interaction between treatment and task also was not significant [F(2, 67.9)=1.11, p=0.30]. Contrasts revealed that the groups did not differ for Tasks 4 or 9, but that errors to criterion was significantly higher for the 1X COC group than for controls on Task 7 [t(93.8)=-2.11; p=0.04], in part due to the differences in rate of improvement across tasks. That is, controls learned Task 4 significantly faster than Task 7 [p=0.04] while 1X COC animals did not improve between these two olfactory-predictive tasks [p=0.9]. For the comparison of 2X COC and controls, the main effect of treatment and the treatment X task interaction were not significant [F(1,34.7)=0.72, p=0.4;

F(2,68.4)=0.5, p=0.6, respectively]. Contrasts comparing controls and 2X COC did not reveal any differences in errors to criterion within any of the olfactory-predictive EDS task; learning rate across the olfactory tasks was similar for controls and 2X COC animals (Figure 3.5).

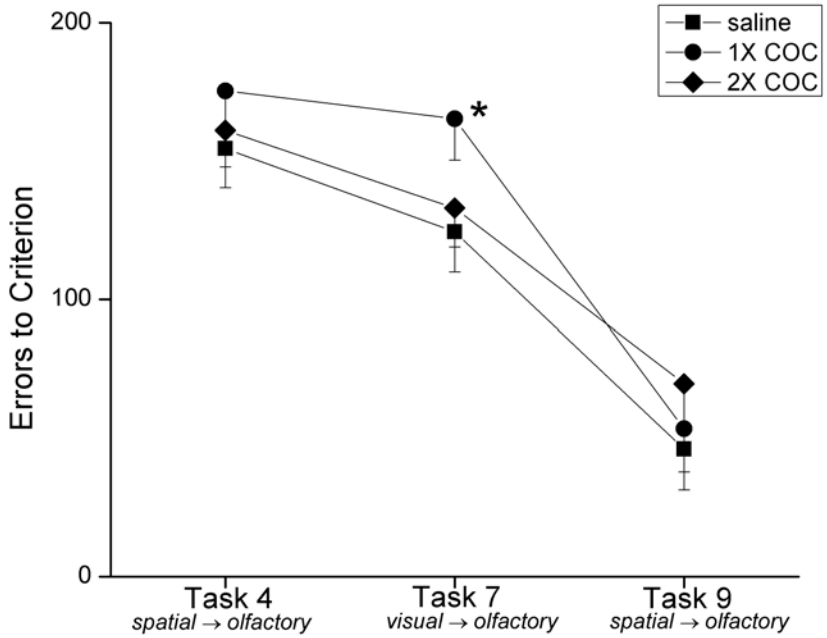

Figure 3.5 Total errors committed on olfactory-predictive tasks. 1X COC animals failed to improve between the first two olfactory tasks, while saline controls and 2X COC animals showed significant improvement. This difference in learning rate produced a significant difference between lower dose COC animals and controls only on Task 7 (*p=0.04).

Perseveration

For Task 7, a shift from visual-predictive to olfactory-predictive cues, the majority of animals (47 out of 52) showed some level of perseveration beyond the minimum score. However, the length of the perseverative phase did not differ by treatments in the Wilcoxon Rank Sum test [all p>0.17].

Block 1 Errors

The analysis comparing the 1X COC and controls for Block 1 errors did not reveal a main effect of treatment [F(1,38)=0.52, p=0.50] nor a significant treatment X

task interaction [F(2, 66.7)=0.93, p=0.40]. Within-task comparisons revealed that 1X COC animals performed similarly to controls on Task 4 and 9 but showed a trend toward greater Block 1 errors than controls within Task 7 [t(65.4)=-1.64; p=0.11] (Figure 3.6). Analyses of improvement rate across the three olfactory-predictive tasks revealed group differences: Block 1 errors dropped significantly between Task 4 and Task 7 for controls [p=0.004] but remained constant across these two tasks for the 1X COC animals [p=0.6].

The analysis comparing the controls and 2X COC groups for Block 1 errors indicated no significant main effect of treatment [F(1,52.4)=0.01, p=0.9] and no overall treatment X task interaction [F(2,50.9)=1.21; p=0.3]. Pairwise comparisons within each of these olfactory-predictive tasks did not reveal significant treatment differences [all p>0.4]. The two groups also did not differ in the rate of decline in Block 1 errors across these three olfactory tasks [all p>0.13].

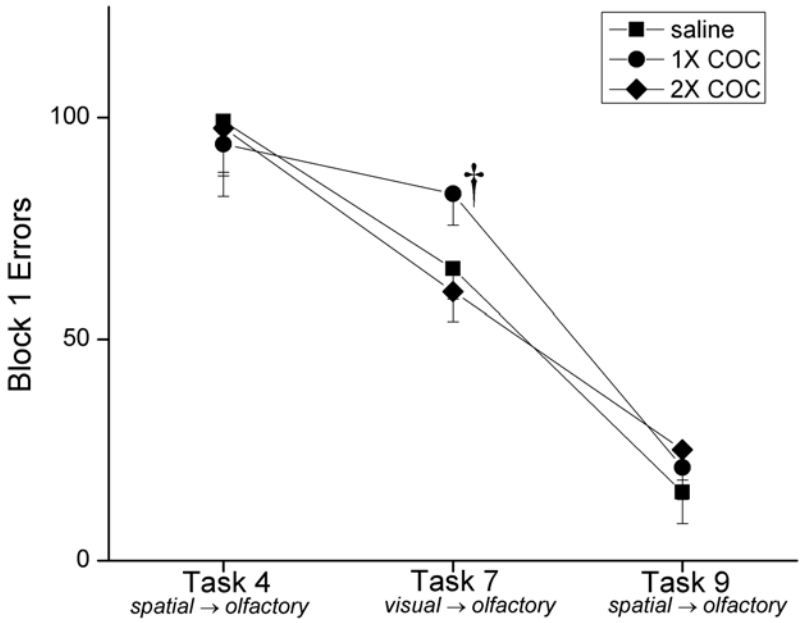

Figure 3.6 Errors committed prior to achieving eight consecutive correct responses (Block 1) on olfactory-predictive tasks. 1X COC animals commit more early learning errors on Task 7 († p<0.10), a trend driven by differences in learning between saline and lower-dose COC animals.

Block 2 Errors

The model comparing controls and 1X COC animals for Block 2 errors revealed a main effect of treatment that approached significance [$F(1,36.1)=3.61$; $p=0.07$]; the treatment X task interaction was not significant [$F(2,70.1)=1.6$, $p=0.2$]. As shown in Figure 3.7, 1X COC animals committed more Block 2 errors than controls on both Tasks 4 and 7 [$t(93.6)=-2.21$, $p=0.03$; $t(95)=-1.86$, $p=0.07$, respectively]; there were no differences between these groups on Task 9. Contrasts indicated that there were no significant differences in slopes across olfactory-predictive tasks.

The controls and 2X COC animals did not differ significantly in the number of Block 2 errors within each of the three olfactory-predictive tasks [all $p>0.4$]. Similarly the rate of decline in Block 2 errors across these three tasks was not different between these two groups [all $p>0.3$].

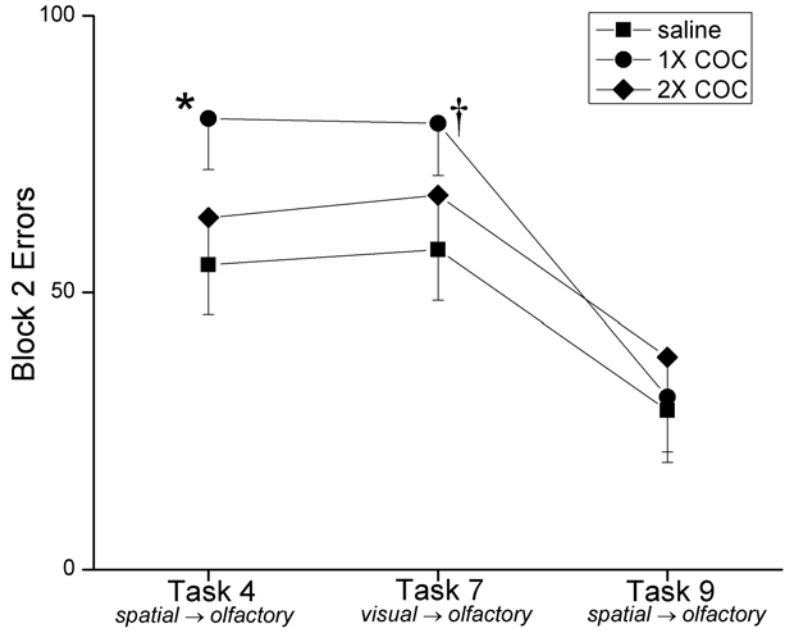

Figure 3.7 Errors committed within Block 2 on olfactory-predictive tasks. The rate of later learning performance across olfactory tasks was not different between COC and control animals. 1X COC animals commit more errors after achieving eight consecutive correct responses on the first two olfactory tasks (* $p<0.05$, † $p<0.10$).

Visual-predictive EDS Tasks (Tasks 2,6,10)

Errors to Criterion

In errors to criterion on the visual-predictive tasks, the comparison between 1X COC and controls revealed a significant main effect of treatment [$F(1,44.1)=4.64$, $p=0.04$] and an interaction between treatment and task that approached significance [$F(2,73.2)=2.95$, $p=0.06$]. Contrasts revealed that the rate of improvement across the three visual-predictive EDS tasks varied for these two treatment groups between both Tasks 2 and 6 [$p=0.03$] and Tasks 6 and 10 [$p=0.08$]. Consistent with this differential rate of improvement, errors to criterion were similar between groups on Tasks 2 and 10 but significantly higher for the 1X COC group than for controls within Task 6 [$t(64.6)=-0.34$, $p=0.001$] (Figure 3.8).

For the analysis comparing the controls and 2X COC groups, there were no significant effects of treatment [$F(1,50.5)=0.60$, $p=0.4$] or treatment X task [$F(2,64.1)=1.13$, $p=0.3$] on errors to criterion. Contrasts were suggestive of treatment differences in slope between Task 2 and Task 6 [$p=0.14$] . Consistent with this contrast, within treatment differences indicated that controls improved significantly between the first two visual-predictive tasks [$p=0.001$] whereas 2X COC animals did not [$p=0.3$]. Likely as a result of this difference in rate of improvement across tasks, the 2X COC animals demonstrated a trend towards slower learning within Task 6 [$t(61.9)=-1.63$, $p=0.11$].

Perseveration

A large proportion of animals, across treatments, had the minimum perseverative score for the visual-predictive tasks. That is, for Task 2, 47% of all animals had a perseveration score of 10; 41% of animals had this score for Task 10. Due to this non-normal distribution, perseverative scores were analyzed with the nonparametric Wilcoxon Rank Sum procedure to assess treatment differences relative

to controls within a single EDS. The Wilcoxon Rank Sum test revealed no significant differences in perseveration between controls and either COC group on either of the two visual tasks analyzed [all p>0.3]. A dichotomous evaluation of "any perseveration" vs. "no perseveration" also revealed no treatment effects on length of this phase.

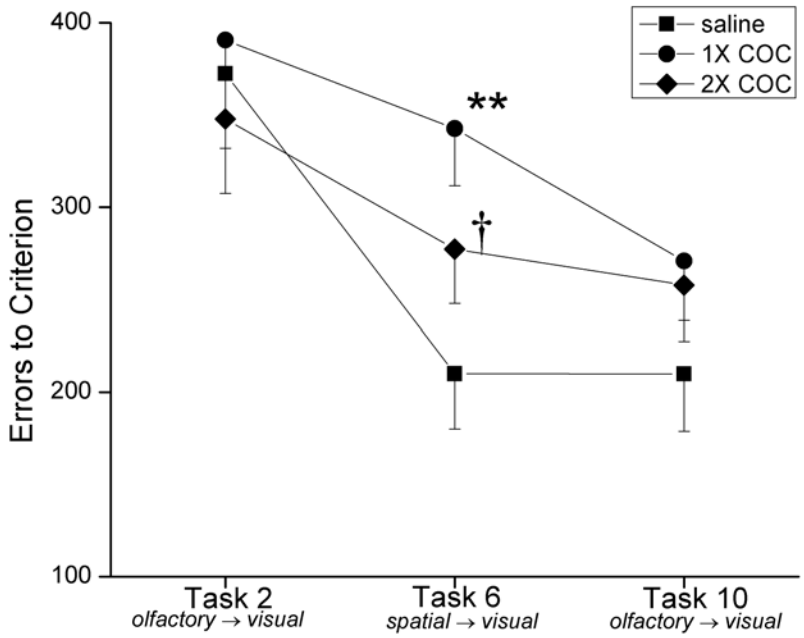

Figure 3.8 Overall errors to criterion committed across visual tasks. Both COC groups failed to decrease error commission rate between Task 2 and Task 6, whereas controls improved significantly. Within Task 6, both COC groups committed more errors than controls (**p<0.01, † p<0.11). Between the later two visual tasks, the 2X COC animals performance was not statistically different.

Block 1 Errors

Analysis of Block 1 for the visual-predictive tasks (Figure 3.9) revealed a significant interaction between treatment and task for the model comparing controls and lower-dose cocaine exposure [F(2,75.1)=3.07, p=0.05]; the main effect of treatment suggested a trend [F(1,41.6)=2.57, p=0.11]. Within task comparisons showed that 1X COC animals committed significantly more Block 1 errors than

controls on Task 6 only [t(73.6)=-3.26, p=0.002]. Contrasts revealed that the rate of decline of Block 1 errors between Task 2 and Task 6 was significantly different for controls and 1X COC animals [F(1,60.1)=4.43, p=0.04]; slopes between these groups were also different between Task 6 and Task 10 [F(1,39.4)=3.96, p=0.05].

The analysis comparing the 2X COC and control groups for Block 1 errors did not reveal a significant main effect of treatment or a significant treatment by task interaction [F(1,51.9=0.2, p=0.6; F(2, 68.2)=1.41, p=0.3]. Analysis of the decline in Block 1 errors between Tasks 2 and 6 was suggestive of a treatment effect [F(1,57)=2.48, p=0.12]. Investigation of within treatment improvement between these two tasks revealed that controls committed fewer Block 1 errors on Task 6 than on Task 2 [p=0.006] while 2X COC animals did not improve [p=0.6].

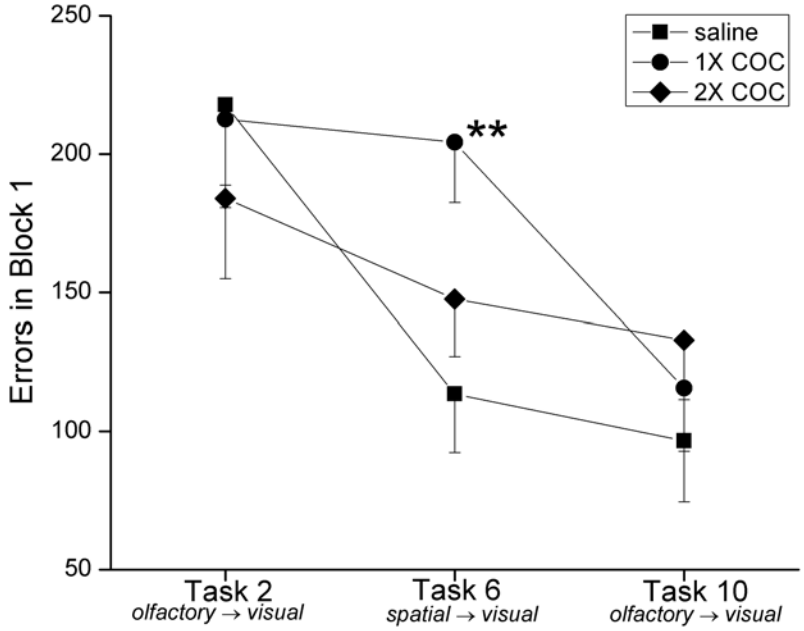

Figure 3.9 Early learning errors committed across visual tasks. The rate of 1X COC performance between Tasks 2 and 6 was statistically different from controls (p=0.04). 2X animals did not statistically change in performance across the visual-predictive tasks (all p>0.4). Within Task 6, 1X COC animals committed more Block 1 errors than controls (** p<0.1).

Block 2 Errors

In the analysis comparing the controls and 1X COC animals for Block 2 errors, the main effect of treatment approached significance [F(1,51.6)=2.74, p=0.10]; the treatment X task effect was not significant [p=0.8]. 1X COC animals committed significantly more Block 2 errors than controls within Task 6 [t(62.1)=2.02, p=0.05], but did not differ from controls in Tasks 2 and 10 (Figure 3.10). There were no significant differences in slopes across visual-predictive tasks between these two treatment groups.

In the analysis comparing controls and the 2X COC animals, the main effect of treatment and the treatment by task interaction were not significant [p>0.4]. Contrasts revealed no differences in slopes for these groups across visual-predictive tasks and pairwise comparisons indicated no within task differences in Block 2 performance.

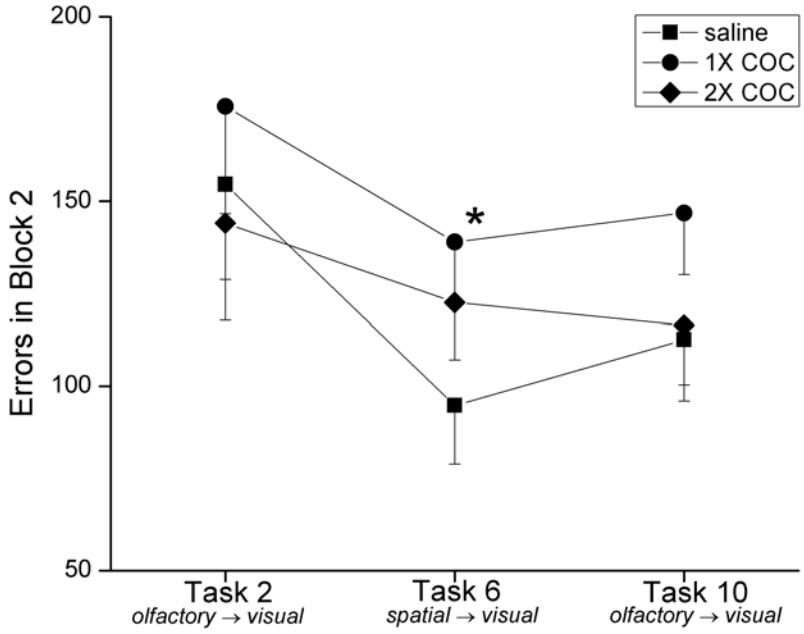

Figure 3.10 Visual-predictive tasks Block 2 errors. 1X COC animals committed significantly more errors than controls after achieving eight consecutive correct responses on Task 6 (*p=0.05). There were no within task differences between 2X COC animals and saline controls.

Spatial-predictive EDS Tasks

Errors to Criterion

In the analysis comparing controls and 1X COC animals for errors to criterion of the spatial-predictive EDS tasks, there were no significant main effects or treatment or significant treatment by task interactions [all p>0.5]. Contrasts revealed no group differences in rate of improvement across spatial-predictive tasks [all p>0.2]; pairwise comparisons indicated no differences in least square means within any spatial task [all p>0.3].

In the analysis of 2X COC animals and controls, the main effect of treatment was not significant [F(1,53.5)=0.04, p=0.8]; the interaction between treatment and task also failed to reach significance [F(2, 61.3)=2.03, p=0.14]. Contrasts suggested a treatment difference in slopes between Tasks 5 and 8 [F(1,41.8)=3.37, p=0.07], reflecting an increase in errors to criterion between tasks 5 and 8 for the 2X COC group but no change for the controls (Figure 3.11).

