# THE ROLE OF ALTERED CATECHOLAMINERGIC ACTIVITY IN THE ATTENTIONAL DYSFUNCTION INDUCED BY LEAD EXPOSURE OR PRENATAL COCAINE EXPOSURE

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# THE ROLE OF ALTERED CATECHOLAMINERGIC ACTIVITY IN THE ATTENTIONAL DYSFUNCTION INDUCED BY LEAD EXPOSURE OR PRENATAL COCAINE EXPOSURE

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Attentional dysfunction is associated with numerous genetic and environmental factors that disrupt brain development. Evidence implicates catecholaminergic systems in attentional processing, suggesting that alterations in catecholamine neurochemistry may underlie these attentional dysfunctions. The goal of the present experiments was to use a pharmacological challenge approach to examine underlying neural changes that relate to attentional dysfunctions induced by two developmental insults, lead (Pb) exposure and prenatal cocaine exposure, and to test the hypothesis that exposure-induced alterations in catecholaminergic systems contribute to these attentional deficits. In each experiment, adult rats were administered a visual attention task which assessed subjects' ability to monitor an unpredictable light cue and maintain performance when presented with olfactory distractors. In the first study, Pb-exposed subjects demonstrated significant impairments in accuracy relative to controls, providing evidence for Pb-induced attentional dysfunction. The alpha-2 adrenergic antagonist idazoxan improved accuracy, specifically in the most attentionally demanding conditions. However, this effect of idazoxan did not differ between Pb-exposed and control subjects, failing to support the hypothesis that the attentional dysfunction was due to alterations in

noradrenergic systems. In the second study, prenatal cocaine-exposed subjects did not differ from controls in their response to idazoxan's effect on errors of commission, but were more sensitive to idazoxan's effects on errors of omission and nontrials. The pattern of effects suggested that the differential treatment response to idazoxan resulted from prenatal cocaine-induced alterations in norepinephrine-modulated dopamine release, reflecting lasting changes in dopaminergic and/or noradrenergic systems underlying attention. The final study was designed to assess whether cocaine exposed and control subjects differ in sensitivity to attentional effects of the D1 dopamine agonist SKF81297. Prenatal cocaine-exposed subjects were more sensitive to the impairing effects of SKF81297 on errors of omission, providing specific evidence that prenatal cocaine exposure produces lasting changes in dopaminergic systems mediating attention. In conclusion, environmental Pb exposure impairs attention; however, these impairments do not appear to reflect alterations in noradrenergic function, suggesting the involvement of other neurochemical systems. In contrast, prenatal cocaine-induced attentional impairments appear to be related to lasting changes in dopaminergic systems underlying attention, suggesting possible targets for pharmacotherapeutic intervention in affected children.

# BIOGRAPHICAL SKETCH

Lorna Elissa Bayer earned an A.B. Magna Cum Laude in Psychology from Cornell University in 1990. During her undergraduate years, she pursued undergraduate research projects on the development of cognition in infancy under the direction of Dr. Elizabeth Spelke of the Cornell University Department of Psychology, and on neurotransmitter interactions and plasticity in the rat septohippocampal pathway under the direction of Dr. Teresa Milner of Weill Cornell Medical College Department of Neurology and Neuroscience, Division of Neurobiology. Prior to her graduate studies, Lorna worked as a Research Specialist at the Cornell University New York State College of Veterinary Medicine on toxicology research projects under the supervision of Dr. Fred Quimby. Lorna's primary academic interests are within the areas of neurobehavioral pharmacology and toxicology, functional neuroanatomy, and the neurobiology of memory, attention and executive function.

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# CHAPTER ONE

# INTRODUCTION

## Attentional systems as a target of toxicity

Deficits in attention are among the most common impairments seen in developmental disorders, and are associated with a wide variety of genetic and environmental factors that disrupt brain development. Attentional dysfunction is a commonly cited consequence of unrelated disorders such as attention deficit hyperactivity disorder (ADHD) (reviewed in Karatekin, 2001), fragile X syndrome (Baumgardner, et al, 1995; Hagerman, 1996; Lachiewicz, et al, 1994; Largo and Schinzel, 1985; Moon, et al, 2006; Turk, 1998), Down syndrome (Brown et al, 2003; Driscoll, et al, 2004; Tomporowski, et al, 1990; Wilding et al, 2002), phenylketonuria (PKU) (Diamond, 2001; Pennington, et al, 1985), and conditions caused by exposure to a variety of substances such as environmental toxins and drugs of abuse. The prevalence of attentional impairments as a symptom of early insult relative to other types of cognitive impairment suggests that the attentional processing system appears to have increased vulnerability to disruption by genetic and environmental factors (Pennington, 1991). This differential vulnerability is likely due to the evolutionary history, developmental time-course, and neurochemical modulation of the prefrontal cortex (PFC), a critical brain region for attentional processing

## Prefrontal control of attentional function

Considerable evidence suggests that the PFC mediates attentional processing, including both selective and sustained attention. Lesions to the PFC in human patients (Chao and Knight, 1995; Knight, et al, 1995; Mennemeir, et al, 1994; Ptito, et al, 1995; Rueckert and Grafman, 1996; Wilkins, et al, 1987**)**, monkeys (Stuss and

Benson, 1986) and rats (Chudasama, et al, 2003; Muir, et al 1996; Passetti, et al, 2002; 2003) cause deficits in sustained attention, and increased distractibility to irrelevant stimuli in auditory, visual, and haptic modalities. Dorsolateral prefrontal patients **(**Eslinger and Grattan, 1993; Milner, 1995; Owen, et al, 1991), marmosets with dorsolateral prefrontal lesions (Dias, et al, 1996; 1997), and rats with lesions to the medial PFC (Birrell, et al, 2000) show deficits in the ability to shift attentional set to a previously irrelevant dimension. Human patients with lesions to the dorsolateral PFC are also impaired on tasks of divided attention (Godefroy, et al, 1996) and on performance of the Stroop task, a task of attentional selection and response inhibition (Stuss, et al., 2001; Vendrell, et al, 1995).

The prefrontal cortex shows selective activation during performance of attentional tasks. Human functional neuroimaging studies have demonstrated specific frontal activation during the performance of the continuous performance task (CPT), a standard test of visual sustained attention and response inhibition (Rezai, et al, 1993). Differences in frontal evoked potentials produced in response to relevant target stimuli versus irrelevant distractor stimuli during visual and auditory attention tasks have also been reported (Baudena, et al, 1995; Zani, 1995). Dorsolateral prefrontal activation has been reported during shifting of attention between spatial locations (Corbetta, et al, 1993), between sorting categories on the Wisconsin Card Sorting Task (Konishi, et al, 1999; Rezai, 1993), and between competing tasks during dual-task performance (D'Esposito, et al, 1995**;** Loose, et al, 2003). Dorsolateral prefrontal activation has also been demonstrated during performance of the Stroop task, with greater activation exhibited on more attentionally demanding trials (Banich, 2000). Single-unit recordings from rhesus monkey dorsolateral PFC have identified a population of neurons that increased activity levels during the delay (or attention-demanding) period of a visual target detection task in which difficulty was manipulated by altering the

size and color contrast of the target (Lecas, 1995). Although these neurons were active during the delay under all conditions, average firing rate was proportional to attentional demand. Firing rate was highest in the difficult blocks, and lowest in the easy blocks, suggesting that these neurons control or code for the level of attention of the animal. Increases in unit activity of neurons in PFC under conditions of enhanced attentional demand have also been reported in rats during performance of sustained visual attention tasks in which visual distractors were presented (Gill, et al, 2000).

The critical role of the PFC in attentional processing may provide insight into the differential vulnerability of this functional brain system. The relatively large size of the human PFC occurred late in evolutionary history, and maturation of this brain region occurs relatively late in brain development; both factors which can increase susceptibility to developmental disruption (Pennington, 1991). Prefrontal control of attention relies on multiple neurotransmitter systems, making this system vulnerable to a variety of different neurochemical insults. Finally, evidence suggests that the dopamine (DA) neurons in this brain area have a greater dependence on precursor availability than those of other brain systems (Tam, et al, 1990), providing an additional mechanism for the greater vulnerability of this region.

**Evolutionary History**

The large prefrontal cortices of primates, especially humans, are a recent development in evolutionary history. Both the absolute and relative size of this brain region have increased significantly over recent evolution. According to Lieberman (1984), recently evolved brain systems tend to demonstrate a higher level of genetic variation both within and across species when compared to evolutionarily older, more highly conserved brain systems. Thus, the prefrontal attentional system's recent

evolutionary history should result in greater variation and, consequently greater vulnerability to developmental pathologies (Pennington, 1991).

**Late development compared to other brain systems**

A second relevant feature of the PFC is its protracted developmental time-course. The PFC is the last brain region to develop in humans and does not reach full maturity until early adulthood (Huttenlocher, et al, 1979, 1982, 1984, 1990; Rosenberg and Lewis, 1994; Sowell, et al, 1999; Thatcher, et al, 1987; Yakovlev, et al 1967). Thus, childhood exposure to toxins may cause alterations in this prefrontal attentional system while sparing other brain systems that are more fully developed at the time of exposure. Recent studies also suggest that the first two years, especially the second half of the first year of life, is an important transition period in prefrontal cortical anatomical development. Studies show that specific changes in and maturation of prefrontal cortical neurons and their associated cognitive functions are occurring during this period (Diamond, 2002). During this period, both the levels of DA (Brown, et al, 1977) and density of DA receptors increase (Lidow and Rakic, 1991) in dorsolateral PFC and significant changes occur in the distribution of tyrosine hydroxylase containing axons in this brain region (Lewis and Harris, 1991; Rosenberg and Lewis, 1995) in rhesus macaques. These neurochemical and anatomical changes parallel the development of PFC dependent cognitive tasks in human infants (Diamond and Doar, 1989). During this same period, longitudinal EEG studies of human infants show an increase in frontal electrical activity correlating with increasing proficiency at the performance of PFC dependent cognitive tasks (Bell and Fox, 1992). Toxin-exposure that occurs during this period may therefore have more marked effects on dorsolateral PFC dependent functions such as attention because it is during this period that the system undergoes critical developmental changes. For

example, the late development of the PFC relative to other brain regions may make this system particularly sensitive to insult caused by exposure to environmental lead (Pb) during post-natal development, the time of maximal Pb-exposure due to increases in hand-to-mouth behavior (Bellinger, et al, 1994).