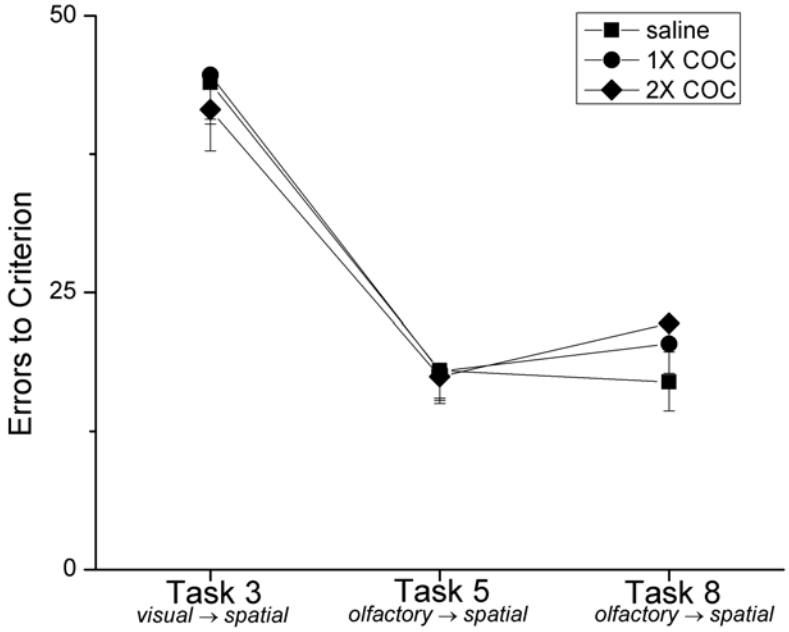

Figure 3.11 Total errors committed across spatial tasks. There were no significant treatment differences in overall errors within or between spatial-predictive tasks.

Errors in Block 1

The analysis comparing controls and 1X COC animals for Block 1 errors indicated no significant effect of treatment and no significant treatment by task interaction [p>0.9]. Contrasts and within task differences showed comparable performance across spatial-predictive tasks between controls and 1X COC animals. The analysis comparing controls and the 2X COC group revealed an interaction between treatment and task that approached significance [F(2,80.3)=2.46, p=0.09]; the main effect of treatment was not significant [F(1,42.5)=0.01, p=0.9]. There were no significant differences in Block 1 errors within each spatial-predictive task, although performance within Task 3 was suggestive [p=0.14]; within this task, 2X COC animals committed fewer Block 1 errors than controls (Figure 3.12). Contrasts did not reveal significant treatment differences in rate of decline of Block 1 errors across spatial-predictive tasks [all p>0.16].

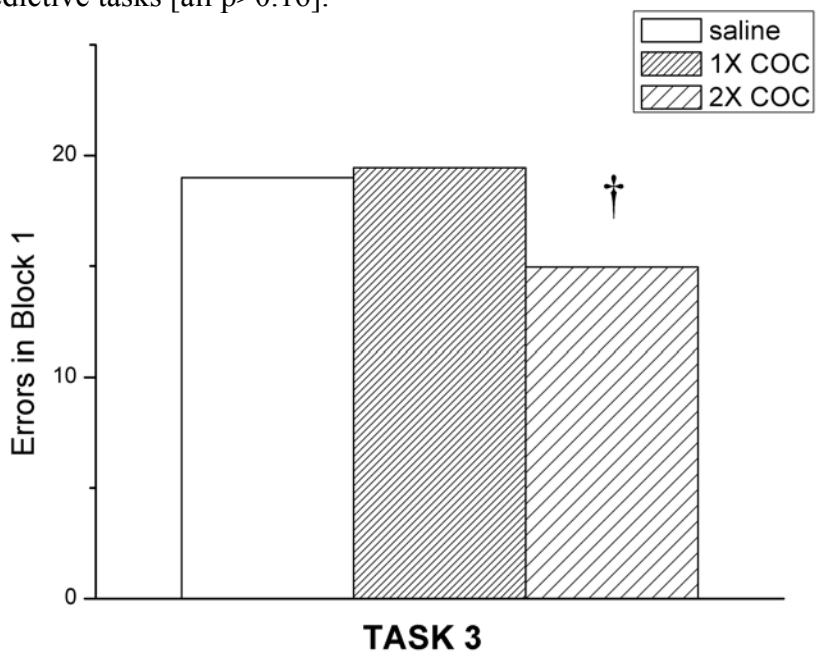

Figure 3.12 Errors committed within Block 1 on Task 3, a spatial-predictive task preceded by a visual-predictive task. 2X COC animals demonstrated a trend towards higher early learning errors on this task only. No differences were observed in Block 1 on the other two spatial-predictive tasks (Tasks 5 and 8).

Errors in Block 2

For the comparison of controls and 1X COC animals for Block 2 errors, the main effect of treatment and the treatment by task interaction were not significant [all $p>0.4$]. Further analyses of rate of decline of Block 2 errors across spatial tasks and mean Block 2 errors within each spatial-predictive task indicated no effect of treatment on either of these outcomes [all $p>0.2$]. Similarly, there were no significant treatment or treatment by task effects in the analysis of controls and 2X COC animals [all $p>0.6$], and no differences between treatments in slopes or mean Block 2 errors on spatial-predictive tasks [all $p>0.4$].

DISCUSSION

The two cocaine exposure regimens used in this study were identical during GD7-14 and differed only between GD15-21, during which time the dams of the "low dose" animals received cocaine (3.0 mg/kg) once daily (1X COC) and dams of the "high dose" animals were administered this dose of cocaine twice daily (2X COC). Nonetheless, the in depth analysis of performance across this series of EDS tasks uncovered the interesting finding that the cognitive effects of these two regimens varied both quantitatively and qualitatively, as delineated below.

The cognitive profile seen in the two cocaine-exposed groups was informed by dividing the learning of EDS tasks into two phases, termed Block 1 and Block 2. Block 1 ("early learning") is a period in which several cognitive processes must work in conjunction in order for an animal to learn a new rule after a switch in task contingencies. Animals must first break their attentional set to the previously predictive cue, followed by a period of testing hypotheses to determine the new rule. Simultaneously, the animals must filter out irrelevant information and to develop these new cue/reward associations. To achieve a consistently high level of performance (e.g. eight consecutive correct responses), animals must also maintain an attentional

set to the newly predictive dimension over time. As illustrated by the performance of the control animals in this experiment, the ability to master extradimensional shifts tended to improve with continual experience on these types of tasks, as the animals became accustomed to frequent changes in the sets of cues which predict reward. This improvement in performance with experience on a given type of task, referred to as "transfer of learning" or "learning to learn", was first suggested by Thorndike and Woodworth (1901), who proposed that the ability to transfer knowledge from one context to another context depended on the "identical elements" of the training task and the learning task (Thorndike & Woodworth, 1901). Transfer of learning generally occurs when previous knowledge is applied to solve a problem in a new situation; effective transfer increases the rate at which the new or similar task is learned (Ormrod, 2004). In the present study, poor early learning performance (i.e. higher Block 1 errors) early in the series was behavior appropriate to the sudden and unpredictable shift in task rules. However, as the series progressed, animals should have learned that frequent and unpredictable changes in task contingencies would occur; they should then be able to adapt to these changes, modify their learning strategies, and acquire the new rule. Inability to "learn to learn," in this study, would manifest as dysfunction early in the learning process across tasks in a given dimension (i.e. that shared "identical elements").

In contrast, during Block 2, the animals had already learned the relevant task rules; thus, errors during this phase were not due to failures in attentional set shifting, associative learning, or "learning to learn", as these processes must have been sufficient for the animal to have achieved eight consecutive correct responses. Instead, the primary function tapped in later learning was that of selective attention, in which the animal had to continuously filter out irrelevant stimuli and maintain an attentional set to the known predictive dimension.

While the behavioral outcomes analyzed here are measures of learning and learning rate, we do not believe that animals exposed to cocaine *in utero* have a deficit in overall learning ability (e.g. associative ability). Rather, because learning requires a number of cognitive processes, the measures of "learning" here reflect differences in the combination of these functions. Thus, it is the pattern of observed behaviors and change in performance over time that provides us with the greatest insight into the nature of the deficit experienced by cocaine-exposed animals. These patterns are delineated below.

Lower-dose (1X COC) cocaine exposure impairs both transfer of learning and selective attention in olfactory-predictive and visual-predictive EDS tasks.

The lower dose regimen exhibited several differences relative to controls. These animals improved less than controls from the first to the second visual-predictive EDS tasks, and improved less between the first two olfactory-predictive EDS tasks; no group differences were seen for the spatial-predictive EDS tasks. This impairment in learning transfer, which resulted in slower mastery of Tasks 6 and 7, was attributable to a reduced rate of improvement in the early learning phase of each of these tasks. The 1X COC animals achieved control-level performance in Block 1 by the end of the series, but the rate of improvement was slower. Note that contrary to the 2X COC animals, as described below, this deficiency in learning to learn was not seen only when the predictive cues were subtle relative to the irrelevant cues, but also when the predictive cues were salient.

Additionally, these 1X COC animals exhibited increased attention to very salient cues, an effect that produced impaired performance when potent and novel cues were irrelevant but superior performance when they were predictive. The olfactory cues presented on Task 1 were novel and highly potent, as animals had never previously experienced almond-anise-maple as an odor set. Despite the presentation

of visual distractors on this task, the cocaine-exposed animals' attention was commanded by these salient olfactory stimuli. Thus, on Task 1 (the first olfactory-predictive task of this series), 1X COC animals acquired the rule faster than controls because their attention was "captured" by the novel and potent olfactory stimuli.

Consistent with this "capturing" of attention by salient stimuli, the 1X COC animals committed significantly more errors than controls later in learning on both visual-predictive and olfactory-predictive tasks, suggesting a specific deficit in selective attention after task contingencies have been acquired. We suggest that the presence of simultaneously presented irrelevant stimuli increased distractibility, such that attention was captured by the most salient stimuli (where salience was determined by both the sensory characteristics of the stimulus and its previous association with reward).

Higher-dose (2X COC) cocaine exposure produces a specific constellation of early learning deficits dependent on predictive modality.

The pattern of effects seen across the three types of EDS tasks (visual-predictive, spatial-predictive, olfactory-predictive) is indicative of a different type of cognitive dysfunction in the rats exposed to the higher dose (2X COC) regimen. These animals exhibited a striking lack of improvement across the three visual-predictive tasks, relative to both the 1X COC and control groups. This impairment in "learning to learn" was specific to the visual-predictive tasks; 2X COC animals improved in the expected pattern across the series of olfactory-predictive and spatial-predictive tasks. The specificity of this disruption (unlike 1X COC animals) provides clues to the nature of the cognitive deficit induced by this higher exposure regimen. The lack of differences on spatial- and olfactory-predictive tasks indicates that the failure to improve across the three visual-predictive tasks was not due to a more general associative deficit. Rather, the pattern of effects observed for 2X COC

animals suggests a disruption in transfer of learning, specifically when the task involves shifting from a relatively dominant or salient predictive cue, to a more subtle predictive cue. We suggest that the olfactory predictors served as powerful salient cues for these 2X COC animals such that they were not distracted or aroused by other more subtle environmental stimuli, leaving transfer of learning intact.

Interestingly, 2X COC animals also tended to commit fewer Block 1 errors on the first shift from a visual-predictive task to a spatial-predictive task (Task 3). This pattern suggests that these cocaine-exposed animals may have formed a weak attentional set to subtle visual cues, which then enabled them to learn the subsequent spatial-predictive task very readily. The weak attentional set to the visual cues may have been due to the subtlety of these cues relative to the olfactory and spatial distractors (irrelevant stimuli). Thus, these 2X COC animals progressed more slowly on EDS tasks in which the previously predictive cue was highly salient (e.g. olfactory and spatial) and the current predictive cues were subtle (e.g. visual), but progressed more rapidly than controls when shifting from a less potent set of cues (e.g. visual) because they formed a weak attentional set under these conditions. Although the superior performance observed here was only a trend, a similar pattern (decreased performance on Task 2 followed by superior performance on Task 3) has previously been observed in a separate cohort of animals exposed to this same 2X COC regimen, validating the causal relationship between the higher dose exposure and this behavioral effect.

Consistent with these conclusions, the 2X COC animals also demonstrated superior performance on Task 1, the first task in which the maple-anise-almond odor triad was used. Again, the higher dose COC animals' attention was captured by the most salient environmental stimuli (here, potent and novel olfactory cues), which facilitated rule acquisition on this task.

Summary of findings

The pattern of performance observed in the present study indicates that the dose of cocaine exposure is an important factor in the constellation of persistent behavioral deficits. Animals exposed to a lower dose of cocaine prenatally were affected both early and later in learning. The impairments of these animals were primarily in transfer of learning (early) and selective attention (late), regardless of predictive modality. In contrast, the 2X COC animals showed a more specific and severe deficit in learning transfer, but only on the visual-predictive tasks. Additionally, these 2X COC animals showed an impaired ability to form an attentional set to the visual cue early in the task series, an effect that was not observed in later tasks.

Synthesis

The present findings concur with the majority of earlier findings that utilized the 2X COC exposure regimen. The present results and previous research in this lab have consistently shown that animals exposed to the 2X COC regimen were impaired on tasks in which olfactory stimuli were irrelevant but not when olfactory stimuli were predictive (Garavan et al., 2000; Gendle et al., 2004). Garavan and colleagues (2000) reported that animals in the 2X COC regimen were specifically impaired on spatial-predictive tasks when previously predictive olfactory cues were simultaneously presented; this disruption could not be attributed to task difficulty, cognitive inflexibility or associative ability but rather suggested impaired attentional control. In this prior study, cocaine-exposed animals (specifically males) were also disrupted in olfactory serial reversal learning, an effect again not due to deficits in associative learning or attentional set-shifting but rather due to impairments in selective attention. In a more recent study from this lab, Gendle and colleagues found that the unpredictable presentation of olfactory distractors disrupted the performance of

cocaine-exposed (2X COC) animals on a visual discrimination task, again reflecting that prenatal cocaine exposure impairs the ability of adult animals to maintain attention to less salient stimuli in the face of competing, dominant cues (Gendle et al., 2004). The over-arching dysfunction reported by these prior studies implicates impaired attentional processes cause by *in utero* cocaine exposure, specifically in that cocaine-exposed animals' attention is "captured" by the most salient stimuli. In the present study, 2X COC animals also demonstrate this dysfunction, which manifests as alterations in attentional set formation dependent on the salience of previously predictive stimuli; upon a shift in task parameters, this produces superior performance when the previously predictive stimuli were subtle (reflecting a failure to form a strong attentional set to prior cues) and inferior performance when the previously predictive stimulus was salient (suggesting "over-attention" to this previously predictive dimension). In addition, the findings regarding the lower dose regimen (the 1X COC group) provide a new contribution to the literature on persistent effects of prenatal cocaine exposure, by elucidating the behavioral disruption (i.e. in the domains of transfer of learning and selective attention) in a lower dose than previously examined.

Clinical Implications

The findings reported here may have important clinical implications. The present study provides compelling evidence that *in utero* cocaine exposure, even to very low doses, produces lasting deficits in attentional processes. In this study, because the distracting stimuli (in all task types) were presented repeatedly over a long period of time, there was the opportunity for animals to habituate to the existence of environmental distractors, an effect reflected by the lack of group differences on the final tasks of each type in the series. However, in the classroom setting in which novel and salient stimuli occur unpredictably, the ability of cocaine-exposed

individuals to essentially "ignore" the irrelevant information may be even more impaired than that reflected here. Thus, the effect of prenatal cocaine on ability to filter out irrelevant stimuli in order to form and/or shift attentional sets may be underestimated in the present report. Additionally, the disruptions in "learning to learn" observed here may have serious implications in a classroom setting, as learning sets are often tapped for applications to similar (but not identical) tasks over time. Taken together, the increased attention to salient stimuli coupled with failure to effectively transfer previously learned sets of information may inhibit classroom success for cocaine-exposed children.

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CHAPTER 4

LOW ALPHA-2 RECEPTOR DENSITY IN PREFRONTAL CORTEX INCREASES VULNERABILITY TO THE LASTING EFFECTS OF PRENATAL COCAINE EXPOSURE ON TRANSFER OF LEARNING AND SELECTIVE ATTENTION

ABSTRACT

The present study was designed to test the hypothesis that the attentional dysfunction produced by prenatal cocaine exposure (COC) is mediated by underlying changes in noradrenergic and/or dopaminergic activity in the prefrontal cortex. Female Long-Evans rats were exposed intravenously once daily to a low dose of cocaine (3.0 mg/kg) or saline from gestational days 8-21; offspring were tested in adulthood on a series of nine extradimensional shift (EDS) tasks. For each task, stimuli from three dimensions (olfactory, visual, spatial) were simultaneously presented on each trial; only one dimension was predictive of reward and the other dimensions were irrelevant and randomly associated with reward. At the end of behavioral testing, the brains were extracted and autoradiography was used to quantify the density of various noradrenergic and dopaminergic receptors in prefrontal cortex (PFC). This chapter presents the results of analyses correlating α2 receptor density in PFC with various behavioral outcomes. Density of α2 receptors in PFC was not different between controls and COC animals. Behavioral testing revealed that COC animals were impaired, relative to controls, in both early and later learning phases; the magnitude of effects was dependent on task type and α2 level of COC animals. The observed pattern of deficits suggested that prenatal cocaine exposure coupled with lower density of α2 receptors in prefrontal cortex impaired both selective attention and the ability to transfer learning across similar tasks, irrespective of the relative salience of predictive and nonpredictive cues, whereas COC coupled with high α2 produced a

more subtle impairment in selective attention (i.e. only on EDS tasks in which the predictive stimuli were subtle relative to distractors). These findings support results from prior studies indicating that COC animals are impaired when required to selectively attend to less salient cues in the face of irrelevant, dominant cues. This study also provides new information that this effect is not directly mediated by alterations in α2 receptor density. Rather, low α2 density in PFC may increase vulnerability to the lasting cognitive effects of prenatal cocaine exposure.

INTRODUCTION

Background

Over the past 25 years, the scientific community has debunked the media myth of a "crack baby epidemic," in which the incidence of cocaine use during pregnancy was greatly exaggerated. In the most recent survey from the Department of Health and Human Services, 4% of women ages 15 to 44 years reported recent use of illicit drugs (Substance Abuse and Mental Health Services Administration, 2007). Although this study did not specifically quantify cocaine use among pregnant women, a prior study from the National Institute for Drug Abuse estimated that 1.1% of women used cocaine while pregnant (Mathias, 1995). While the incidence of cocaine use during pregnancy is not nearly as high as suggested by the popular press in the late 1980s and early 1990s, the societal impact of such drug use is significant both economically and socially (National Institute on Drug Abuse, 1998; National Institute on Drug Abuse, 2001).

Recent studies of humans exposed to cocaine *in utero* have suggested a cognitive profile of possible cocaine-related effects that is much more subtle than originally suggested, with dysfunction specifically in the domains of attention, arousal regulation and reactivity to stressors (Mayes, 1999). A number of prospective studies in school-aged children have revealed associations between prenatal cocaine exposure

and impaired selective and sustained attention, inhibitory control, and arousal (Bendersky, Gambini, Lastella, Bennett, & Lewis, 2003; Delaney-Black et al., 1998; Delaney-Black et al., 2000; Dennis, Bendersky, Ramsay, & Lewis, 2006; Noland et al., 2005; Savage, Brodsky, Malmud, Giannetta, & Hurt, 2005). However, because cocaine use tends to be associated with other factors that may independently affect child development, including poor nutritional status, lack of pre- and post-natal care, and poly-drug use (Mayes, 1999), the findings from human studies are inconclusive. That is, the developmental effects of these confounding factors and those of cocaine cannot be disentangled or experimentally controlled in humans, so no causal link between *in utero* cocaine exposure and cognitive dysfunction has been established from these findings.

Animal Models of Prenatal Cocaine Exposure

Many researchers have turned to investigating animal models of prenatal cocaine exposure, which provide much more control over nutrition, postnatal environment, and stressors in both mothers and progeny. Collective evidence from rodents, rabbits and nonhuman primates exposed to cocaine *in utero* has indicated deficits in selective and sustained attention (Mayes, Molfese, Key, & Hunter, 2005). Cocaine-exposed animals have been shown to be specifically disrupted when a subtle but relevant stimulus was simultaneously presented with a potent irrelevant stimulus and when task contingencies varied unpredictably (Chelonis, Gillam, & Paule, 2003; Gabriel & Taylor, 1998; Garavan et al., 2000; Gendle et al., 2003; Gendle et al., 2004; Mayes et al., 2005; Morgan et al., 2002; Romano & Harvey, 1998). Recent evidence has suggested that the disruption of attentional set shifting and formation was highly specific to the parameters of the shift; cocaine-exposed animals were drawn to salient stimuli, which affected both the strength of the attentional set formed and the ease of shifting upon change in task rule (see Chapter 2). Cocaine-exposed animals also

demonstrated persistent dysregulation of arousal states, as evidenced by hyper-excitability to changes in environmental circumstances and increased reactivity to committing an error (Gendle et al., 2004; He, Bai, Champoux, Suomi, & Lidow, 2004). The extent of these observed behavioral impairments have been shown to be dependent on dose, timing and duration of prenatal cocaine exposure.

Brain Regions and Transmitter Systems Underlying Arousal and Attention

While recent work has provided insight into the specific behavioral dysfunction caused by gestational exposure to cocaine, less is known about the underlying changes in neural systems that may mediate these effects. There are several lines of evidence to suggest that the attentional and affective changes produced by prenatal cocaine are caused by catecholaminergic changes in the prefrontal cortex (PFC) and cingulate gyrus (CG) (Meyer & Quenzer, 2005; Viggiano, Ruocco, & Sadile, 2003). Studies in both rats and primates suggest that the medial prefrontal cortex is critical to adaptively modifying behaviors in response to environmental change and shifting attention between perceptual domains. Birrell and Brown (2000) reported that lesioning medial frontal cortex (and, to a lesser extent, CG1 and CG2) in rats produced deficits in shifting between response rules when the new task contingency required that attention be directed to a different perceptual dimension (Birrell & Brown, 2000). Non-human primates with lesions of the lateral prefrontal cortex were not affected in acquisition of visual discrimination or intradimensional shift tasks, but demonstrated inferior performance on the EDS (Dias, Robbins, & Roberts, 1996). These prefrontal processes are differentially regulated by both dopamine and norepinephrine, such that adaptive behavioral output requires synergy between neuromodulatory systems (Bouret & Sara, 2005; Robbins & Roberts, 2007). For example, α2 and D1 receptors in the PFC are functionally linked, such that an upregulation of D1 receptors (and therefore increased DA transmission) can be offset

by an upregulation of presynaptic α2 receptors (which decreases NE output), normalizing cognitive function (e.g. arousal and attention) (Steere & Arnsten, 1997). Co-regulation of NE and DA neuromodulatory systems represents a "fine-tuning" mechanism to produce appropriate catecholaminergic outputs yielding adaptive behavioral responses (Vanderschuren, Wardeh, De Vries, Mulder, & Schoffelmeer, 1999).

Investigations of neural changes in cocaine-exposed animals

These catecholaminergic systems are directly affected by cocaine, which increases synaptic monoamine concentration by blocking transport and reuptake of NE and DA. As discussed above, disruption of these catecholamines in PFC produces deficits in arousal regulation and attentional function similar to those cognitive impairments observed in cocaine-exposed animals; thus, it is logical to investigate the direct effects of *in utero* cocaine exposure on these systems. Changes in prefrontal neural networks have been observed in animals prenatally exposed to cocaine, but the findings have been inconsistent, due to the dose, route of administration and timing/duration of exposure as well as the neural outcomes investigated. One critical experimental issue is route of drug administration: the commonly used subcutaneous route of administration produces necrotic lesions at the injection site which increases maternal stress, upregulating both DA and NE in the prefrontal cortex and thereby independently influencing the neural substrates underlying attention and arousal processes (Mactutus, Booze, & Dowell, 2000). In addition, studies using the SC route generally employ much higher doses of cocaine than those using the IV route (generally 40 mg/kg with the SC route compared to 3.0 mg/kg with the IV route). IV administration, using low-dose exposure, has been shown to most accurately mimic human recreational use, and thus provides the most appropriate model system for studying behavioral and neural changes causes by prenatal cocaine. Therefore, the

literature reviewed here will only include those studies that utilized an IV-exposure procedure, which served as the rationale for specific neural endpoints investigated in the present study.

Dopaminergic function and cocaine-associated changes in DA processes

Dopamine projections extend from the ventral tegmental area (VTA) to restricted cortical areas, including the mPFC, ACC, and entorhinal cortex (Devoto, Flore, Pira, Longu, & Gessa, 2004; Meyer & Quenzer, 2005). Dopamine is of critical importance for goal-directed behaviors, especially in the mediation of attention and reward (Viggiano et al., 2003), relaying arousal signals, and producing appropriate behavioral responses (Robbins et al., 1998; Robbins & Roberts, 2007). The relationship between dopaminergic activity in PFC and PFC function is an "inverted-U," where moderate stimulation of D1-like receptors (D1 and D5) reduces "noise" by suppressing processing of irrelevant information, an effect eliminated when D1 stimulation too high or too low (Brennan & Arnsten, 2008). Several research groups have reported alterations in dopaminergic activity caused by *in utero* cocaine exposure (Bayer, Brown, Mactutus, Booze, & Strupp, 2000; Bayer, Kakumanu, Mactutus, Booze, & Strupp, 2002; Elsworth, Morrow, & Roth, 2001; Gabriel & Taylor, 1998; Mayes, 2003; Morrow, Elsworth, & Roth, 2001; Stanwood, Washington, & Levitt, 2001; Stanwood & Levitt, 2004; Stanwood & Levitt, 2007); however the specific nature of this effect is unclear. Data from the lab of Roth and colleagues suggest normal DA release under basal conditions but excessive DA release during times of stress (Elsworth et al., 2001; Morrow et al., 2001). Consistent with these data, two studies involving pharmacological challenges during testing on attention tasks indicates excessive DA activity in neural systems subserving attention and/or arousal following *in utero* IV cocaine exposure (Bayer et al., 2000; Bayer et al., 2002). The finding that animals exposed to low doses of IV cocaine during gestation also have

excessive DA activity in the PFC and/or ACC fits into the current framework of an "inverted-U" relationship between DA release and attention/arousal properties, by reflecting the upper end of the spectrum.

One finding, however, does not readily fit into this framework; namely, that prenatal cocaine exposure in rabbits has been found to disrupt coupling of D1 receptors and their associated G-protein, which may be the cause of the elongated dendritic projections also found in these animals, as DA activity normally curtails dendritic growth (Harvey, 2004; Romano & Harvey, 1998; Stanwood et al., 2001). Thus, there is some uncertainty about the nature of the change in dopaminergic systems following prenatal cocaine exposure. However, in light of the strong evidence that either insufficient or excessive DA disrupts PFC function, it is likely that disruptions in the DA system contribute to the observed behavioral dysfunction in cocaine-exposed animals. Clearly this relationship requires considerable additional investigation in order to fully elucidate how dopaminergic release is affected by *in utero* cocaine exposure and how such changes affect attention and arousal processes.

Noradrenergic function and cocaine-associated changes in NE processes

The locus coeruleus is the group of neurons that produces norepinephrine; these neurons send widespread axons throughout the cortex as well as hippocampus and cerebellum with relatively homogeneous cortical distribution (Meyer & Quenzer, 2005; Devoto, Flore, Pira, Longu, & Gessa, 2004). *In vivo* electrophysiology has shown that noradrenergic activation improves selectivity of evoked neuronal responses, which suggests a functional relationship between NE and a number of cognitive functions, including learning and memory, perception and attention (Bouret & Sara, 2005). Coereulo-cortical noradrenergic projections are also involved in modulating attention, primarily by permitting or enhancing signals that transmit novel stimuli in the environment (Aston-Jones, Rajkowski, & Cohen, 1999; Robbins et al.,

1998). NE has been specifically implicated in EDS performance, where stimulation of NE release improves EDS performance and inhibiting NE release produces a deficit on these same tasks (Bouret & Sara, 2005; Devauges & Sara, 1990; McGaughy, Ross, & Eichenbaum, 2008).

Like DA, the relationship between NE activity in PFC and those cognitive functions modulated by PFC follows an "inverted-U." Very low levels of arousal (e.g. sleep) reduces both tonic and phasic firing of NE cells in the LC and very high levels of arousal (e.g. stress) produces high tonic firing and low phasic firing – both of these conditions yield disruption in the ability to filter distracting stimuli (Arnsten, Scahill, & Findling, 2007; Aston-Jones et al., 1999; Lapiz & Morilak, 2006). When animals are alert but not overstressed, both tonic and phasic firing of NE cells in the LC is optimal (Brennan & Arnsten, 2008). Moderate release of NE engages high affinity postsynaptic α2A adrenoceptors, which couple to Gi and inhibit cAMP signaling (Aston-Jones et al., 1999; Brennan & Arnsten, 2008; Ramos & Arnsten, 2007). This change in receptor signaling strengthens network connectivity with neurons that have similar stimulus characteristics, thereby reducing the tendency to respond to non-target stimuli in both rats and monkeys (Aston-Jones et al., 1999; Brennan & Arnsten, 2008).

A large body of evidence specifically implicates prefrontal postsynaptic α2 receptors in the modulation of attention. Alpha-2A receptors are found on or near postsynaptic densities of dendritic spines of PFC pyrimdal cells and receive information from excitatory cortical networks (Arnsten et al., 2007; Ramos & Arnsten, 2007). In primates, pharmacological stimulation of postsynaptic α2A (with guanfacine) has been shown to improve PFC function by reducing distractibility and strengthening inhibitory control (Arnsten & Contant, 1992; O'Neill, Fitten, Siembieda, Ortiz, & Halgren, 2000; Steere & Arnsten, 1997). Conversely, blocking postsynaptic α2A with yohimbine (α2 antagonist) in the PFC of monkeys produces a constellation

of symptoms reflecting attention deficit, including impaired impulse control and decreased working memory needed to overcome distractors (Li & Mei, 1994; Ma, Qi, Peng, & Li, 2003). Taken together, these studies indicate that prefrontal postsynaptic α2 receptors regulate executive functions and are critical for the regulation of attention (Arnsten et al., 2007).

While the evidence suggesting a role of NE in PFC function is compelling, less work has focused on investigating the contribution of NE disruption to behavioral impairments directly associated with prenatal cocaine. However, several lines of evidence suggest that alterations in this system may be involved in cocaine-associated deficits in attention and arousal. Recent studies have revealed morphological changes in the noradrenergic system, both *in vivo* and *in vitro*; cocaine-exposed progeny show cell-specific inhibition of neurite outgrowth (initiation, elongation and branching) from the LC (Dey, Mactutus, Booze, & Snow, 2006; Snow, Smith, Booze, Welch, & Mactutus, 2001; Snow et al., 2004). Cocaine exposure has been shown to produce an upregulation of α2 density in PND35 day old males across brain areas (e.g. hippocampus, parietal cortex, amygdala, and hypothalamus); females were similarly affected but only in parietal cortex and amygdala (Booze et al., 2006). However, in adult animals (PND395), there were no differences observed in overall α2 receptor density between controls and cocaine-exposed animals in prelimbic and cingulate cortices (Ferris et al., 2007). These prior studies have focused on the role primarily of postsynaptic α2 receptors, but there is also some evidence that prenatal cocaine exposure may also effect the α2 autoreceptor in the LC. Roth and colleagues have recently shown a decrease in α2A density on NE neurons of the LC associated with *in utero* cocaine exposure, as well as elevated NE turnover in the PFC (Elsworth et al., 2007).

Thus, the nature of the alterations in the NE system following prenatal cocaine exposure is unclear. However, since a large body of evidence indicates that either insufficient or excessive NE impairs PFC function, it is reasonable to assume that disruption of this neurotransmitter system by prenatal cocaine may contribute to the constellation of behavioral abnormalities observed in exposed animals. The relationship between NE modulation of PFC function and subsequent disruption of these processes by prenatal cocaine is an area of research that demands further investigation.

Need for Present Study

Most of these previous studies of prenatal cocaine have investigated either neural *or* behavioral outcomes in cocaine-exposed subjects, rather than examining both outcomes in the same animals. The available studies that have looked at either neural or behavioral changes in cocaine-exposed animals have often used different doses and routes of administration, further complicating integration of neural and behavioral data. Thus, the body of knowledge on the relationship between prenatal cocaine, neural changes and behavior is lacking on two counts: (a) we have little information on whether cocaine-exposed animals exhibiting neural alterations would have attentional impairments, and (b) when behavioral deficits are observed, we do not know the nature of the underlying neural changes. The present study aimed to fill this gap in the literature by assessing densities of DA and NE transporters and several DA (D1, D3) and NE (α1, α2) receptors in cortical and subcortical areas and behavioral measures of attention and arousal regulation in the same animals. As discussed above, there is a growing body of evidence that norepinephrine is necessary for attentional processes, functions also disrupted in animals prenatally exposed to cocaine. Additionally, there is evidence suggesting that NE systems are affected by cocaine exposure *in utero.* Therefore, we aimed to investigate whether changes in

attention and/or arousal regulation in cocaine-exposed animals are correlated with changes in noradrenergic receptors in PFC.

The present chapter presents the results of a subset of the neural data and a subset of the administered behavioral tests. Specifically, it presents the findings relating to correlations between low-dose cocaine exposure, performance on a series of attentional set-shifting tasks and density of α2 adrenoceptors in prefrontal cortical areas. These analyses relating behavior to receptor density were conducted on the same animals described in Chapter 3. Specifically, the present report presents behavioral and neural correlations for a subset of animals (controls and 1X COC animals) from this earlier chapter.

METHODS

Subjects

Male and female Long–Evans rats were obtained from a commercial supplier (Harlan Sprague–Dawley, Indianapolis, IN) at approximately 11 weeks of age. The health of this animal colony and their housing conditions were monitored according to guidelines set forth by the National Institutes of Health and the American Association for the Accreditation of Laboratory Animal Care. All breeding and surgical procedures were conducted at the University of South Carolina (Columbia). The animal facility at the University of South Carolina was maintained at 21± 2°C, 50% ± 10% relative humidity, and had a 12-hr light/dark cycle, with lights on at 7:00AM. Food (Prolab RMH 1000, PMI Nutrition International, Brentwood, MO) and water were available *ad libitum*.

Catherization and Mating

A sterile intravenous catheter (22 gauge; Becton/Dickson, General Medical Corporation, Grand Prairie, TX) with a Luer-lock injection cap (Medex, Kensington, MD) was implanted into the jugular vein of nulliparous female Long-Evans rats; this

served as the IV port for either cocaine or saline after conception. Details of the catheters and surgical procedures used can be found in Mactutus, Herman, and Booze (1994).

After recovery from surgery (4-8 days), the females were group-housed (n=3) with a male rat. Conception (Gestational Day 0; GD0) was confirmed with a sperm-positive lavage.

Drug Administration

Drug injection procedures were conducted as described in (Mactutus, Herman, & Booze, 1994). Briefly, the dams were divided into three treatment groups; only data from controls and lower-exposure cocaine groups will be discussed here. The catheterized dams received once daily IV saline injections from GD 1-7. Cocaine hydrochloride (Research Triangle Institute, NC) or saline was then administered as a bolus injection of 3.0 mg/kg once per day from GD 8-21, at a volume of 1 ml/kg (15s), followed by flushing (15s) the catheter with 0.2 mL of heparinized (2.5%) saline. The drug was dissolved daily immediately prior to injection. Food and water were provided *ad libitum* for the duration of drug administration. The 3.0 mg/kg dose per injection was selected based on prior research that suggested that this dose produced a pharmacokinetic profile in the periphery and psychological responses that appropriately modeled human recreational use (Booze, Lehner, Wallace, Welch, & Mactutus, 1997; Evans, Cone, & Henningfield, 1996).

Offspring Care

After birth, litters were culled to four males and four females; on postnatal day (PND) 21, one male and one female offspring from each of 24 litters were transported under environmentally controlled conditions to Cornell University for behavioral testing, with 12 animals in each treatment X sex group (n=48). All animals were housed in same sex pairs and acclimated to a reversed day/night schedule (lights off at

5:30AM, lights on at 8:30PM EST) for three weeks prior to behavioral testing. Behavioral testing occurred six days a week (Sunday-Friday) for two hours a day for the duration of the study; all behavioral testing occurred during the animals' active (dark) cycle.

Food Restriction

Animals were placed on a food restriction schedule on approximately PND28 to accustom them to the feeding regimen used during behavioral testing. Females were initially restricted to a daily allotment of 18 grams of rat chow (Pro-Lab Rat/Mouse/Hamster Chow); males were allowed 21 grams of chow per day. During this acclimation period, animals were allowed five hours in individual feeding cages to consume their food allocation. Once behavioral testing began, the amount of food reward received during testing was subtracted from the animals' daily allotment of chow described above to normalize caloric intake. Animals were allowed three hours immediately after testing to consume the remainder of their food individually before being returned to their home cage, for a total of five hours to consume allowed food. On non-testing days (Saturdays), animals were given five hours in their individual cages to eat their food allowance. Tap water was provided *ad libitum* throughout the study.

Food allotment was monitored and adjusted by individuals unaware of treatment conditions of the individual animals. Animals with response patterns indicative of low motivation during testing sessions had daily chow intake reduced as needed to increase motivation and still maintain a healthy body weight.

Apparatus

Behavioral testing was conducted in 12 custom-built Plexiglas automated operant chambers, each housed in a sound-attenuating wooden enclosure. The chambers consisted of a rectangular waiting area (26.5 cm x 25 cm x 30 cm) with a

smaller testing alcove extending from one wall. A motorized guillotine-type door controlled entrance into the alcove and prevented responses between trials. Within each of the three walls of the alcove was a recessed funnel-shaped port. The left and right ports were at an approximate 45° angle to the center port. A set of infrared phototransistors was located at the alcove entrance and at the opening of each of the three ports; breaking the infrared beam signaled trial initiation or a nosepoke, respectively.

A green light-emitting diode (LED) was located above each of the three ports in the alcove; illumination of one of these LEDs served as either the predictive cue or the visual distractor in the present report. Additionally, the narrow end of each port was connected by tubing to three bottles containing liquid odorants (anise, almond, and maple scents), attached to a board placed outside of the box (9 bottles total). Compressed air was forced through the scented liquids, allowing scented air to be emitted into the testing chamber during the task. These odors served as the olfactory stimuli in the present report.

Behavioral Testing Procedure

Each animal was assigned to one of the 12 testing chambers such that each chamber was balanced across treatment groups; each chamber was designated for rats of one sex only and each rat used the assigned apparatus for the duration of the study. Animals began nose-poke training on PND53 (for details of training procedure, see Hilson & Strupp, 1997). Briefly, during this training, all animals learned to make a one-second nose-poke into the response ports; for all tasks, this 1-second nose-poke constituted a 'choice.' Correct responses were rewarded with a 45-mg Noyes food pellet delivered directly onto the alcove floor from a pellet dispenser.

Following successful completion of these training phases, behavioral testing began. For all tasks, a daily testing session consisted of 200 response trials (trials on

which the animal entered the alcove within 60 seconds after the door was raised) or two hours, whichever came first. Before being tested on the task series described in the present report, all animals had mastered a visual sustained attention task, a visual-predictive/olfactory distraction task, and an olfactory serial reversal task. Animals began the EDS series described below on approximately PND180.

Extra-Dimensional Set (EDS) Shifting Tasks

The EDS series here is identical to that presented in Chapter 3: an initial olfactory-predictive task with visual distractors followed by nine subsequent "shifts" in which the predictive dimension (olfactory, visual, spatial) was switched with each successive task. For each task, the rat's entry into the testing alcove at trial onset produced the immediate illumination of one of three LEDs and the emission of three odors (one from each port). The odor triad was always anise-almond-maple, and the port from which each was emitted varied pseudo-randomly. The presentation of stimuli on each trial also included a spatial dimension (i.e. left, center, or right port). All animals completed the ten tasks of the EDS series in the same order, as described below (Table 4.1). Reward contingencies for these tasks are detailed in Table 4.2.