**Modulation by multiple neurotransmitter systems**

The PFC receives diffuse ascending projections from the main sources of the forebrain monoaminergic and cholinergic neurotransmitter systems. Noradrenergic afferents from the locus coeruleus (LC) project to PFC (Porrino, et al, 1982; Gatter, et al, 1977). Norepinephrine (NE) concentration and turnover is highest in PFC relative to other cortical areas (Brown, et al, 1977; 1979), and the coeruleocortical noradrenergic projection plays a critical role in the modulation of attentional regulation, as discussed below. The PFC receives dopaminergic innervation from the ventral tegmental area (A10), the retrorubral area (A8), and the medial third of the substantia nigra (A9) (Williams and Goldman-Rakic, 1998). Like NE, DA levels in PFC are the highest of any cortical region (Brown, et al, 1977; 1979), and dopaminergic activity is critical to prefrontal control of executive functions such as attention, as discussed below. The PFC also receives widespread innervation from basal forebrain cholinergic neurons, and there is a wealth of evidence for the role of this afferent system in facilitating sustained attention and selection of attentional targets, particularly under conditions of high attentional demand (Sarter, et al, 2001; 2005). Serotonergic neurons from the dorsal raphe nucleus also project to the PFC (Porrino, et al, 1982; Gatter, et al, 1977). This modulatory system appears principally to mediate functions such as impulsivity and response inhibition (Robbins, 2005); however, some evidence suggests it may play a role in attentional processing under certain circumstances (Winstanley, et al, 2003).

Functional interactions occur among these monoaminergic and cholinergic systems, and conditions that selectively alter the neurochemistry of one or more of these systems during early development may result in long-lasting changes in the prefrontal control of attention. For example, cocaine binds to the DA, NE, and serotonin transporters, increasing concentrations of these neurotransmitters in the synapse; the CNS effects of cocaine are reported to be due principally to DA transporter binding (Meyer, 1993; Ritz, et al, 1987; 1990; reviewed in Harvey, 2004). Prenatal cocaine-induced attentional impairments may be related to specific and lasting changes in one or more of these monoaminergic systems.

**Greater dependence on precursor availability**

An additional potential reason for the particular susceptibility of the prefrontal attentional system is its comparatively greater dependence on precursor availability. The mesocortical dopaminergic neurons that mediate attentional function have higher basal firing rates and greater DA turnover than other brain DA systems (Tam, et al, 1990). This property makes these neurons more susceptible to small reductions in precursor availability than neurons in other DA systems. Thus, selective deficits in these DA dependent attentional functions could conceivably result from conditions that cause changes in precursor availability which are insufficiently great to alter other brain systems. For example, this mechanism has been proposed for the specific attentional and executive deficits resulting from the moderate phenylalanine/tyrosine imbalance still demonstrated in children treated for PKU (Diamond, 2001).

The reasons described above make attentional systems particularly vulnerable to insult caused by variety of disorders or toxic agents. The experiments described in this volume examine attentional deficits caused by exposure to two specific

developmental insults, environmental Pb-exposure and prenatal cocaine exposure, and examine the role of catecholaminergic attentional systems in these dysfunctions.

**Evidence for disordered attention following Pb-exposure**

Pb-induced deficits have been reported in studies of sustained attention (Dudek and Merecz, 1997**;** Harvey, et al, 1988**;** Hunter et al, 1985; Minder et al, 1994, Needleman, et al, 1979; 1990; Walkowiak, et al, 1998), selective attention (Bellinger, et al, 1994; Minder, et al, 1994; Raab, et al, 1990), and concentration **(**Faust and Brown, 1987). (See chapter 2 for review.) Interpretation of these studies are complicated by a variety of limitations including methodological constraints and sociodemographic confounding, highlighting the importance of animal model studies of Pb-exposure. Deficits in sustained attention have been identified in rats following developmental Pb-exposure (Morgan, et al, 2001; Stangle, et al, 2007). However, no previous animal model studies have specifically demonstrated effects of Pb on selective attention or the ability to withstand distraction, although these deficits were associated with Pb-exposure in several studies of human children (Bellinger et al, 1994; Minder et al, 1994; Raab et al, 1990). In addition, the specific neural changes that underlie these attentional deficits have not been established.

**Evidence for disordered attention following prenatal cocaine exposure**

A number of studies suggest that prenatal cocaine exposure causes attentional impairments in children (Azuma and Chasnoff, 1993; Beckwith, et al, 1995; Karmel and Gardner, 1996; Leech, et al, 1999; Mayes, et al, 1998; Richardson, et al, 1996). (See chapters 3 and 4 for review.) Again, many of these studies are difficult to interpret due to confounding factors such as polydrug abuse and poor postnatal care, highlighting the importance of animal model studies of prenatal cocaine exposure.