Table 4.1 Order of tasks in Extra-Dimensional Shift Series

Task Number	Predictive Dimension
Task 1	Olfactory
Task 2	Visual
Task 3	Spatial
Task 4	Olfactory
Task 5	Spatial
Task 6	Visual
Task 7	Olfactory
Task 8	Spatial
Task 9	Olfactory
Task 10	Visual

Table 4.2 Reward contingencies for extra-dimensional shift tasks

	LED ILLUMINATED	ODOR EMITTED	SPATIAL LOCATION	Correct Response is:
Visual-predictive (Tasks 2,6,10)	Relevant	Irrelevant	Irrelevant	illuminated LED
Olfactory-predictive (Tasks 1,4,7,9)	Irrelevant	Relevant	Irrelevant	maple odor
Spatial-predictive (Tasks 3,5,8)	Irrelevant	Irrelevant	Relevant	center port

Animals were tested on a given task in the series until the learning criterion was reached. Based on prior evidence that asymptotic performance on the visual-predictive task was 80-85% correct (relative to 90-95% on the spatial- and olfactory-predictive tasks), it was deemed optimal to designate the learning criterion on this task at 80% correct in a single session, whereas the criterion for the other two task types was designated as 88% correct for a single session.

After completion of the EDS series, animals were tested on a "Surprising Reward Omission" task for approximately 40 days, after which they were euthanized and their brains were extracted.

Tissue Preparation

After the completion of all behavioral testing (approximately PND400), animals were injected with sodium pentobarbital (130mg/kg IP) and transcardially perfused with 50 mL of 0.9% cold NaCL (37°C) and 150 mL 0.9% NaCL at room temperature. Their brains were immediately extracted; the cerebellum was blocked from the cerebral cortex and both sections were fixed to glass slides and frozen for storage (-80°C). The tissues were packed in liquid nitrogen and shipped to Columbia, South Carolina for receptor autoradiography.

At the University of South Carolina, the brains were cryostat sectioned

(-20°C, 20 μm thick) in the standard coronal plane and thaw mounted onto glass slides. Sections were then collected using stereotaxic coordinates for the prelimbic region, hippocampus, cingulate gyrus, and nucleus accumbens (for details see Ferris et al., 2007). These sections were then stored at –80°C for 24 hours prior to processing, then switched to –20°C storage. For α2 adrenergic receptor autoradiography, tissue sections from the prelimbic area, CG1 and CG2 were examined for paraiodoclonidine (PLS) total binding and RX821002 (RBI) nonspecific binding; images were produced to quantify optical density data. In addition to the α2 measures, autoradiography was also done for D1 and D3 receptors as well as NET density. (Details of the autoradiography and imaging procedures can be found in Ferris et al., 2007.)

Dependent measures

Receptor Dependent Measures

Distributions of α2 receptor density in the prelimbic, CG1 and CG2 regions were evaluated individually and found to be positively correlated ($p>0.9$). Because of the similarity of density within each of these regions and the functional relationship they share, we averaged them to provide a single measure of "PFC" α2 density. Assessment of the continuous α2 data revealed the presence of very high and very low values (across treatment) that influenced the accuracy of a regression line. To minimize the influence of these data points, these α2 values were classified categorically (high or low density), which provided a more readily interpretable outcome.

Behavior Dependent Measures

Total errors to criterion provided a measure of learning rate. Learning on each task was also divided into two phases demarcated by the point at which an animal achieved eight consecutive correct responses; these two phases were designated "Block 1" and "Block 2." Varying strings of correct responses (strings of five, 10,

and 12 correct responses) were compared for the demarcation point to ensure that any observed differences were not solely due to the point of demarcation. Results of these additional analyses confirmed that the string of 8 consecutive correct responses provided the most sensitive demarcation for revealing group differences, although the basic patterns were the same for the other demarcation points.

Separate analyses were conducted for each type of EDS task (e.g., olfactory-predictive, visual-predictive, spatial-predictive); no direct comparisons were made between tasks with different predictive dimensions. We decided *a priori* that we would test for treatment differences for each task, regardless of whether the main effect of treatment or the treatment X task interaction was significant, in order to avoid missing effects. In addition to comparing group differences for each task, we were also interested in treatment-related differences in the rate of improvement across each task type (i.e., "learning to learn"). Observed group differences should be viewed as tentative, and in need of replication by future studies.

Statistical Models

We hypothesized that the deficits in attention observed in cocaine-exposed animals were mediated by underlying alterations in noradrenergic and/or dopaminergic activity in PFC, specifically by changes in the density of noradrenergic and/or dopaminergic receptors in this region. This hypothesis, with respect to α2 receptors, was evaluated with four subtests, each designed to address one aspect of the relationship between PFC α2-receptor density, prenatal cocaine exposure, and behavior. These subtests were designed to proved a statistical answer to each of the following questions:

1. Is α2 density in the PFC associated with *in utero* cocaine exposure?

2. Is overall performance (e.g. errors to criterion) or performance within specific learning phases (e.g. blocks) associated with α2 density in PFC irrespective of treatment?
3. Is there a relationship between the behavioral outcomes (outlined above) and cocaine-exposure, regardless of PFC density? (This is the question evaluated in Chapter 3.)
4. Is there an interaction between α2 and cocaine-exposure on behavioral outcomes, such that a specific combination of α2 and cocaine-exposure would have a differential effect on overall learning or learning within each phase? To investigate this final question, we classified each animal into a separate group based on α2 level (high or low) and cocaine treatment (saline or 1X COC).

Statistical Procedures

All statistical analyses were conducted with SAS v9.1 (SAS Institute, Cary, NC) for Windows XP Professional. A General Linear Model was used for models in which there was one observation per animal; all other models used a repeated measures analysis of variance (ANOVA) to assess statistical significance for each of the dependent behavioral measures. The ANOVA model accounted for both the correlation induced by using littermates and multiple testing on each rat. Outliers in both fixed and random effects were examined for influence; if a model did not meet the criteria for parametric analysis, a nonparametric procedure was used to reduce the effect of extreme data points.

All the models involving prenatal treatment, behavior, and α2 density initially had low and high subgroups (demarcated by the overall median value for the combined population) for both the controls and the cocaine-exposed animals. However, there were no significant differences between controls with low α2 density

and controls with high α2, for any measure [all p>0.3]. Therefore, controls were combined across α2 level for comparison with each of the two subgroups of COC animals for the analyses presented below. For these models, rather than having a main effect of treatment (COC and control), α2 density (low and high) and the interaction of these two variables, the models presented below include only a 3-level "group" factor, which refers to low α2 COC, high α2 COC, and controls (low α2 and high α2 combined).

RESULTS

The behavioral and autoradiography procedures described above were conducted on animals from three exposure groups (as discussed in Chapter 3): controls, lower dose cocaine (1X COC) and higher dose cocaine (2X COC). However, due to a problem in transporting a subset of extracted brains to the autoradiography facility at University of South Carolina, a large number of 2X COC brains were destroyed. Thus, although we had behavioral data with these higher dose animals, we had no neural data with which to correlate it. Therefore, this report will present results that only pertain to controls and 1X COC groups.

Alpha-2 density

The main effect of prenatal treatment on α2 density in the PFC was not significant [$F(1,41)=0.04$, $p=0.8$]. Controls and cocaine-exposed animals showed nearly a complete overlap in range of α2 receptor density (Figure 4.1).

Correlation between behavioral outcomes and α2 density (controls and COC groups combined)

For all behavioral analyses, α2 in PFC was classified as "high α2" or "low α2" based on the median value (435. 25 fmol/mg). The main effect of α2 density was not significant for either errors to criterion or errors within each block, for any of the task types analyzed (Task 1, olfactory-predictive EDS, visual-predictive EDS, spatial-

predictive EDS) [all p>0.2]. The interaction between α2 level and task type also was not significant for all outcomes on all task types [all p>0.2].

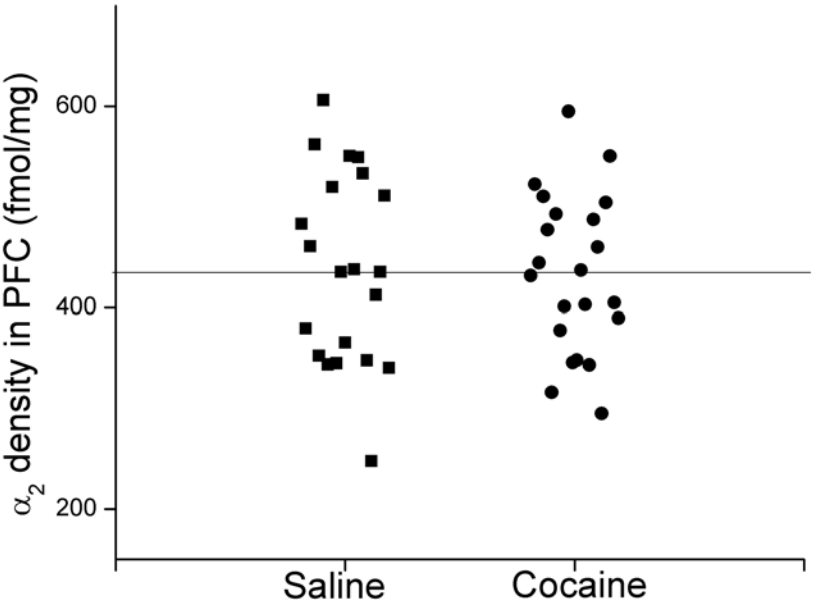

Figure 4.1 The distribution of α2 density in PFC by treatment. Reference line represents the median value: 435.25 fmol/mg. Subsequent categorical classification of "high" or "low" α2 density was based on this median value.

VISUAL-PREDICTIVE EDS TASKS (Tasks 2, 6, 10)

Errors to Criterion

In errors to criterion on the visual-predictive tasks, the comparison between COC animals and controls across α2 level revealed a significant main effect of treatment [F(1,39.4)=5.85, p=0.02]; the treatment by task interaction was not significant [F(2,63)=2.05, p=0.14]. Contrasts revealed that the rate of improvement between Tasks 2 and 6 varied for these two treatment groups (F(1,45.7=2.98, p=0.09) (Figure 4.2). Consistent with this different rate of improvement, cocaine-exposed animals were similar to controls on Task 2 but committed more errors to criterion on Task 6 [t(57.3)=-3.52, p=0.0009] and Task 10 [t(60.7)=-1.68, p=0.10].

These differences between controls and COC-exposed animals varied as a function of the α2 level in the cocaine-exposed animals. There was a significant main effect of group (control, high α2 COC, low α2 COC) [F(2,40.9)=3.84, p=0.03]. The interaction between group and task failed to reach significance [F(4, 60.9)=1.4, p=0.2]. Contrasts revealed that cocaine-exposed animals with low α2 density improved at a different rate than controls between Task 2 and Task 6 [F(1,43.7)=3.8, p=0.06]. Again, consistent with this differential rate of improvement, the low α2 COC group were not different from controls on Task 2, but committed significantly more errors than controls on both Tasks 6 [t(55.8)=-3.76, p=0.0004] and 10 [t(59.8)=-2.43, p=0.02].

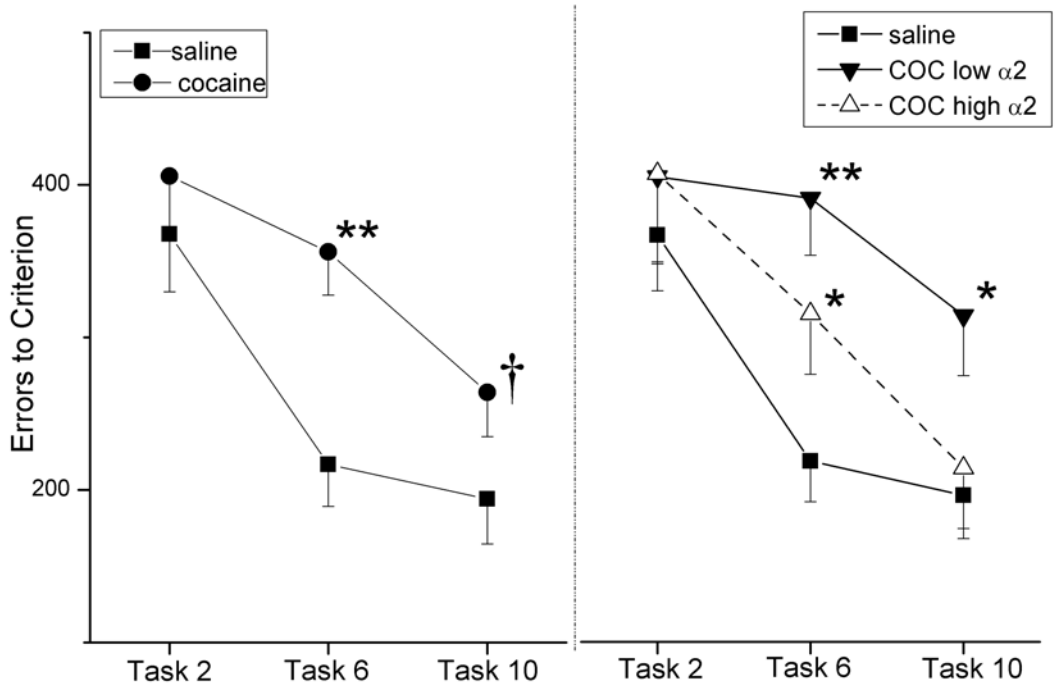

Figure 4.2 Errors to criterion on visual-predictive tasks. COC animals commit more errors than controls on Task 6 and Task10. This effect is enhanced in animals with low α2 receptor density in PFC. Cocaine-exposed animals with high α2 density are impaired relative to controls only on Task 6. († p<0.10; * p<0.05; ** p<0.01)

High α2 density cocaine-exposed animals demonstrated a different pattern of learning across visual-predictive tasks than low α2 COC animals (Figure 4.2). Contrasts indicated no difference between controls and high α2 COC animals in rate of improvement across visual-predictive tasks, but within Task 6 high α2 COC committed more total errors than controls [t(51.4)=-2.03, p=0.05]. These high α2 COC animals reached control level performance by Task 10.

Block 1 Errors

The analysis comparing the COC animals and controls (across α2 level) for Block 1 errors did not reveal a main effect of treatment [F(1,26.1)=2.34, p=0.14] nor a significant treatment by task interaction [F(2,54.7)=1.5, p=0.2]. Contrasts were suggestive of treatment differences in learning rate between Task 2 and Task 6 [F(1,50.4)=2.5, p=0.12]. Further analysis revealed that cocaine-exposed were not different from controls on Tasks 2 and 10 but committed significantly more errors in Block 1 within Task 6 [t(63.1)=-2.65, p=0.01] (Figure 4.3).

When considering the effect of α2 level on Block 1 performance, the main effect of group (control, low α2 COC, high α2 COC) bordered on significant [F(2,31)=2.62, p=0.09]; the group by task interaction was not significant [F(4, 57.4)=1.25, p=0.3]. Contrasts revealed that controls and low α2 cocaine animals improved at different rates between Task 2 and Task 6 [F(1, 48.9)=4.31, p=0.04]. As shown in Figure 4.3, Block 1 performance on Task 2 was similar between groups, but low α2 COC animals committed more Block 1 errors than controls on both Tasks 6 [t(63)=-3.72, p=0.0004] and 10 [t(64.5)=-1.67, p=0.10]. The controls and high α2 COC animals did not differ significantly in the number of Block 1 errors within each of the three visual-predictive tasks [all p>0.5].

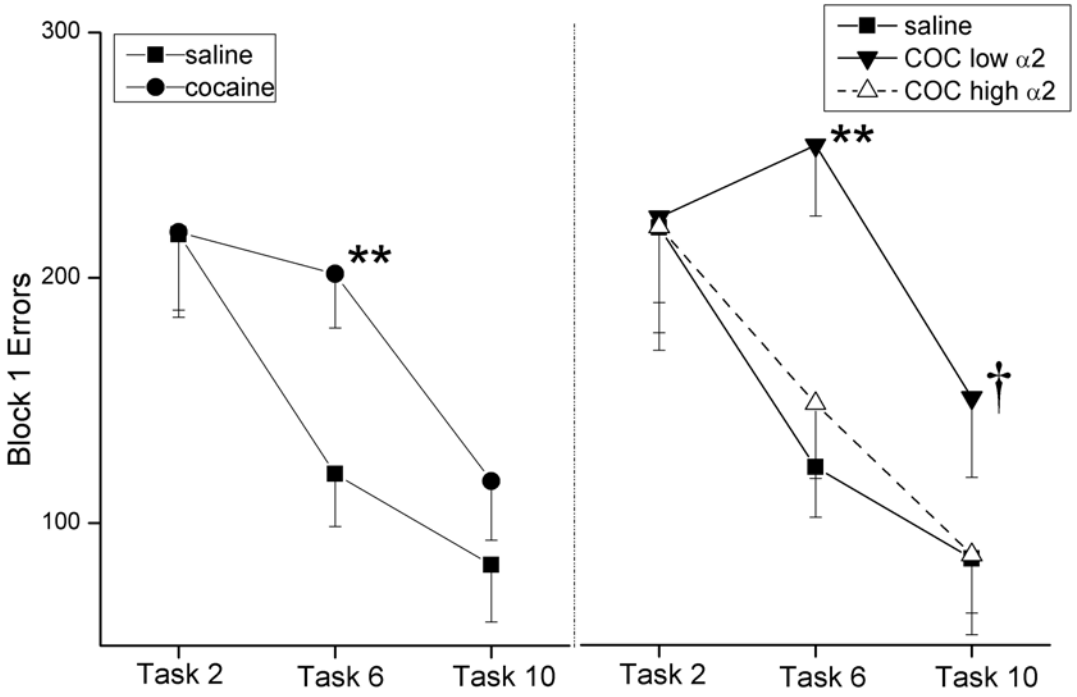

Figure 4.3 Errors committed in Block 1 of visual-predictive tasks. Cocaine-exposed animals commit significantly more errors than controls prior to achieving eight consecutive correct responses. This effect is driven specifically by performance of cocaine-exposed animals with low α2 density in PFC, who commit more Block 1 errors than controls on both task 6 and task 10. (**p<0.01; †p<0.10)

Block 2 Errors

The analysis comparing controls and COC animals across α2 level for Block 2 errors revealed a significant main effect of treatment [$F(1,43.5)=4.23$, $p=0.05$]; the treatment by task interaction was not significant [$F(2, 49.3)=0.05$, $p=1$]. Contrasts indicated that there were no significant differences in rate of Block 2 errors across visual-predictive tasks [all $p>0.7$]. As shown in Figure 4.4, COC animals and controls were not different on Task 2, but COC animals committed significantly more Block 2 errors on Tasks 6 [$t(52.4)=-2.13$, $p=0.04$] and 10 [$t(56)=-1.99$, $p=0.05$].

When evaluating the cocaine-exposed animals divided by α2 level, neither the main effect of group nor the group by task interaction reached significance [$F(2,43.9)=2.15$, $p=0.13$, $F(4, 47.2)=0.28$, $p=0.9$] . Contrasts revealed no differences

in rate of Block 2 errors between groups across visual-predictive tasks. Pre-planned comparisons within each task revealed that both cocaine-exposed groups were not different from controls on Task 2, but both low α2 COC and high α2 COC committed more errors than controls on Task 6 [t(50.5)=-1.81, p=0.08; t(52)=-1.62, p=0.11, respectively]. Low α2 COC animals also committed more Block 2 errors than controls on Task 10 [t(54.9)=-2.23, p=0.03], but high α2 COC animals were not different from controls on this final visual-predictive task (Figure 4.4).