Prenatal cocaine-induced deficits in both focused or sustained attention (Gendle, et al 2003; Morgan, et al, 2002) and selective attention (Bushnell, et al, 2000; Gabriel and Taylor, 1998: Garavan, et al, 2000; Gendle, et al, 2004; Kosofsky and Wilkins, 1998; Romano and Harvey, 1996) have been reported in adult animals, long after the period of exposure. Again, the neural bases of these attentional dysfunctions are not well understood.

**Specific role of catecholamines in attention**

Considerable evidence implicates the coeruleocortical NE system in various aspects of normal attentional function (Arnsten, 1998; Arnsten and Li, 2005; Aston-Jones, et al, 1999; Ramos and Arnsten, 2007; Robbins, 1997; 2002). Lesions to ascending noradrenergic neurons in rats increase distractibility in maze tasks (Roberts, et al, 1976) and impair performance on tasks of visual attention, specifically under conditions of highest attentional demand such as unpredictable target stimulus onset and presentation of distractors (Carli, et al, 1983; Cole and Robbins, 1992). LC activity and cortical NE release have been demonstrated to reduce cortical signal–to-noise ratio (reviewed in Aston-Jones, 1988; 1997; Foote, et al, 1980; 1983; Sara, et al, 1988), and enhance cortical cue and delay related firing relative to spontaneous firing (Sawaguchi, et al, 1990). Activity of LC neuron was closely correlated with performance on visual focused attention tasks in monkeys (Aston-Jones, et al, 1999). At the lowest levels of LC activity, vigilance performance was poor (Rajkowski, et al, 1998). Moderate levels of tonic LC firing were associated with LC phasic activity which was selectively activated by attentional target stimuli, but showed no activation or was inhibited by distractors. This mode of LC activity corresponded with higher levels of task performance, suggesting that this phasic LC firing may facilitate focused and selective attention (Aston-Jones, et al, 1994). In contrast, high levels of tonic LC

activity decreased the phasic responses to target stimuli and correlated with poor task performance (Aston-Jones, et al, 1994). Thus, LC activity produces an inverted U-shaped effect on attention, with low levels of firing resulting in insufficient arousal and attention, moderate levels of tonic firing increasing attentional task performance, and high levels of tonic activity of LC neurons impairing vigilance and selective attention.

This relationship between LC activity and attentional performance is consistent with behavioral pharmacology studies of noradrenergic modulation of attention. Idazoxan (IDZ), an alpha-2 adrenergic antagonist, increases firing of the LC and thus increases coeruleocortical NE activity (Aghajanian, et al, 1977; Dennis, et al, 1987). Administration of this drug to rats prior to performance of a visual selective attention task specifically altered distractibility as a function of baseline performance (Bunsey and Strupp, 1995); more distractible subjects showed IDZ-induced performance improvements, while less distractible subjects were impaired by IDZ. This finding supports the above-stated hypothesis that coeruleocortical activity and endogenous NE release modulate attention according to an inverted U-shaped curve, in which an optimal level of NE facilitates attentional performance and both sub-optimal and supra-optimal levels produce relative impairments. This attentional effect is postulated to be mediated in part by prefrontal alpha-2 adrenergic receptors (Arnsten, 1998; Ramos and Arnsten, 2007), the receptors for which NE has the highest affinity (O'Rourke, et al, 1994). Alpha-2 receptors in PFC are localized both pre- and post-synaptically (Aoki, et al, 1994; Aoki, et al, 1998; Venkatesan, et al, 1996); presynaptic alpha-2 receptors mediate the NE-mediated negative feedback of the LC, whereas postsynaptic alpha-2 receptors are implicated in the NE modulation of prefrontal functions (Arnsten, 1998; Arnsten and Li, 2005; Ramos and Arnsten, 2007). Thus, alpha-2 selective agents may either increase or decrease attentional task performance

in subjects, depending on both the subjects' levels of endogenous NE activity and the dose-related ratio of binding to presynaptic vs. postsynaptic receptors. Consistent with this hypothesis, alpha-2 adrenergic agonists have been shown to increase delay-related neuronal activity (Li, et al, 1999) and decrease distractibility during performance of working memory tasks in aged monkeys (Arnsten and Contant, 1992), to impair performance on visual attention tasks in rats (Sirvio, et al, 1994), to impair attention on the CPT (Coull, et al, 1995) and on visual target detection tasks of selective attention (Coull, et al, 2004; Smith and Nutt, 1996) in healthy human volunteers, improve CPT performance in patients with schizotypal disorder (McClure, et al, 2007), and improve performance on the Stroop task in patients with Korsakoff's amnesia, relative to the patient's level of NE loss (Mair and McEntree, 1986). Guanfacine, an alpha-2 adrenergic agonist used in the treatment of ADHD, normalizes sustained attention in a rat model of ADHD (Sagvolden, 2006), impairs sustained attention in rats with lesions of the NE projections to PFC (Milstein, et al, 2007), and improves ADHD patient performance on both standard ADHD symptom rating scales (Hunt, et al, 1995; Scahill, et al, 2001; Taylor and Russo, 2001) and on more specific tests of attention such as the CPT (Scahill, et al, 2001) and the Stroop task (Taylor and Russo, 2001). Alpha-2 antagonists have been reported to decrease delay-related neuronal activity in monkeys (Li, et al, 1999), to narrow the focus of selective visuo-spatial attention in human subjects (Smith, et al, 1992), and to improve accuracy in performance of a visual attention task in rats at low drug doses when signal brightness was reduced, but to impair performance at higher drug doses when target stimulus onset was unpredictable (Sirvio, et al, 1993; 1994).

NE has a lower affinity for the alpha-1 adrenergic receptor subtype (Mohell, et al, 1983), which also appears to play a role in the NE modulation of attention. Activity at this receptor subtype has been demonstrated to impair PFC dependant

cognitive functions in monkeys (Arnsten, et al, 1999; Mao, et al, 1999), although this evidence comes from tasks of working memory rather than attention. In contrast, a study of the effects of the alpha-1 agonist ST-587 during the performance of a visual attention task in rats demonstrated a reduction in premature responses and an increase in accuracy under conditions of highest attentional demand (short cue duration) associated with the lowest drug dose, but not at higher doses. Low doses of the alpha-1 antagonist prazosin failed to alter task performance alone, but blocked the accuracy enhancing effects of the agonist; a higher dose of prazosin caused an impairment in accuracy on this task. (Puumala, et al, 1997) These data suggest that NE activity at the alpha-1 receptor may also demonstrate a similar inverted-U shaped relationship with attentional performance.

Extensive evidence also links dopaminergic activity to PFC dependent cognitive functions (reviewed in Arnsten, 1998; Arnsten and Li, 2005; Chudasama and Robbins, 2006; Robbins, 2000; 2005; Robbins and Roberts, 2007). Although the majority of the evidence for this DA modulation of prefrontal function is from studies of working memory, a number of studies do provide support for a role for DA in attentional function. Extracellular PFC DA was elevated during performance of a task of visual attention in rats (Dalley, et al, 2002), and DOPAC/DA ratios in right frontal cortex were significantly correlated with accuracy on another variant of the same task (Puumala and Sirvio 1998). Systemic administration of the selective D1 antagonist SCH23390 impaired accuracy and increased errors of omission on this same task (Passetti, et al, 2003). Depletion of PFC DA increased distractibility and facilitated attentional set-shifting in marmosets, likely due to an impairment of attentional set acquisition (Crofts, et al, 2001). Treatment of rats with tolcapone, a catachol-o-methyl transferase inhibitor which has been shown to elevate stimulated prefrontal DA but not NE release, enhanced attentional set shifting in rats (Tunbridge, et al,

2004). Intra-PFC infusions of a variety of DA receptor subtype specific agonists and antagonists also altered attentional set shifting performance in the rat (Floresco, et al, 2006; Ragozzino, 2002). Treatment with the DA D2 receptor antagonist sulpiride induced deficits in attentional set shifting in healthy human volunteers (Mehta, et al, 1999). Similarly, the DA insufficiency demonstrated by unmedicated Parkinson's patients was associated with deficits in extra-dimensional set-shifting ability which were ameliorated by treatment with l-dopa medication (Downes, et al., 1989) and recurred with l-dopa withdrawal (Cools, et al, 2001).

Like NE, dopaminergic modulation of attentional function also appears to follow an inverted-U shaped curve. Electrophysiological and behavioral pharmacological studies have established that moderate DA activity in PFC is commensurate with optimal functioning of this brain region, whereas both suboptimal (Sawaguchi, et al, 1988; 1994) and supraoptimal (Murphy, et al., 1996; Wang, et al, 2004; Zahrt, et al., 1997) DA activity impairs PFC-dependent functions. This effect appears to be modulated principally by DA activity at the D1 DA receptor subtype, the predominant DA receptor subtype in PFC (Lidow, et al, 1991). Although the majority of the evidence for this DA modulation of prefrontal function is from studies of working memory, there is some evidence that this inverted U-shaped relationship also exists between PFC DA activity and attentional function. Prefrontal microinfusions of the DA D1 receptor partial agonist SKF38393 prior to performance of a visual attention task selectively improved accuracy in rats with low baseline levels of performance, whereas microinfusions of the DA D1 antagonist SCH23390 selectively impaired accuracy in rats with high baseline levels of performance. (Granon, et al, 2000). This finding was consistent with the attentional enhancement produced by prefrontal microinfusions of two doses of the selective full D1 agonist SKF81297 in rats performing a task containing both sustained attention and working

memory components (Chudasama and Robbins, 2004). Notably, although two drug doses produced enhancement of attention, the highest dose also altered accuracy on the memory component of the task as a function of baseline performance; this dose impaired performance at the short delay and improved performance at the long delay. This finding suggests that although prefrontal DA activity regulates both working memory and attention by an inverted U-shaped function, optimal DA levels for regulating these two cognitive functions may differ. Additional studies suggest that D2 and D4 DA receptor specific agents may also alter attention as a function of baseline performance. Intra-PFC microinfusions of a selective D4 agonist impaired performance on an attentional set-shifting test in the rat, while microinfusions of a D4 selective antagonist improved set-shifting (Floresco, et al, 2006). Systemic treatment with the DA D2 receptor antagonist sulpiride impaired response accuracy on a task of visual attention in normal rats (Harrison, et al, 1997), but ameliorated accuracy deficits in rats with lesions to PFC (Passetti, et al, 2003). As prefrontal microinfusions of sulpiride in normal rats had no effect on response accuracy in this task (Granon, et al, 2000), the authors hypothesized that the drug's attention impairing effects in normal rats were due to blockade of D2 receptors in striatum, while the attentional enhancements in the PFC lesioned rats resulted from an attenuation of striatal dopaminergic hyperactivity induced by the PFC lesions.