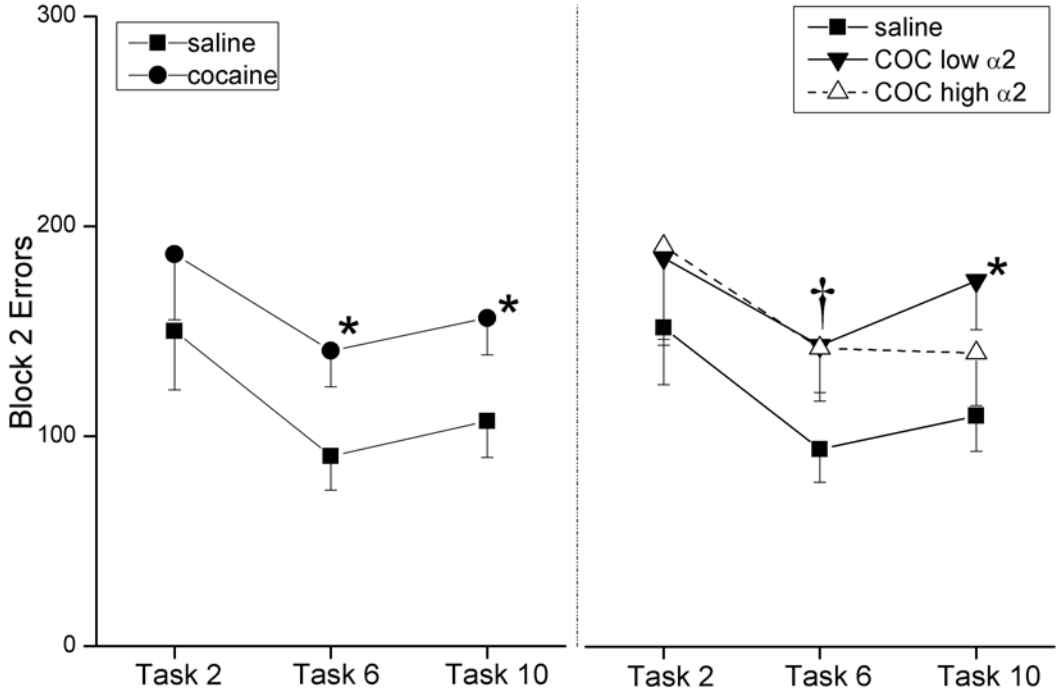

Figure 4.4 Errors committed in Block 2 of visual-predictive tasks. Cocaine-exposed animals commit significantly more errors after learning task contingencies (Block 2) across visual tasks. This effect reaches significance only for Task 6 when cocaine-animals are collapsed across α2 level. Both high and low α2 animals are impaired within task 6, an effect which persists in the low α2 levels to the last visual-predictive task.

OLFACTORY-PREDICTIVE EDS TASKS

Task 1

In the analysis of Task 1 performance, the distribution of residuals for all outcomes (errors to criterion, Block 1, Block 2) did not meet the assumptions for parametric analysis. A nonparametric Wilcoxon Rank Sum procedure was used to minimize the effects of very high data points. The cocaine-exposed animals were not different from controls on any outcome [all $p>0.2$]. Evaluating groups based on α2 level revealed that neither group of cocaine-exposed animals were significantly different from controls in total errors committed on Task 1 [low α2: Wilcoxon $p=0.14$, high α2: Wilcoxon $p=0.7$]. Cocaine-exposed groups were also not different from cntrols on Block 1 errors [low α2: $p=0.3$, high α2: $p=0.8$] or Block 2 errors [low α2: $p=0.4$, high α2: $p=0.9$].

Tasks 4, 7, 9

Errors to Criterion

In the analysis comparing COC and control animals across α2 level, the main effect of treatment on errors to criterion was not significant [$F(1,28.7)=2.08$, $p=0.16$] (Figure 4.5). The interaction between treatment and task also did not reach significance [$F(2, 57.9)=1.01$, $p=0.4$]. Contrasts revealed no treatment differences in rate of learning across olfactory tasks (all $p>0.17$). Further analysis revealed that cocaine-exposed animals committed significantly more errors on Task 7 than controls [$t(70.9)=-1.97$, $p=0.05$] but were not different within Tasks 4 and 9. For the comparison of controls and COC animals subdivided by α2 level, both the main effect of group and the group by task interaction were significant [$F(2,28.4)=3.88$, $p=0.03$, $F(4, 57.5)=4.57$, $p=0.003$, respectively] (Figure 4.5). Contrasts revealed that low α2 COC animals improved at a slower rate than controls from Task 4 to Task 7 [$F(1,57.7)=7.6$, $p=0.008$]. Consistent with this differential rate of improvement,

errors to criterion was similar between these groups on Tasks 4 and 9 but significantly higher for the low α2 COC animals than for controls on Task 7 [t(68.4)=-4.12, p=0.0001]. There were no differences between the high α2 COC animals and controls in total errors to criterion on any of the three olfactory-predictive tasks.

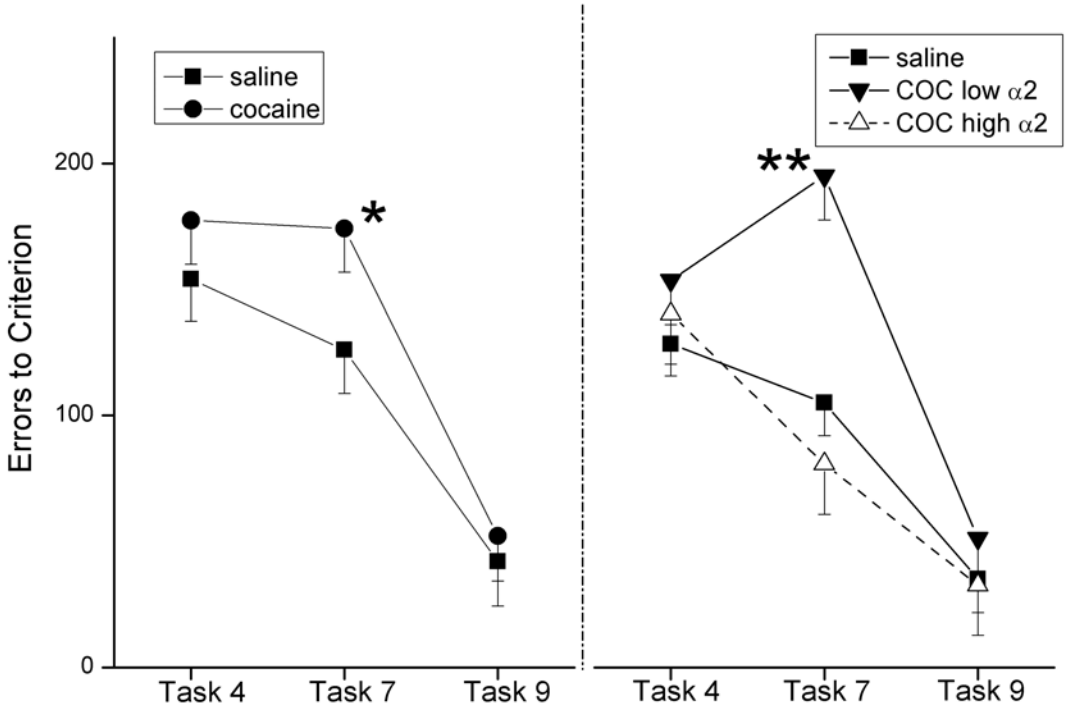

Figure 4.5 Errors to criterion on olfactory-predictive tasks. Across α2 level, cocaine-exposed animals commit significantly more errors than controls within Task 7 (p=0.05). This difference is driven by performance specifically of low α2 cocaine exposed animals, which are different from both controls and high α2 COC animals on Task 7 (p<0.0001).

Block 1 Errors

The analysis comparing the COC and control animals, across α2 level, for Block 1 errors did not reveal a main effect of treatment [F(1,25.4)=0.78, p=0.4] nor a significant treatment by task interaction [F(2,53.9)=1.88, p=0.16]. Contrasts revealed that rate of Block 1 learning was significantly different for controls and COC animals between Task 4 an Task 7 [F(1, 54.4)=3.69, p=0.06] (Figure 4.6). Likely as a result of

this difference in rate of improvement across tasks, the cocaine-exposed animals committed more Block 1 errors than controls within Task 7 [t(68)=-1.82, p=0.07].

Analysis of Block 1 errors for the olfactory-predictive tasks (Figure 4.6) revealed a significant interaction between group and task for the model comparing controls, high α2 COC, and low α2 COC animals [F(4,53.5)=2.72, p=0.04]; the main effect of group was not significant [F(2, 25.1)=1.69, p=0.2]. Contrasts indicated that low α2 COC animals and controls performed at different rates between Task 4 and Task 7 [F(1,54)=8.82, p=0.004]. Further analysis revealed that all groups performed similarly on Tasks 4 and 9, but low α2 COC animals committed significantly more Block 1 errors than controls within Task 7 [t(66.7)=-3.13, p=0.004].

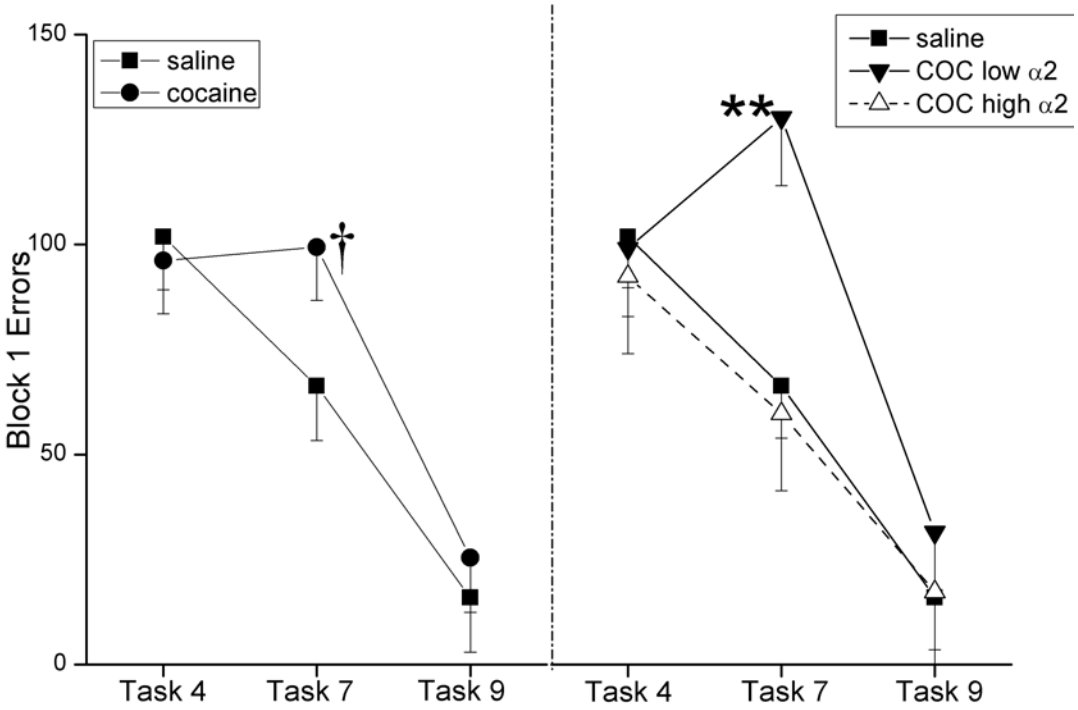

Figure 4.6 Block 1 errors on olfactory-predictive tasks. Cocaine-exposed animals commit significantly more errors than controls on Task 7 when α2 level is low (p=0.004).

Block 2 Errors

In the analysis comparing controls and cocaine-exposed animals across α2 level for Block 2 errors, there were no significant main effects of treatment or the treatment by task interaction [all p>0.16]. Contrasts revealed no group differences in rate of improvement across olfactory-predictive tasks, but COC animals committed significantly more Block 2 errors than controls within Task 4 [t(90.5)=-2.31, p=0.02] (Figure 4.7). In the analysis that divided COC animals by α2 level, both the main effect of group and the group by task interaction were significant [F(2,33.6)=3.36, p=0.05, F(4,64.2)=2.72, p=0.04, respectively]. Contrasts revealed that low α2 COC animals committed more Block 2 errors than controls on both Tasks 4 [t(90.7)=-2.37, p=0.02] and 7 [t(90.8)=-2.42, p=0.02]. The high α2 COC animals performance on Task 4 was suggestive of a similar trend [t(90.6)=-1.56, p=0.12], but performance on tasks 7 and 9 were not different from controls.

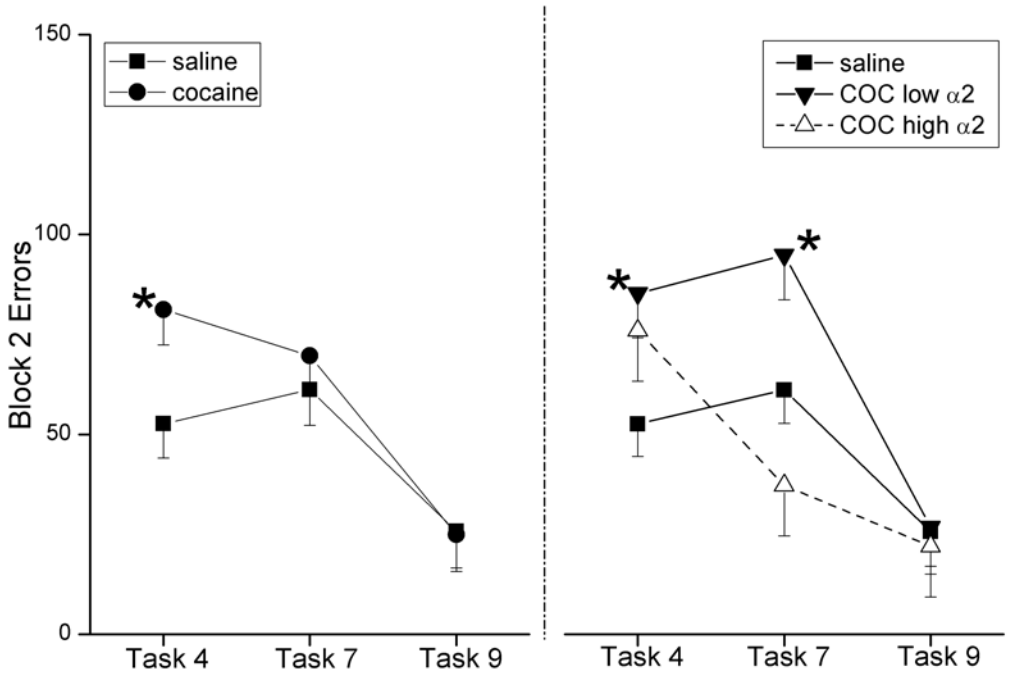

Figure 4.7 Block 2 errors on olfactory-predictive tasks. Cocaine-exposed animals commit more Block 2 errors than controls on Task 4 (p=0.02), an effect seen in both high and low α2 COC animals (p=0.02, p=0.12). This difference persists into Task 7 only for the low- α2 COC exposed animals (p=0.02).

SPATIAL-PREDICTIVE TASKS (Tasks 3, 5, 8)

Errors to Criterion

In the analysis comparing controls and cocaine-exposed animals (across α2 level) for errors to criterion, neither the main effect of treatment nor the treatment by task interaction was significant [$F(1,30.6)=2.43$, $p=0.13$, $F(2, 60.7)=0.25$, $p=0.8$, respectively]. Contrasts indicated no group differences in rate of improvement across spatial-predictive tasks [all $p>0.5$] and no differences within each spatial-predictive task. Figure 4.8 shows the relationship between groups when dividing COC animals by high and low α2 levels. There were no main effects of group or group by task interaction [all $p>0.3$]. Contrasts revealed no significant differences between groups in rate of learning across spatial-predictive tasks and no within task differences. Errors within each block are not presented for this task type because the duration of these phases was insufficient to uncover interpretable differences between treatments.

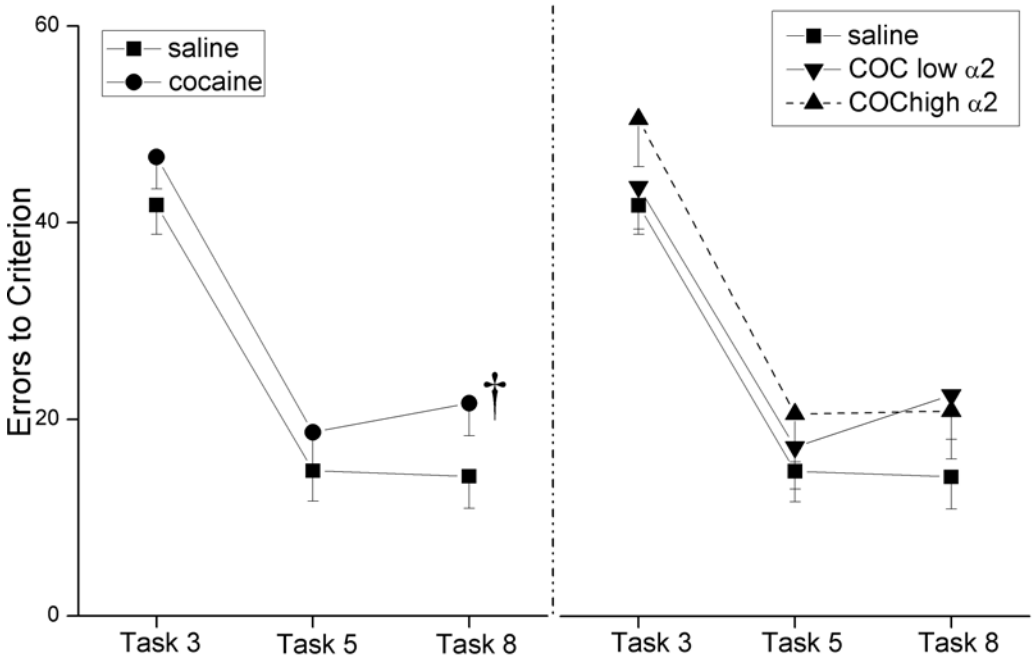

Figure 4.8 Errors to criterion committed on spatial-predictive tasks. Cocaine exposed animals commit more errors than controls on Task 8 (p=0.11).

DISCUSSION

The results of the present study corroborate prior reports of attentional dysfunction caused by prenatal cocaine exposure and further extend our knowledge of the specific behavioral deficits induced by a very low dose of cocaine (which models recreational use). While selective attention deficits caused by prenatal cocaine exposure have been previously reported, the findings presented herein suggests such disruption at a lower dose than previously investigated. Further, this study reveals new information indicating that prenatal cocaine exposure produces impairments in transfer of learning. Additionally, the present findings provide new evidence suggesting that α2 receptor density in the prefrontal cortex modifies the extent of behavioral impairment caused by *in utero* cocaine exposure. Cocaine-exposed animals exhibited two types of impairments, relative to controls. First, whereas controls were proficient in transferring learning across the three tasks of each type (e.g., visual-predictive, olfactory-predictive, spatial-predictive), thereby learning each successive task of a given type progressively more quickly, the cocaine-exposed animals were less proficient in transferring learning. Thus, the cocaine-exposed animals improved less across the three visual-predictive tasks and the three olfactory-predictive tasks than controls, due to a lesser degree of improvement in the early learning phase. They also exhibited an elongated later-learning phase on both task types, indicative of impaired selective attention.

Additional insight was provided by the analyses that included density of α2 receptors in PFC. In the present report, *in utero* cocaine exposure had no effect on the density of α2 receptors in the prefrontal cortex. However, the analyses correlating the density of α2 receptors in PFC and performance on this EDS task series revealed that the density of these receptors in PFC was a significant predictor of the severity of the effects of prenatal cocaine exposure (i.e. a modifying factor). Specifically, cocaine

exposed animals that also had low density of α2 receptors in the prefrontal cortex were significantly more impaired on this series of EDS tasks than cocaine-exposed rats with high α2 levels. The low α2 COC animals exhibited impaired transfer of learning on EDS tasks ("learning to learn") relative to saline-treated control rats, regardless of whether the newly predictive cues were very salient relative to the irrelevant cues or whether they were relatively subtle. They also exhibited impaired performance during the later learning stages, indicative of impaired selective attention. In contrast, for the high α2 COC group, deficits were observed only in the selective attention domain (learning to learn was unaffected) and only on the EDS tasks in which the newly predictive cues were subtle relative to the distractors (not when they were relatively salient); the deficits in these areas were less severe and more transient.

Low-dose cocaine exposure coupled with low alpha-2 receptor density in prefrontal cortex impaired both transfer of learning and selective attention in visual-predictive and olfactory-predictive EDS tasks.

Low dose cocaine exposure coupled with low α2 density in the PFC produced a unique pattern of performance relative to controls. These animals improved less than controls between the first and second visual-predictive EDS tasks, and also improved less between the first two shifts to tasks in which the olfactory cue was predictive. These differences in rate of improvement in the early learning phase of each of these tasks, which also produced slower mastery of Tasks 6, 7 and 10, is attributable to an impairment in learning transfer. As illustrated by control animal performance in this experiment, performance on EDS tasks tended to improve as animals became accustomed to frequent changes in cue/reward associations and habituated to the presence of irrelevant environmental stimuli. This improvement in performance with experience is referred to as "transfer of learning" or "learning to learn." This phenomenon was first suggested by Thorndike and Woodworth (1901),

who argued that previous knowledge is applied to solve a problem in a new situation, which thereby increases the rate at which a new or similar task is acquired (Thorndike & Woodworth, 1901). The failure of low α2 COC animals to improve their rate of early learning between tasks of the same dimension is indicative of a deficit in learning to learn. Note that this deficiency in learning transfer was seen both when the predictive cues were subtle relative to the irrelevant cues (e.g. visual-predictive EDS tasks) as well as when the predictive cues were salient (e.g. olfactory-predictive EDS tasks).