**Effects of Pb- exposure on noradrenergic systems**

The critical role described above of the coeruleocortical noradrenergic system in the modulation of attention makes this system a plausible cause of the attentional deficits induced by environmental Pb-exposure. Unfortunately, studies of effects of Pb-exposure on NE function report disparate results, likely due to the numerous

methodological differences across studies. (See chapter 2 for review.) Previous studies have been inconsistent with regard to both the presence and direction of Pb effects on various measures of NE function. However, a number of groups have reported Pb-induced increases in measures of NE in forebrain (Rossouw, et al, 1987; Silbergeld and Goldberg, 1975) and frontal, parietal, and occipital cortex (Freedman, et al, 1990). In addition, Pb-induced increases and decreases in measures of NE function have been reported in midbrain **(**Dubas, et al, 1978; McIntosh, et al, 1989), cortex (Devi, et al, 2005; Prasanthi, et al, 2005; Rajanna, et al, 1997; Rossouw, et al, 1987), hippocampus ( Devi, et al, 2005; Collins, et al, 1984; Kumar and Desiraju, 1990; Prasanthi, et al, 2005), and striatum **(**Dubas, et al, 1978; Jason and Kellogg, 1981; Kumar and Desiraju, 1990). Prior studies have also observed alterations in sensitivity to the effects of NE drugs (Carter and Leander, 1980; Grover, et al, 1993; Rafales, et al, 1981; Reiter, et al, 1975). These studies have not assessed the specific functional consequences of the noradrenergic alterations.

**Effects of Prenatal Cocaine Exposure on Catecholaminergic systems**

Alterations in these underlying catecholaminergic systems may also mediate the attentional impairments induced by prenatal cocaine exposure. The available evidence concerning effects of prenatal cocaine exposure on central catecholaminergic activity is also inconsistent, due to the wide variation in both doses of cocaine and routes of administration. (See Chapters 3 and 4 for review) However, evidence from studies utilizing low dose IV exposure regimens that closely mimic recreational cocaine use in humans present a more consistent picture of the effects of prenatal cocaine exposure on catecholaminergic function. IV prenatal cocaine exposure has been reported to alter noradrenergic receptor number (Booze, et al, 1994; 2006; Elsworth, et al, 2007), inhibit the outgrowth of neurites in LC neurons (Snow, et al,

2001), and cause changes in measures reflecting NE levels and NE turnover (Booze, 2006; Wang, et al 1996, Elsworth, et al, 2007), including stress induced NE turnover in PFC and down-regulation of LC autoreceptors (Elsworth, et al, 2007). Studies of IV prenatal cocaine exposure have also demonstrated prenatal cocaine-induced decreases in regional $D_1$ receptor G-protein coupling (Friedman, et al, 1996; Levitt, et al, 1997; Wang, et al, 1995) and increases in the amount of DA released in response to a stressor or a pharmacological challenge, but not alterations in basal DA levels or release (Du, et al, 1999; Elsworth, et al, l999; Morrow, 2001; Wang, et al, 1995; 1996), or DA $D_1$ receptor number (DeBartolomeis, 1994; Leslie, et al, 1994). Again, however, these studies have not assessed the specific cognitive/behavioral consequences of the catecholaminergic alterations.

**Linking neurochemistry/pharmacology with function**

Pb-exposure and prenatal cocaine exposure both cause deficits in attentional function and have been demonstrated to cause alterations in measures of catecholaminergic activity. However, prior to the studies described in the current volume, there was no evidence to directly link these toxin- or drug-induced catecholaminergic changes to the exposure-induced deficits in attention. The studies described in this volume examine the functional significance of these catecholaminergic changes. These studies use pharmacological challenges to test the hypothesis that the alterations in attentional function caused by environmental Pb-exposure and prenatal cocaine exposure are due to alterations in the catecholaminergic neurochemical systems underlying attention. In these studies, exposed and control subjects were administered a range of doses of specific catecholaminergic drugs, and tested on an attentional task requiring subjects to monitor an unpredictable light cue in the presence of olfactory distractors. Dose-effect curves were generated for each

subject, and curves for exposed and control subjects were compared. A shifting of the dose-effect curves in exposed subjects relative to controls was the critical measure used to indicate altered sensitivity to the effects of the catecholaminergic drug on attentional task performance in the exposed subjects, and to provide evidence for exposure-induced changes in the catecholaminergic systems mediating attentional function. Chapter 2 examines the nature of the attentional dysfunction induced by environmental (Pb)-exposure and the role of noradrenergic activity in these Pb-induced attentional impairments, using a challenge with the alpha-2 noradrenergic antagonist idazoxan. Chapters 3 and 4 examine the roles of noradrenergic and dopaminergic systems in prenatal cocaine-induced attentional dysfunctions, using idazoxan and the selective DA D1 receptor agonist SKF81297 respectively. It is critical to identify the specific neurochemical changes that underlie these attentional deficits in order to develop drug therapies that may ameliorate the dysfunction.