Further, the low α2 COC animals exhibited increased attention to salient stimuli, an impairment magnified in Block 2 of learning. In Block 2, animals had already acquired relevant task rules, such that errors during this phase could not be attributed to failures in associative learning, attentional set shifting, or learning to learn. Rather, the primary function tapped in this latter learning phase was selective attention, in which the animal had to filter out irrelevant stimuli and maintain focused attention to the predictive dimension. Consistent with this capturing of attention by salient stimuli, the cocaine-exposed animals with low α2 in the prefrontal cortex committed more errors later in learning on both visual-predictive and olfactory-predictive tasks. We suggest that the attention of these low α2 COC animals was captured by the most salient stimuli (where salience is dependent by both the sensory characteristics of the stimulus and its previous association with reward).

Cocaine exposure coupled with high alpha-2 receptor density produced impairments in selective attention dependent on predictive modality.

Cocaine-exposed animals with high α2 in the prefrontal cortex exhibited a remarkably different pattern of performance than their low α2 counterparts. Specifically, rate of early learning on both visual-predictive and olfactory-predictive tasks was not different between controls and high α2 COC animals, suggesting that

transfer of learning remained unaffected in this subgroup. However, these animals committed significantly more total errors than controls specifically on visual-predictive tasks, in which the predictive dimension was subtle and irrelevant stimuli were salient, an effect driven by differences in later learning (Block 2). As discussed above, errors in Block 2 suggest impaired selective attention rather than deficits in associative ability or attentional set-shifting. The specificity of this disruption (i.e. later learning only on the most difficult task type) provides clues to the importance of prefrontal α2 receptors in relaying information relative to the potency of environmental stimuli.

Proposed Neural Mechanism Underlying Behavioral Dysfunction

The present findings indicate that density of α2 receptors in the prefrontal cortex, within the normal range, modifies the degree of dysfunction produced by prenatal cocaine exposure. At present, one can only speculate why this is the case, but a tentative theory is presented below. Before presenting this conceptualization, one caveat should be noted. Alpha-2 receptors are found both presynaptically and postsynaptically in the prefrontal cortex (MacDonald & Scheinin, 1995). The autoradiography procedure used here did not distinguish between pre- and post-synaptic receptors, so the density measures presented represent a total sum of both receptor locations. Although we are unable to distinguish the proportion of pre- versus post-synaptic α2 receptors quantified in this study, the present conceptualization pertains primarily to presynaptic α2 receptors, because the pattern of results found here is consistent with the dual findings that (a) COC rats exhibit excessive NE release in response to arousing or stressful conditions, and (b) presynpatic α2 receptors are important for moderating NE release in response to highly stressing or arousing conditions (Elsworth et al., 2007).

The pattern of effects observed here suggests that α2 receptor density in PFC does not directly mediate these behavioral alterations produced by prenatal cocaine exposure but rather acts as a modifier of the dysfunction. Although we did not find any direct evidence that changes in NE activity underlie the attentional changes seen in the COC animals, recent evidence suggests that prenatal cocaine may produce increased NE release, particularly under times of arousal, which may explain the behavioral differences observed in cocaine-exposed animals with different levels of α2 receptors. It seems plausible that the degree of norepinephrine release in the cocaine-exposed animals is higher in the low α2 COC animals than in high α2 COC animals because low α2 cocaine-exposed animals cannot effectively regulate increased release, whereas the higher number of adrenergic autoreceptors in the high α2 COC group serve to "turn off" NE release. Alpha-2 receptor density does not differentially affect control performance, however, most likely because these animals do not have excessive NE release which renders α2 density to be of less consequence.

In normal animals, frequent and unpredictable changes in task contingencies produce phasic activation of LC neurons, which increases the release of NE to prefrontal areas and permits or facilitates behavioral adaptation to the new parameters, an effect which has particular consequences for early learning (Aston-Jones et al., 1999; Bouret & Sara, 2005). While these moderate levels of NE release have beneficial effects on performance, the increased release induced by cocaine-exposure coupled with the failure to regulate release due to low presynaptic α2 density may result in excessive (supra-optimal) NE levels, thereby disrupting early-learning performance. Thus, a reduction in density of α2-adrenergic receptors may disrupt autoinhibitory control of NE neurons in animals exposed to cocaine *in utero*, an effect that may be most consequential during conditions of stress or high arousal (e.g. by novel and salient stimuli) (Elsworth et al., 2007). This proposed mechanism also

sufficiently explains why cocaine-exposed animals with high α2 are not disrupted early in the learning process – they have sufficient levels of presynaptic α2 receptors to regulate the release of NE, maintaining optimal levels of this neurotransmitter and facilitating behavioral adaptation.

Conclusions

The present study revealed that the density of α2 receptors in the prefrontal cortex acts as a modifier of the effects of prenatal cocaine exposure on learning transfer and selective attention. One interpretation of this finding is that cocaine-exposed animals with low α2 receptor density may experience increased NE release into the PFC (relative to animals with cocaine exposure and high α2 receptor density), which may account for the pattern of findings in the present study. COC animals with low α2 were most impaired early in learning, a deficit evident on both visual-predictive and olfactory-predictive tasks. In contrast, high α2 COC animals exhibited less widespread behavioral disruption, with early learning performance similar to controls on all task types; this suggests that they are less affected by increased NE release, perhaps because the level of presynaptic α2 receptors is sufficient to allow them to effectively regulate NE release. As all cocaine-exposed animals showed impairments in the later phase of learning, irrespective of α2 density, we suggest that failures in selective attention in this later phase of learning may be sensitive to even subtle elevations in NE, such that both groups demonstrated some degree of impairment. Further research is needed to elucidate changes in the noradrenergic processes (e.g. release to prefrontal cortex), which may more directly underlie the observed cocaine-related impairments in transfer of learning and selective attention on EDS tasks. Still, this study provides persuasive evidence that the α2 receptor may be a valid target for future pharmaceutical interventions that aim to alleviate the affects of prenatal cocaine exposure on transfer or learning.

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CHAPTER 5

CONCLUSIONS AND FUTURE DIRECTIONS

Synthesis of Findings from this report

The results presented in this report reveal that *in utero* exposure to cocaine produces a specific constellation of behavioral impairments, dependent on timing, dose and duration of drug exposure. Specifically, the cognitive processes disrupted by cocaine include the domains of selective attention, attentional set formation and transfer of learning. For both studies, cue salience, as determined by both the sensory characteristics of the stimuli and prior experiences (i.e. previous association with reward), was a critical predictor of performance and differentially affected the acquisition of task rule and maintenance of appropriate attention for COC animals. Briefly, the deficits observed here were:

1. Animals exposed early in gestation (i.e. both early COC and "full" COC exposure groups) exhibited increased distractibility in later learning on tasks in which irrelevant stimuli were very salient relative to predictive cues.
2. COC animals, regardless of timing and duration of exposure, exhibited increased attention to salient stimuli, which disrupted attentional set shifting when previously predictive stimuli were salient and disrupted set formation when prior cues were subtle.
3. Animals exposed to a lower dose of cocaine (i.e. 3.0 mg/kg once daily GD8-21) demonstrated increased distractibility in later learning irrespective of predictive modality. When considering α2 density as a moderating variable, 1X COC animals with low α2 density in prefrontal cortex were impaired later in learning for both olfactory-predictive and visual-predictive tasks. In contrast, 1X COC animals with high α2 density in PFC showed increased

distractibility only on visual-predictive tasks in which irrelevant stimuli were potent.

4. Animals exposed to a lower dose of cocaine showed a pattern of early-learning performance reflective of impaired learning transfer, irrespective of predictive modality. Correlating this effect with α2 receptor density revealed that only low α2 COC animals demonstrated this impaired learning transfer, which occurred on both olfactory-predictive and visual-predictive tasks. High α2 COC animals' learning rate was not different from controls.
5. When olfactory stimuli were relatively novel (i.e. a different odor triad than that presented on prior serial reversal tasks), higher dose COC animals (i.e. 3.0 mg/kg once daily GD8-15, twice daily GD16-21) demonstrated altered transfer of learning only on the visual-predictive tasks.

The exposure regimen used in these studies represents an accurate model of human recreational use, as the pharmacological profile produced by this IV procedure has been shown to be similar to that observed in humans. Further, the animals used here were not affected by the confounding factors often observed in humans (e.g. maternal malnutrition, lack of pre- and post-natal care, polydrug use) and in other animal models of COC exposure (e.g. necrotic skin lesions, malnutrition, maternal stress), lending additional credence to our findings. The investigations presented herein provide a significant contribution to the available literature on neurotoxicology and neuropharmacology as they confirm previous findings of COC exposure producing deficits in attentional processes as well as provide a more specific cognitive profile by elucidating the task conditions that produce the most robust effect. The present report also provides new information regarding transfer of learning. Further, this serves as the first study directly correlating behavioral and neural outcomes in an animal model of prenatal cocaine exposure.

Clinical Relevance of Investigating Timing and Duration of Exposure

Much of the information from humans regarding timing and duration of exposure comes from maternal self-report, which indicates that the level of drug use tends to decrease across trimesters (Snow et al., 2004). Richardson and colleagues have reported that the percentage of women who report cocaine use in the first trimester is 22-24%, a statistic that is reduced significantly in the 2nd and 3rd trimesters (3-4% and 4-5%, respectively) (Richardson, Conroy, & Day, 1996). Of those who reported using cocaine while pregnant, average monthly usage also decreased across trimesters, from 3.3 grams in the first trimester to 0.1 and 0.2 grams in the 2nd and 3rd trimesters, respectively. Thus, most cocaine-abusing pregnant women decrease drug use over time, with highest levels occurring early in gestation. From a developmental standpoint, this timing is particularly relevant, since the neurochemical pathways that are affected by cocaine (i.e., NE and DA systems) are at peak development around the fifth week of gestation (Ferris et al., 2007; Snow et al., 2004). Therefore, modeling prenatal cocaine exposure at different periods in gestation can provide great insight into the developmental processes that may be disrupted and mediate observed behavioral alterations.

In the study presented in Chapter 2, animals exposed to cocaine limited to early (GD8-15) or late gestation (GD16-21) or extending throughout pregnancy (GD8-21) were all similarly affected in the formation and shifting of attentional sets dependent on the salience of environmental cues. This does not represent a deficit in attentional set shifting per se, as the cognitive change resulted in inferior performance relative to controls under certain circumstances but superior performance under other conditions. Rather, the dysfunction in these animals, regardless of timing and duration of exposure, seems to be an increased attention to salient stimuli, which disrupted attentional set shifting when previously predictive stimuli were salient and disrupted

set formation when prior predictive cues were subtle. This is consistent with previous research from this lab, and others, which suggests that cocaine-exposed animals' attention is "captured" by the most salient cues (Garavan et al., 2000; Gendle et al., 2004). A similar pattern of behavior has been observed in primates with prefrontal dopaminergic depletion, who demonstrated no improvement across a series of intradimensional shift tasks but were superior to controls on EDS tasks –both effects apparently due to a failure to form an attentional set to less arousing stimuli (Crofts et al., 2001; Robbins & Roberts, 2007; Roberts et al., 1994; Rogers, Andrews, Grasby, Brooks, & Robbins, 2000).

The study discussed in Chapter 2 also uncovered specific deficits associated with timing of cocaine exposure. When cocaine exposure occurred early in gestation (regardless of total duration of exposure, i.e. both early and "full" COC groups), these animals demonstrated a pattern of performance reflective of impaired selective attention, an effect not observed when COC was limited to the later gestational period. This is in contrast to the previously reported "sensitive period" to cocaine exposure, in which neuropharmacological changes specific to a later period of development were observed (i.e. alterations that were observed when cocaine was limited to late gestation and when it was administered for the duration of gestation). Stanwood and colleagues noted morphological and neurochemical effects in the anterior cingulate cortex in the rabbit specifically upon exposure during the equivalent of the second trimester in humans (Stanwood, Washington, & Levitt, 2001; Stanwood & Levitt, 2004). These researchers described dose-dependent and permanent effects in regions of high dopaminergic innervation, including the anterior cingulate cortex. Specifically, cocaine-exposed animals showed a functional decoupling of the D1 receptor and its associated G-protein and elongated dendritic projections in the ACC; organization of monoaminergic systems remained unaffected in rabbits with cocaine

exposure limited to early gestation (Stanwood et al., 2001). However, while Stanwood and colleagues uncovered a constellation of neural alterations dependent on late cocaine-exposure, they failed to examine any behavioral changes that may correspond with neural alterations. Thus, it is plausible that the neural changes reported by Stanwood and colleagues are not necessarily in the same systems as those that underlie the behavioral disruption reported in Chapter 2. Clearly, more research is needed to elucidate the effects of timing and duration of cocaine exposure in inducing changes in other brain areas that may underlie the behavioral effects observed here.

Clinical implications for impaired transfer of learning in COC animals

Chapter 3 provided new information regarding the ability of cocaine-exposed animals to "learn to learn" across the task series, an impairment not previously reported in COC animals. The EDS series taps, in effect, "cumulative learning," in which prior experiences and learned rules can facilitate the acquisition of new rules in subsequent, similar tasks (Strupp, Bunsey, Levitsky, & Hamberger, 1994). In this task series, transfer of learning over time – the accumulation and application of information, concepts and strategies that comes from prior experiences – is critical in "early learning," a phase of learning that taps not only associative proficiency but also the shifting of attention between relevant domains and ability to selectively attend to relevant stimuli. While disruption in learning transfer is classically correlated with IQ deficits in humans (Strupp et al., 1994), the present findings do not implicate "learning" per se as the disrupted function in COC animals. Rather, the observed deficits in "learning to learn" seem to be mediated by the attentional demands of the task. That is, while lower-dose COC animals were impaired on both olfactory- and visual-predictive task types, the effect was transient for both task types. In contrast, the 2X animals were showed a loss of learning transfer only on the task that placed the

greatest demand on attention (i.e. visual-predictive tasks with olfactory distractors); the effect on 2X animals persisted through the end of the task series.

These findings have particular clinical relevance for cocaine-exposed children of school age. The ability to take what is learned in one context and apply it to another context is critical to successful classroom performance. In the classroom setting, the information being taught may often lack apparent meaning or logic (Bransford, Brown, & Cocking, 1999), but by exploring underlying concepts and generating connections to previously learned knowledge, the new information becomes meaningful. For example, the skill of writing letters must be applied when learning to write words (vertical transfer), while the skill of writing words must be applied when writing sentences (near transfer). Thus, transfer of learning is considered an active and dynamic process, in which individuals must actively choose and evaluate strategies, consider available resources and respond to feedback (Bransford et al., 1999; Ormrod, 2004). Cocaine-exposed children that are delayed or impaired in "learning to learn" may exhibit poor classroom performance, not because of inability to learn new information but rather due to the inability to apply previously learned knowledge and experiences to the context of new information. Increasing our understanding of the specific cognitive domain affected in children exposed to cocaine prenatally may provide an opportunity for educators to modify teaching techniques to compensate for impaired transfer of learning.

Correlations between low COC exposure (1X) and alpha-2 receptor density in PFC: alpha-2 as a modifier of cocaine's effects

The findings presented in this report are the first to directly correlate neural and behavioral outcomes in the same cohort of COC animals. We had observed (in Chapter 3) that prenatal cocaine significantly impaired transfer of learning and selective attention in the low-dose animals, irrespective of α2 level. To expand upon

these findings, in Chapter 4 we considered three additional aspects of the relationship between neural outcomes, behavior and cocaine status. First, we determined that cocaine did not directly influence the density of α2 receptors (i.e. COC does not inhibit or facilitate development of this receptor type in PFC), since both COC and control animals' α2 density was within the same range. Secondly, analysis of the relationship between α2 density and behavior, irrespective of cocaine exposure, revealed that the level of α2 was not a significant predictor of performance on EDS tasks. However, evaluation of cocaine's effects on behavior coupled with information about the α2 status of the individual animals revealed that α2 level did not significantly influence the performance of control animals (i.e. both high and low α2 controls performed similarly on all tasks), but was an important variable in determining the pattern of effects in COC animals (i.e. low α2 COC was different from high α2 COC). Taken together, these analyses indicate that α2 receptors in PFC do not directly mediate the disruption in selective attention and transfer of learning observed in cocaine-exposed animals but do moderate behavioral deficits in COC animals.

In Chapter 4, we suggested that the primary neural disruption underlying observed behavioral deficits in COC animals was an increased release of norepinephrine from the locus coeruleus. Activity of the LC has been correlated with performance on selective attention tasks, such that moderate LC firing improved attention to predictive stimuli in the face of distracting cues, improvement thought to be due to reducing signal-to-noise ratio (Aston-Jones, Rajkowski, & Cohen, 1999; Aston-Jones & Cohen, 2005). The proposed mechanism underlying the observed disruption in attentional processes (i.e. increased NE release from LC) would differentially impact cocaine-exposed individuals with differing α2 levels. These long-lasting alterations in brain pharmacology or cell signaling caused by prenatal

cocaine exposure, then, would result in hypo- or hyper-responsiveness to pharmacological challenges depending on α2 receptor density, complicating the development of a suitable pharmaceutical intervention (Stanwood & Levitt, 2004).

Less clear from these findings is the role of norepinephrine in modulation of selective attention. In Chapter 4, both high and low α2 COC groups were disrupted in later learning on EDS tasks, although the effect was more widespread and severe (i.e. occurring in both visual- and olfactory-predictive tasks) for the low α2 COC animals. Previous research in this lab has suggested that one explanation for disruptions in performance on tasks in which greatest attentional demand is increased "emotionality" or reactivity to errors. For example, cocaine-exposed rodents were specifically disrupted on trials following an error on tasks in which distracting stimuli are presented with predictive cues (Gendle et al., 2003; Gendle et al., 2004; Morgan et al., 2002) and in tasks with unpredictable timing and onset of stimuli (Gendle et al., 2004; Morgan et al., 2002). It may be that the cocaine-related effects on arousal regulation may be attributed to changes in either (or both) noradrenergic and dopaminergic systems, for which density of α2 may not be a relevant indicator of functional disruption. Alternatively, rather than being related to increased arousal, it is possible that the observed failures in selective attention may be sensitive to even subtle elevations in NE, such that both low α2 and high α2 COC subgroups demonstrated some degree of impairment

Limitations and considerations for future research

The investigations presented herein provide a significant contribution to the literature regarding the direct relationship between behavioral disruption and underlying neural changes in cocaine-exposed subjects. However, each of the investigations presented in this report represented the first of its kind (i.e. regarding timing/duration of exposure, doses investigated, and neural changes, as well as the use

of a 3-choice EDS task), and thus additional studies will need to be conducted to verify the conclusions of this report.

In addition to independent verification of the present findings, one valuable point for future investigations will be to evaluate the behavioral and neurobiological differences between cocaine-exposed males and females. Behavioral evidence in rodents suggests that the effects of prenatal cocaine exposure are sex-dependent, with COC males showing specific disruptions later in a testing session and specifically on trials following an error, while COC females demonstrated an decrease in task participation particularly later in a testing session (Gendle et al., 2003). A previous drug challenge study from this research group also demonstrated a sex-dependent pattern of behavior upon administration of idazoxan, which decreased accuracy in COC males but improved performance in COC females (Bayer, Kakumanu, Mactutus, Booze, & Strupp, 2002). It is possible that these sex-related behavioral differences in response to the α2-antagonist are associated with baseline differences in noradrenergic activity (where females may have had sub-optimal NE activity, such that IDZ raised noradrenergic activity to optimal levels). Neurochemically, D1 and α2 receptor densities are differentially affected by COC exposure in male and female rodents as observed in both adolescence and adulthood, with as high as a 34% difference in density in some regions (Booze et al., 2006; Ferris et al., 2007). Thus, studies such as that presented in Chapters 3 and 4, which did not have sufficient sample size to uncover sex-dependent differences in EDS performance or neural alterations, may mask functionally important differences in cocaine-exposed males and females. This is an important factor to consider in the design of future studies.

Another important consideration in application of the present research was the particularly confounding effect of prior experiences on subsequent behavior. To better gauge deficits specifically in extradimensional set shifting, it may be beneficial to

present this task series early in the overall set of behavioral tests. This would eliminate confounders of "previous association with reward" and "habituation to irrelevant stimuli," at least for the earlier tasks in the EDS series. By presenting the EDS tasks early in the experiences of these animals, and ensuring that they had not been previously exposed to the odor set employed, researchers would be provided with a more readily interpretable outcome of disruption due to cue salience per se versus those impairments due to true failures in attentional set shifting.

While the exposure protocol used in the investigations presented here is an excellent model for human recreational use of cocaine during pregnancy, several modifications to this procedure may be considered in future investigations. First, the restriction of cocaine exposure to only developmental periods after gestational day 7 serves as a significant limitation to this IV method. Disruption of developmental events occurring prior to GD7, including neuronal proliferation and maturation, are relevant factors when extrapolating from animal models to human recreational use of cocaine. Further, the failure of the current model to represent "crack" cocaine use, which also produces pharmacologically active pyrolysis products, restricts the application of these findings to only the subset of cocaine-users who self-administer intravenously or intranasally. Additionally, because pregnant women rarely use cocaine in isolation (Mayes, 1999), it may be most clinically relevant to model poly-drug use in order to determine the extent of behavioral and neural disruption likely experienced by cocaine-exposed children.

Conclusions

The long-lasting effects of prenatal cocaine exposure on transfer of learning and selective attention are both subtle and highly specific. The extent of observed disruption is dependent not only on dose of exposure, for which there has been considerable prior research, but also on the timing and duration of drug exposure.

While deficits in selective and sustained attention have been shown to be associated with *in utero* cocaine exposure, there has been little investigation on the disruptions of this cognitive process beyond simple measures of overall learning rate. The present study has revealed the critical importance of learning phase and has elucidated the specific cognitive processes underlying acquisition of extradimensional shift tasks. This research has provided insight into the importance of cue salience, prior experience, and task design in uncovering the subtle dysfunction caused by prenatal cocaine exposure. Additionally, this research has provided new and important information regarding the importance of noradrenergic function in behavioral outcomes. While α2 receptors do not directly mediate cocaine's effects, as originally suggested, they play a significant role in the regulation of norepinephrine release and subsequent behavioral adaptation. The findings reported here provide a significant contribution to the literature on neuropharmacology and neurotoxicology of *in utero* cocaine exposure and will be of critical import in designing pharmaceutical interventions to alleviate persistent and subtle disruptions in attentional processes documented in these drug-exposed individuals.

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APPENDIX A

Specifics of Phase Demarcation:

To determine the points at which each animal shifted to a new phase of learning, the reviewers had to evaluate a separate graph for each day on each task for each animal. There were 72 total animals evaluated in the present report, each of which was on a given task for 200 response trials a day for approximately 60 days (total time on EDS series varied by animal). Thus, the reviewers had to evaluate literally thousands of graphs in order to accurately demarcate phase endpoints. In order to ensure consistency between independent reviewers, a specific list of rules was generated that coders followed when making decisions.

Rules followed by reviewers when demarcating phase endpoints

1. For the EDS series, phases were only examined for visual-predictive (Tasks 2, 6, 10) and olfactory-predictive (Tasks 1, 4, 7, 9) tasks. The spatial-predictive tasks were acquired quickly for all animals, such that demarcation of individual phases of learning was not interpretable. Additionally, perseveration could only be evaluated for tasks that were not preceded by spatial, due to the issue of side bias as discussed in Chapter 3. There was no perseverative score quantified for Task 1, as this did not represent a true extra-dimensional shift.
2. For determining all phases: two independent coders, blind to treatment conditions, look at graphs for each animal for the duration of each EDS. Later learning phases (i.e. those after perseveration) may require a larger bin size than perseverative phases.
3. In determining any phase, if there was not agreement between the two coders as to when a phase begins or ends, a third coder (also blind to treatment) should independently evaluate the full EDS for that animal and make decisions of <u>all</u> phases (not just at the point of discrepancy). This third persepective was then compared to the original two coders to resolve the discrepancy.

4. A "discrepancy" between coders' values was when the difference in count value was greater than 10. Any difference greater than ten should be reevaluated by both coders (and the third independent coder) to reach an agreement.
5. Demarcation points for phases were as follows:

    a. Perseveration: when responses to the *previously* correct dimension was either below the upper bound of chance or when both coders agree on a qualitative end to this phase.

    NOTE: When an animal was in perseveration phase, responses to the currently predictive dimension are at chance level. Therefore, there was an overlap in phases, where perseverative trials are included in chance phase.

    b. Chance: the number of trials between when perseveration ends and early post-chance beings. That is, it is the number of trials between the 90% Confidence Interval of chance-value (with bin size=60). For the data presented in this report, chance was between 23.3% and 44.6% (the 90% CI around 33%) of presponses to the currently predictive dimension.
    c. Early Post-Chance: when responses to the correct dimension was consistently above the upperbound of the 90% CI for chance and below a halfway point between this upper value and the criterial value. For analysis of data in the present report, early post-chance was between 44.6% and 66% of responses to the currently predictive dimension.
    d. Late Post-Chance: when response to the currently predictive dimension was consistently above the halfway point and the criterial value. For analysis of data here, late post-chance was between 66% and 80% (for visual tasks) or 88% (for olfactory tasks).
    e. Criterial: the first bin in which an animal was performing consistenly above criterion until the end of the first full day with average performance at this criterial value.

    Note on criterial phase: This definition of criterial phase is different from that previously used in this lab. Garavan, et al. (2001) defined criterial phase as the number of trials from the first <u>bin</u> greater than or equal to criterion until the end of testing. This "first bin > criterion to end" outcome was also evaluated in the present study, but criterial phase as defined above (i.e. "consistently > criterion to end") was considered to be a more sensitive and interpretable indicator of an individual's ability to perform at a consistently high level.

6. If there were five bins in a row at the same proportion-of-response value, coders chose the first bin in the line as the place at which the phase began or ended.

7. The conversion from "bin count" to "trials in phase" was dependent on bin size. As a rule, trials-in-phase= count – (1/2 bin size).
8. Perseverative phases were evaluated on two levels, each decided independently by both of the coders. First, coders determined a qualitative end to perseveration. That is, they decided where, with their best judgment, the animal had "given up" the previously predictive dimension either in favor of another dimension or with no apparent dimensional preference. Second, coders determined where the percentage to the previously predictive dimension crosses the upper bound of chance. Note that these two points were not necessarily in the same place (i.e. an animal can "give up" the previously predictive dimension but still make responses to that dimension in a range above chance). If an animal seemed to be going to the previously predictive dimension and another dimension alternately, with both remaining above chance, perseveration was no longer occurring. It was assumed that all animals would perseverate to some extent as a way to determine the previously predictive dimension was no longer correct. Thus, it was not possible to have no perseveration (persev score = 0); rather if an animal did not appear to be perseverating beyond the first bin, they were assigned a perseveration score of 10.
9. For perseveration, coders also evaluated the raw data for the first 50 response trials (or more if necessary) to see a more specific picture of response patterns.
10. Decreases in performance at the beginning of a day were disregarded when determining if an animal was "consistently" above a given value. Settling-in effect may make have been responsible for observed disruption in performance early in a task, such that that performance level would not reflect actual knowledge of task parameters.
11. The first 60 bins of a given day also factor in some trials from the previous day. Because of the way bins were determined (i.e. a moving average of 60 trials), bins moved across days. That is, "count 201" actually reflected an average of trials 141-200 on day 1 and trial 1 on day 2. Thus, count 201

Perseveration

The duration of any perseverative phase was expected to be short. Using a bin size that was too large would effectively eliminate the existence of any perseverative trials, however using window that was too small could overestimate trials due to perseveration. Based on the findings of previous studies in this lab (Garavan 2000), we determined that a bin size of 20 trials would be most likely to produce appropriate quantification of the duration of perseverative phase. Below is a representative example of the evaluated perseverative graphs. Figure A.1 is from B3, an animal that demonstrated perseveration to the previously predictive stimulus on Task 7. Task 7 was an olfactory predictive task preceded by a visual-predictive task.

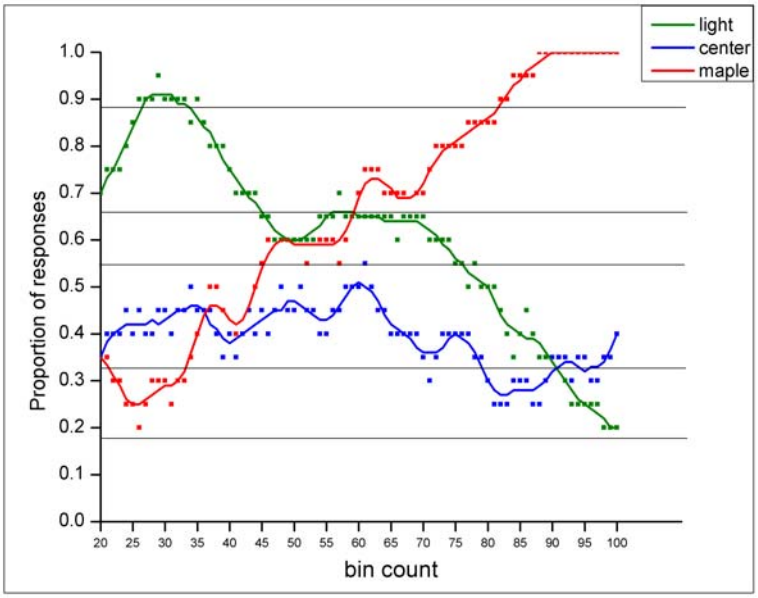

Figure A.1 Representative graph of perseveration. This is the first 100 bins of performance for B3 on Task 7, an olfactory-predictive task preceded by a visual-predictive task.

The green data points represent average responses (with bin size=20) to the visual stimulus upon change in task contingencies, while the red line indicates the proportion of responses to the maple odor. This example indicates a clear pattern wherein B3 persistently responds to the light cue for the approximately the first 50 bins, after which he learns that the maple odor is predictive of reward and begins persistent responding the correct stimulus. In this example, the end of perseveration was not considered to be where responses to light falls below 55.8% (the upperbound of chance), but rather where responses to maple increase as responses to light decrease, even though the proportion of responses to both dimensions is above chance level.

Side Bias

Figure A.2 reveals that animals did adopt a side bias to the center port, even when they had never had "center" as the previously predictive dimension. The figure below represents early learning performance for B36 on Task 2, a visual predictive task preceded by olfactory-predictive task; it is of note that the first task in which the center port was predictive of reward occurs *after* the task presented in the figure (Task 3).

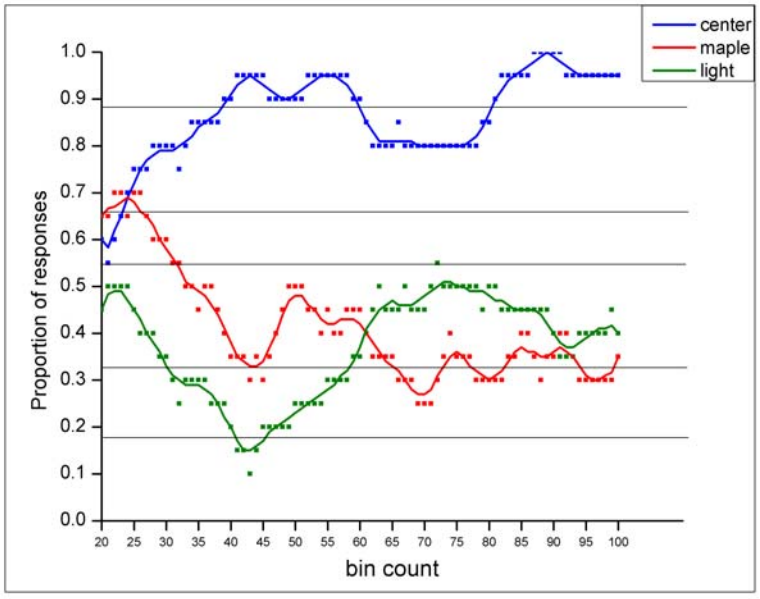

Figure A.2 Graph of performance on Task 2 representing a side bias to the center port. At this point, animals had never previously experienced center as the predictive dimension.

Summary of Phase Analysis Results for Visual Tasks

Duration of chance phase (Figure A.3)

There was no significant main effect of treatment [F(2,50.8)=0.75, p=0.5] and no treatment by task interaction [F(4, 106)=0.62, p=0.6]. There were no differences in slopes between treatments (all p>0.3). Within task differences indicate that 1X COC animals committed more errors than controls in chance phase only within Task 6 [t(168)=-1.71, p=0.09].

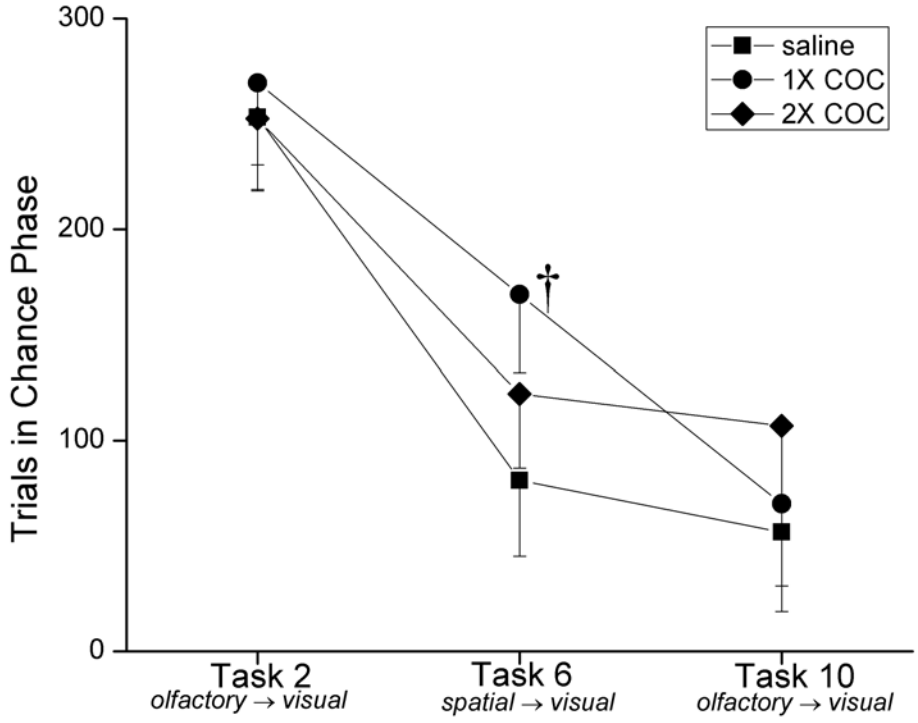

Figure A.3 Duration of chance phase across visual predictive tasks. There were no differences in slopes between treatments 1X COC animals committed more errors than controls in chance phase only on Task 6 (p=0.09).

Duration of early post-chance (Figure A.4)

There was a significant main effect of treatment [F(2, 59.3)=3.27, p=0.4] and a borderline significant interaction between treatment and task [F(4, 114)=2.10, p=0.08]. Contrasts indicated that controls and 2X COC progressed from Task 6 to 10 at a different rate [F(1,113)=2.94, p=0.09]. That is, 2X COC animals had a longer early post-chance phase on Task 10 than Task 6, while controls shortened this phase between the latter two visual tasks. Pairwise comparisons indicated that 1X COC animals committed significantly more errors than controls within Task 6 and Task 10 [t(164)=-2.55, p=0.01, t(168)=-1.83, p=0.07, respectively). 2X COC animals committed more errors than controls only within Task 10 [t(168)=-2.3, p=0.02].

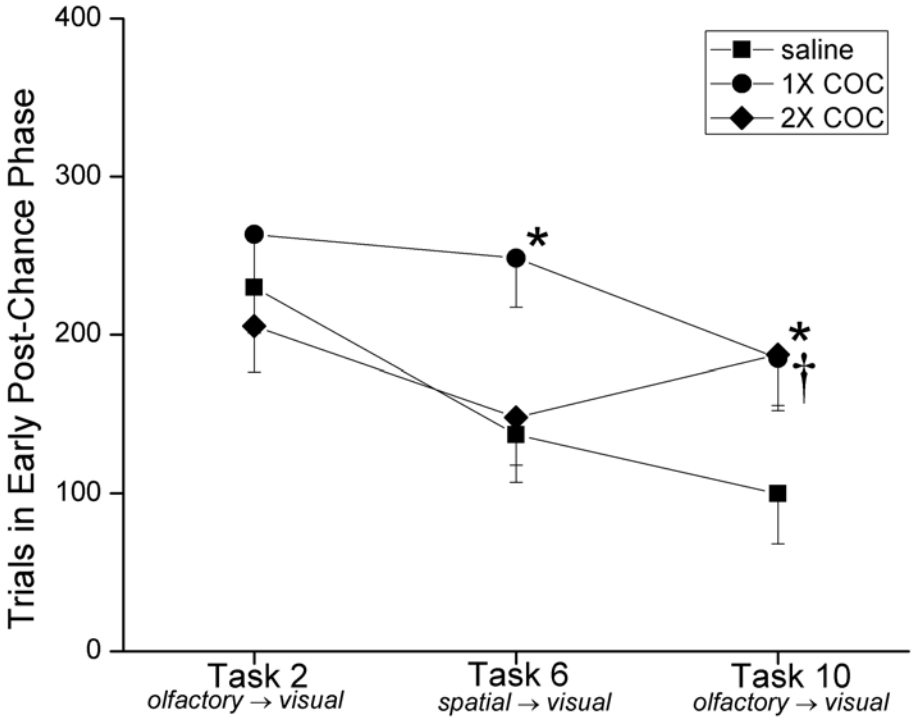

Figure A.4 Duration of early post-chance across visual tasks. COC animals' rate of learning across visual tasks was significantly different from controls. 1X COC animals had a significantly longer early post-chance phase on both Tasks 6 and 10 ($p<0.07$). 2X COC had a slope that was different from controls Task 6 to Task 10 ($p=0.09$), a trend that resulted in a treatment difference within Task 10 ($p=0.02$).

Duration of late post-chance (Figure A.5)

Both the main effect of treatment and the treatment X task effect were non-significant [all f<1, all p>0.5]. There were no significant differences in slopes between groups, although the contrast between controls and 2X COC from Task 6 to Task 10 was suggestive of a trend [F(1,107)=2.53, p=0.11]. Pairwise comparisons suggested a similar trend between controls and 2X COC animals specifically within Task 6 [t(141)=-1.58, p=0.12].

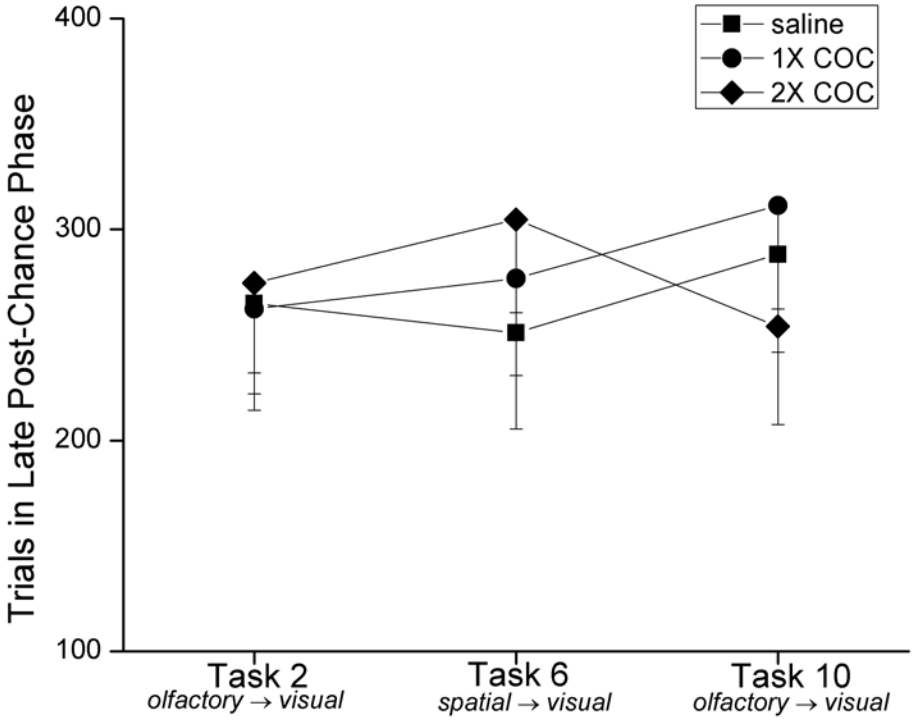

Figure A.5 Trials in late post-chance across visual tasks. There were no significant treatment differences within or across these tasks.