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# CHAPTER 2

## CHRONIC POST-WEANING LEAD-EXPOSURE IMPAIRS ATTENTION BUT DOES NOT ALTER SENSITIVITY TO THE EFFECTS OF IDAZOXAN ON DISTRACTION TASK PERFORMANCE

## ABSTRACT

The present two studies were conducted to examine the effects of lead (Pb)-exposure on sustained and selective attention and to test the involvement of noradrenergic (NE) systems in Pb-induced attentional dysfunction. In study 1, Pb-exposed and control rats were tested on a distraction task which assessed the ability of the subjects to monitor an unpredictable light cue and maintain performance when presented with olfactory distractors. Pb-exposed subjects exhibited impaired accuracy on this task relative to controls. In study 2, Pb-exposed and control rats were administered the alpha-2 adrenergic antagonist idazoxan (IDZ: 0, 0.5, 1.0, 1.5 mg/kg) and tested on a version of the same distraction task. Analyses revealed specific attentional effects of IDZ: The highest dose of the drug (1.5 mg/kg) improved accuracy, specifically in the most attentionally demanding conditions; i.e., trials with either the briefest light cue or those in which an olfactory distractor was presented. The specificity of the drug effects rules out nonspecific alterations in performance as the basis of IDZ effects. Furthermore, the analyses did not reveal a differential effect of IDZ in Pb-exposed and control rats. These results provide evidence for Pb-induced impairments in sustained or focused attention, but fail to support the hypothesis that alterations in the coeruleocortical NE pathway contribute to this Pb-induced attentional dysfunction.

# INTRODUCTION

Recent recalls of lead-contaminated children's toys and other consumer products demonstrate that environmental lead (Pb) contamination continues to pose a serious health risk. Attentional impairments, such as the deficits in sustained and selective attention commonly associated with attention deficit hyperactivity disorder (ADHD), are frequently cited as a consequence of exposure to Pb in children. However, much of this evidence for Pb-induced attentional dysfunction comes from studies which report correlations between Pb exposure levels and parent/teacher behavior ratings (Baloh et al, 1975; Benetou-Marantidou et al, 1988; Fergusson, et al, 1988; Needleman et al, 1979; Silva, et al, 1988; Thomson, et al, 1989; Tuthill, 1996; Yule, et al, 1984). These studies are difficult to interpret due to both their lack of specific information on the cognitive processes altered by Pb and the presence of confounding sociodemographic factors.

The relatively few studies that have specifically examined attentional processes in children have yielded inconsistent results. Mixed results have been reported for both simple and choice reaction time tasks; sustained and selective attentions contribute to performance on these tasks respectively. Some studies have observed an association between lead exposure and simple (Dudek and Merecz, 1997; Hunter et al, 1985; Minder, et al, 1994, Needleman, et al, 1979) or choice reaction time (Minder, et al, 1994; Raab, et al, 1990), while other studies have found no Pb-associated deficits in simple (Winneke, et al, 1990, 1994) or choice reaction time (Dudek and Merecz, 1997; Winneke, et al, 1989; 1990). Inconsistent results have also been reported for the Continuous Performance Test (CPT), a task that reveals deficits in children with ADHD. One study observed an association between Pb exposure and performance on this task (Walkowiak, et al, 1998); while two earlier studies found no association

between Pb-exposure and any measure of performance (Hansen, et al, 1989; Harvey, et al, 1988). A fourth study failed to find an association between Pb-exposure and overall task performance, but did report a significant association between Pb-exposure and the ability to "focus-execute", which the authors interpret as an index of selective attention (the ability to select and respond to critical information) (Bellinger, et al, 1994). Faust and Brown (1987) reported a borderline effect of early Pb-exposure on the subdivision of concentration but no effect on the subdivision of attention in a neuropsychological test battery. Finally, Padich et al (1985) found no association between Pb-exposure in 18-month old infants and attentional measures in a structured problem-solving task.

The inconsistent results reported above likely reflect a variety of factors including timing of Pb-exposure, whether complete longitudinal information concerning lifetime lead exposure was available, the sensitivity of the testing instrument in detecting subtle effects, and the range of lead exposures in the study population. In addition, these inconsistencies may also be due to variation in the extent to which lead exposure in these studies is confounded with sociodemographic variables associated with cognitive development such as quality of the home environment, as well as the methods used to assess and statistically control for these factors. (For detailed discussion of this issue, see Morgan, et al, 2001).