Duration of criterial phase (Figure A.6)

There was a main effect of treatment [F(2,172)=4.22, p=0.02], but no significant treatment by task interaction [F(4, 172)=1.21, p=0.3]. Contrasts revealed that controls and 2X COC performed at significantly different rates between Task 2 and Task 6 [F(1, 172)=3.48, p=0.06]. The difference in performance rate was suggestive of a trend between Tasks 6 and 10 for controls and 1X COC animals [F(1,172)=2.48, p=0.12]. Within Task 2, 2X COC had a significantly shorter criterial phase than controls [t(172)=2.24, p=0.03]. Within Task 6, 1X COC had a longer criterial phase than controls [t(172)=-2.15, p=0.03].

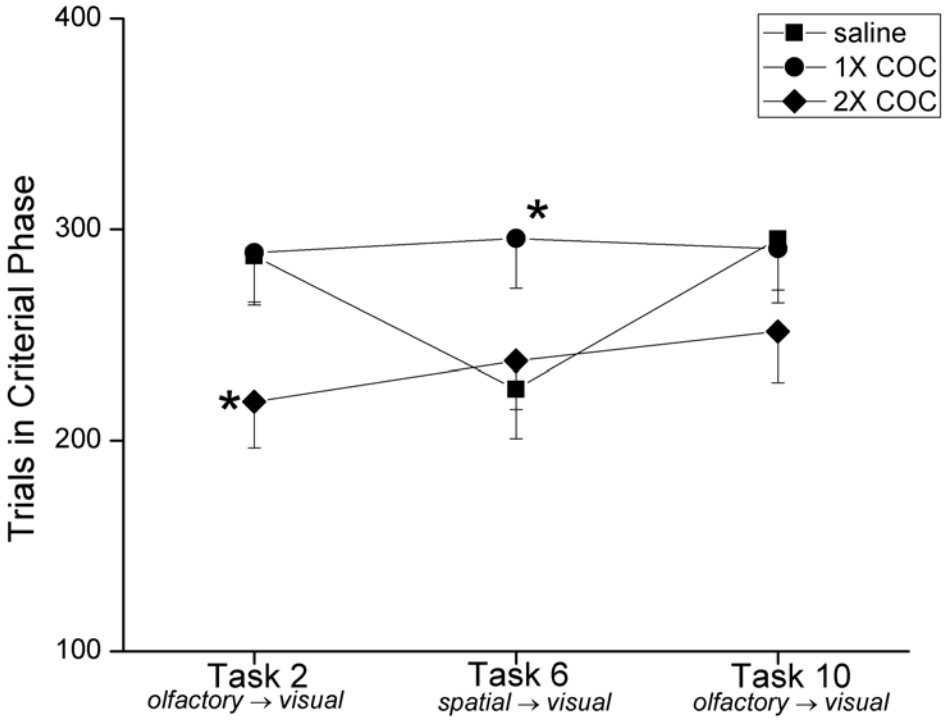

Figure A.6 Trials in criterial phase across visual tasks. 1X COC committed more errors than controls within Task 6. Both COC groups failed to improve in criterial phase duration across visual tasks, although 2X COC were asymptotic at shorter phase duration. 2X COC had a shorter criterial phase relative to controls on the first visual-predictive task (p=0.03), but were not different from controls on Tasks 6 and 10. 1X COC were only different from controls within Task 6 (p=0.03).

Synthesis of Phase Analysis for Visual-Predictive Tasks

The pattern of performance in chance and early-post chance phase were similar. In both phases, control animals improved between the first two visual tasks and then hit a "floor" in improvement from Task 6 to Task 10. In contrast, 1X COC did not improve in these phases at as fast a rate as controls from Task 2 to Task 6; this lack of effect was magnified within early post-chance. From Task 6 to Task 10, 1X COC improved, but since they were performing worse than controls within Task 6, they had more "room for improvement" before hitting a "floor" in learning ability. The duration of these phases for 2X COC was more similar to controls, but in both chance phase and early post-chance phase, they failed to reach the speed of controls by the final visual task. These early learning phases, when taken together, are indicative of impaired learning transfer.

This pattern is in marked contrast to those observed in late post-chance and criterial phase. In late post-chance, there were no differences in phase duration or in rate of change of duration between any of the groups. In the criterial phase, controls showed the expected pattern across visual tasks. That is, controls were significantly better on Task 6 (the spatial → visual shift) than on Tasks 2 or 10 (both olfactory → visual shifts). We suspect that this was because the spatial dimension was easier to "let go," such that it did not serve as inherently distracting for controls in the criterial phase of learning. However, both COC groups did not improve in this phase; both slopes remain essentially flat. The difference in learning rate for 1X COC translates into a difference in criterial phase duration during this second visual task, which may reflect a deficit in selective attention. The superior performance of 2X COC animals in the criterial phase of the first visual task is less straightforward and perhaps may reflect some "over-attention" to these subtle cues as a way of minimizing arousal.

Summary of Phase Analysis Results for Olfactory Tasks

Duration of chance phase (Figure A.7)

Neither the treatment main effect nor the treatment by task interaction were significant [F(2, 77)=0.40, p=-0.7; F(4,57.1)=0.98, p=0.4, respectively]. Contrasts revealed that controls and 1X COC performed at significantly different rates between Task 4 and Task 7 [F(1,59.2)=3.04, p=0.08]. Within task comparisons revealed that controls and 1X COC animals had significantly different duration of chance phase within Task 7 [t(61.5)=-2.24, p=0.03].

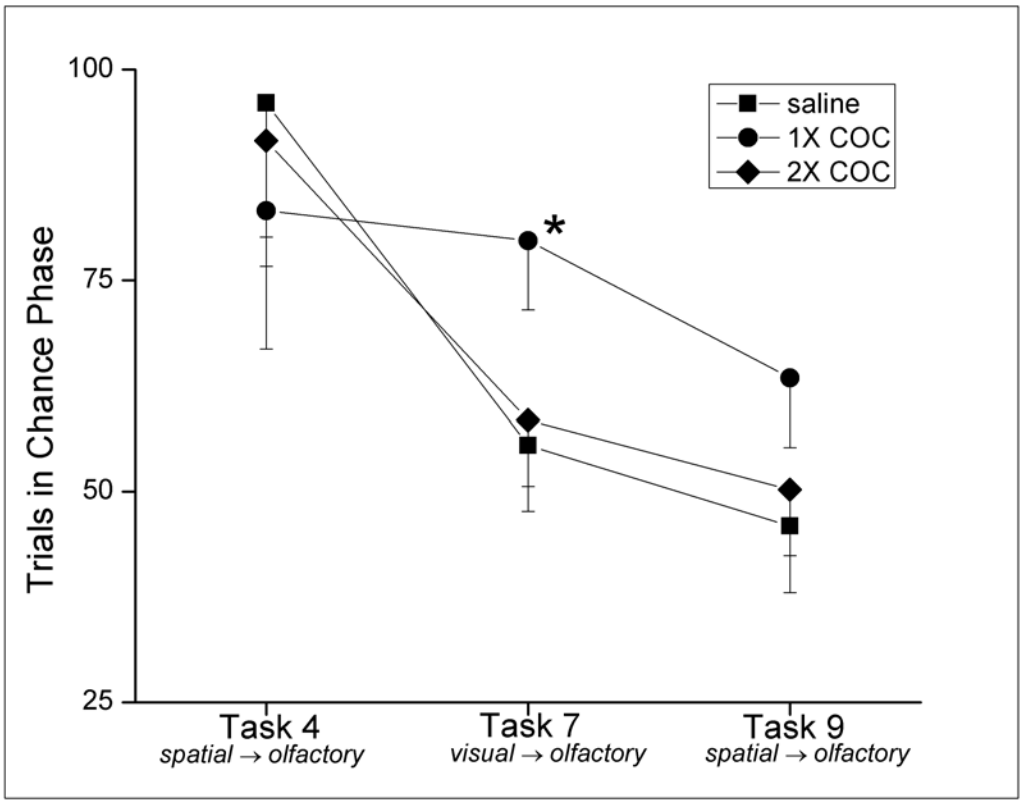

Figure A.7 Trials in chance phase across olfactory-predictive tasks. Controls and 2X COC animals performed at the same rate across these tasks. 1X COC failed to improve at the same rate as controls between Task 4 and Task 7 (p=0.08), yielding a within task difference on Task 7 (p=0.03).

Duration of early post-chance phase (Figure A.8)

For duration of early post-chance, neither the treatment nor treatment X task interaction fixed effects were significant [all $F<0.7$, all $p>0.6$]. There were no significant differences in slopes [all $p>0.2$] and all within task comparisons were not significant [all $p>0.4$].

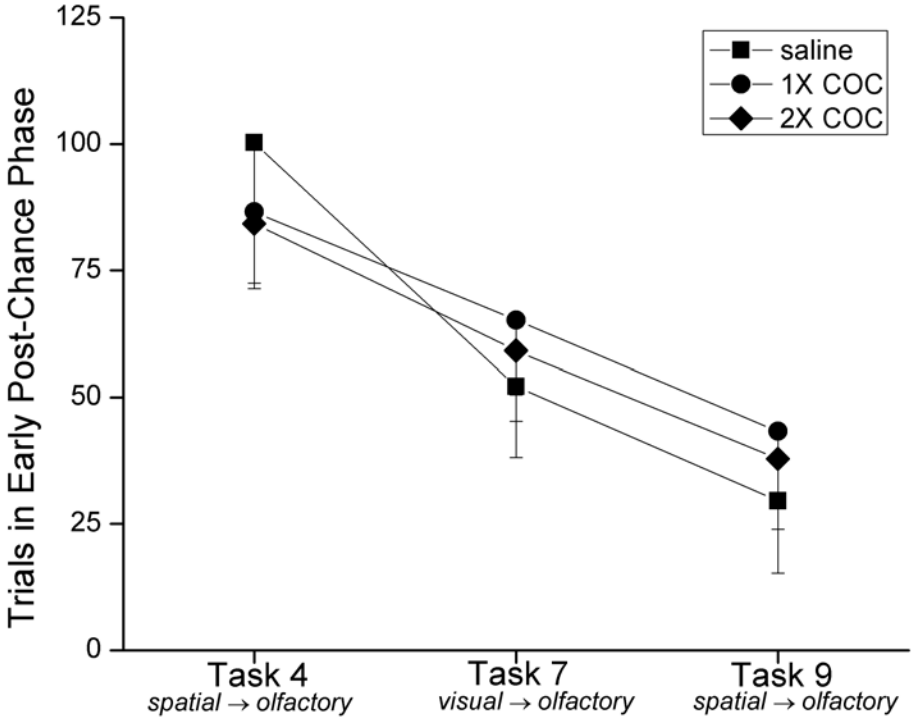

Figure A.8 Trials in early post-chance phase across olfactory-predictive tasks. There were no differences in duration of this phase between treatments. All animals progressed through early post-chance at the same rate.

Duration of late post-chance phase (Figure A.9) and criterial phase (Figure A.10)

There were no significant main effects of treatment or interaction between treatment and task [all p<.2], and no significant differences in slopes [all p>0.7]. Within Task 4, the difference in performance was between 1X COC and controls was suggestive of a trend [t(119)=-1.61, p=0.11] (Figure A.9). Figure A.10 reveals that there were no treatment differences in criterial phase duration within or across tasks [all p>0.4].

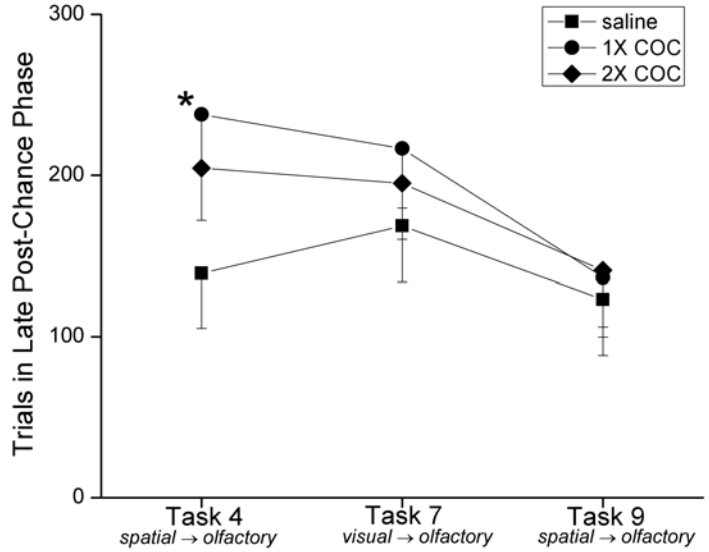

Figure A.9 Duration of late post-chance phase across olfactory tasks.

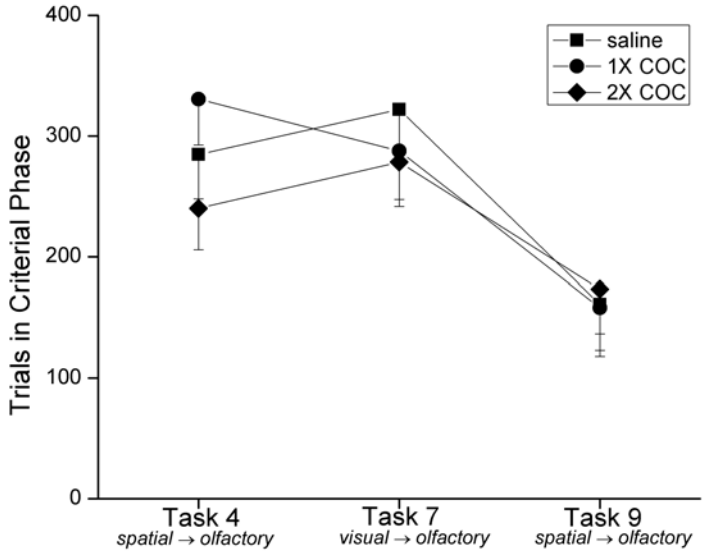

Figure A.10 Trials in criterial phase across olfactory-predictive tasks.

Synthesis of phase analysis findings for olfactory-predictive tasks

The above graphs reveal that the treatment differences in learning phases for olfactory-predictive tasks are again best described by dividing total learning into "early" and "late" learning phases. This distinction is not as clear cut as in the visual-predictive tasks, but we still see some patterns that suggest a "blocks" analysis approach appropriately represents learning phases. 1X COC animals clearly demonstrate a different pattern of chance level performance across the olfactory tasks, an effect reflected in Block 1 analysis described in Chapter 3. Further, the pattern seen in criterial phase (and to some extent late post-chance phase) reflects the observed "Block 2" results, again lending support to "Block 2" interpretation as errors committed after an animal knows the task rule.

Conclusions to draw from phase analysis for both visual and olfactory tasks

For the visual-predictive tasks, it is clear that the chance and early post-chance phases represent the same cognitive dysfunction, namely impaired transfer of learning. While this is less clear for the olfactory tasks, early learning patterns (especially for 1X animals) are distinct from those in later phases of learning. Thus, it makes sense to combine these early phases for both of these task types. Rather than just adding the phase durations together (to have one chance/earlypost value), we looked at blocks of learning. The problem with simply adding the two early phases together to get a duration of "early learning" is that the endpoint of earlypost chance is not readily interpretable. That is, functionally, what does it mean when an animal is consistently above 66.7% correct? What cognitive processes are in play? The blocks outcome provides a more interpretable endpoint – when an animal achieves eight consecutive correct responses, it "knows" the correct rule, any errors made after that point can be directly attributed to failures in selective attention. In point of fact, late post-chance

trials are most likely split between Blocks 1 and 2, which does not dull any treatment effect because there were no specific effects of treatment within late post-chance for either task type. The borderline difference between 1X and controls on the first olfactory task can also be observed in criterial phase, although it does not reach significance. Thus, not much is lost in the way of interpretable patterns by classifying some latepost trials as "block 1" and others as "block 2" While some latepost is in Block 2, the pattern of block 2 most represents that observed in the criterial phase of learning, an effect observed in both visual and olfactory tasks, further lending credence to our interpretation of Block 2 as primarily tapping selective attention, which is the primary cognitive process in play during criterial phase.

Explaining different pattern of effects on EDS tasks for 2X animals

Chapter 2 (the "sensitive period" study) and Chapter 3 (the "dose/response" study) both used the same cocaine-exposure regimen: saline GD1-7, IV cocaine HCl (3.0 mg/kg) once daily GD8-15, IV cocaine HCl (3.0 mg/kg) twice daily GD16-21. This group was referred to as the "full exposure" group in Chapter 2 and the "2X exposure" group in Chapter 3. Despite being exposed to the same dose, timing and duration of cocaine exposure, and being tested on the same behavioral paradigm, the patterns of results for these two groups are quantitatively and qualitatively different.

Full exposure (2X) animals in the Chapter 2 study demonstrated a pattern of performance on all task types suggestive of a failure in attentional set formation when the previously predictive stimuli was subtle and an impairment in attentional set shifting when the prior relevant dimension was salient. There were no differences in the Chapter 2 study between full exposure animals and controls in rate of learning on any task type. However, in Chapter 3, the most compelling finding of the 2X animals was the difference in slopes between this COC group and controls, particularly on the visual-predictive task. This led to a conclusion that 2X animals showed a complete lack of learning transfer across this difficult task.

The patterns observed in the two studies are not entirely inconsistent. Indeed, the olfactory tasks look very similar for both Chapter 2 and Chapter 3 comparisons of 2X/full with controls. Further, both studies demonstrated 2X animals perform better than controls on the first visual to spatial shift (Task 3); this superior performance in both cases reflects that these animals did not form a strong attentional set to the visual cue such that the subsequent spatial task was not a true "shift" in attention. However, the present study has found a robust deficit in transfer of learning for visual tasks that has not been previously reported with these 2X animals. Additionally, the two studies present different patterns of performance on Task 1: in Chapter 2, full animals were

superior to controls early in learning; in Chapter 3, 2X animals were impaired relative to controls later in learning. These differences can not be reconciled by considering drug administration differences, changes in testing equipment or lab personnel, sample size or statistical analysis; the patterns on the visual-predictive task are just clearly different. Thus, we explored some methodological reasons why the two groups aren't comparable.

We attribute these inconsistencies to differences in animals' prior experiences between the two studies. That is, in the "sensitive period" study, the odor triad strawberry-rose-lilac was used for both the preceding serial reversal tasks and the extra-dimensional shift tasks. Animals in that study were exposed to these same odors each day for several months prior to the start of the EDS series. Such persistent presentation may have effectively "dulled" the salience of these odors on subsequent tasks, both when they were predictive *and* when they were intended to be distracting. As a result, the familiar odors were less compelling and did not fully "capture" the attention of the animals. In the present study, however, a different odor triad was employed for the EDS series as that used for the serial reversal tasks (anise-almond-maple vs. strawberry-rose-lilac). The use of less familiar odors in the EDS series here avoids the problem of over-training; the olfactory dimension then served as more salient in these tasks.

Another inconsistency between the two studies was the designiation of criterial performance. In the Chapter 2 study, the criterion for visual-predictive tasks was 88% correct whereas in the Chapter 3 study criterion was set at 80%. Animals spent much longer on the first visual-predictive task in the Chapter 2 study compared to time on the same task presented in Chapter 3. This increased amount of time on Task 2 and higher level of performance may have effectively eliminated the cocaine-associated effect on transfer of learning in the previous study.

Taken together, these methodological issues can explain the lack of similarity regarding the patterns of performance on the visual-predictive tasks and Task 1 between the two studies.