Due to these limitations, it is important to assess the putative effect of lead on attention using animal models, where lead exposure can be studied in the absence of these sociodemographic factors which tend to co-occur with lead in the human situation and which themselves are risk factors for impaired cognitive functioning. Two studies have found simple reaction time unimpaired in Pb-exposed monkeys, (Rice, 1988, Hopper, et al, 1986); however, in both of these studies the cue was presented immediately after trial onset, limiting the demands the task placed on

attention. Two studies of reversal tasks with irrelevant cues reported systematic responding to the irrelevant cues by Pb- exposed monkeys, which the authors interpreted as impairments in selective attention (Rice, 1992; Rice and Gilbert, 1990). However, these findings can also be explained as a Pb-induced associative deficit (see Garavan, et al, 2000; Hilson and Strupp, 1997). Studies from our laboratory have identified deficits in sustained attention in rats following both developmental (Morgan, et al, 2001; Stangle, et al, 2007) and chronic post-weaning (Driscoll, 2003) Pb-exposure. However, whether these effects extend to selective attention is still unclear, although selective attention deficits were associated with Pb-exposure in several studies of children (Bellinger, et al, 1994; Minder, et al, 1994; Raab, et al, 1990). Clearly, additional animal-model studies are needed to assess the specific nature of the attentional functions that are altered following exposure to Pb.

In addition to identifying the nature of the attentional dysfunction induced by Pb-exposure, identification of the neurochemical basis of these observed attentional deficits is necessary for the development of therapeutic intervention strategies. The locus coeruleus (LC) is essential for various aspects of normal attentional processing, including selective and focused attention (see Bunsey and Strupp, 1995 for review; Arnsten, et al, 1992; 1996; 1997; 2005; Aston-Jones, 1988; 1997; 1999; Bushnell, 1998; Clark, et al, 1987a, 1987b; Ramos and Arnsten, 2007; Robbins, 1997; Sara, 1985; Sara, et al, 1988) and scanning attentiveness (Aston-Jones, 1999), suggesting alterations in the coeruleocortical noradrenergic (NE) neurotransmitter system as a good candidate for the basis of Pb-induced attentional impairments. However, the available evidence concerning effects of Pb-exposure on NE function is inconsistent. Previous studies have been inconsistent with regard to both the presence and direction of Pb effects on various measures of NE function including levels of NE in urine (Payton, et al, 1993; Tang, et al, 1995), plasma (Carmignani, 2000; Chang, et al,

1996a; 1996b; Goldman, et al, 1980), and whole brain (Flora, et al, 2007; Golter and Michaelson, 1974; Rafales, et al, 1981; Sauerhoff and Michaelson, 1973; Saxena and Flora, 2006; Tandon, et al, 1984) and regional NE levels (Bailey and Kitchen, 1986; Baksi and Hughes, 1982; Collins, et al, 1984; Devi, et al , 2005; Dubas, et al, 1978; Freedman, et al, 1990; Grant, et al, 1976; Hrdina, et al, 1976; Jason and Kellogg, 1977; 1981; Kumar and Desiraju, 1990; McIntosh, et al, 1988; 1989; Meredith, et al, 1988; Prasanthi, et al, 2005; Silbergeld and Goldberg, 1975; Sobotka and Cook, 1974; Sobotka, et al, 1975; reviewed in Moore, et al, 1986); levels of VMA in urine (Silbergeld and Chisolm, 1976; Silbergeld and Goldberg, 1975; Tang, et al, 1995) and brain (Silbergeld and Chisolm, 1976); adrenergic receptor number (Rajanna, et al, 1997; Chang, et al, 1996a; Roussouw, et al, 1987) and affinity (Roussouw, et al, 1987); NE turnover (Collins, et al 1984; Goldman, et al, 1980; Michaelson, et al, 1974); NE uptake (Agrawal, et al, 1986; Silbergeld and Goldberg, 1975); tyrosine hydroxylase levels (McIntosh, et al, 1988; 1989; Meredith, et al, 1988; Schumann, et al, 1977; Wince and Azarro, 1978; reviewed in Moore, et al, 1986); regional NE terminal density (Freedman, et al, 1990); in vitro NE release (Tomsig, et al, 1993); NE induced inosital incorporation (Rajanna, et al, 1997); and behavioral response to NE drug challenge (Carter and Leander, 1980; Fregoneze, et al, 1997; Grover, et al, 1993; Rafales, et al, 1981; Reiter, et al, 1975). Differences in exposure period, age at testing, lead level, and brain region tested make comparisons across studies difficult to draw, and may be the likely basis for the disparity in findings. Moreover, these studies have not assessed the specific functional consequences of these alterations.

The current series of investigations were designed to shed additional light on both the nature and the basis of Pb-induced deficits in attention. Experiment 1 was designed to ascertain the effects of chronic post-weaning lead exposure on both sustained and selective attention, using a visual attention task in which olfactory

distractors were presented unpredictably on some trials. Experiment 2 used a pharmacological challenge design to test the hypothesis that alterations in the NE system contribute to Pb-induced attentional deficits.

## EXPERIMENT 1

## MATERIALS AND METHODS

### Subjects

Female Long-Evans rats were obtained from Harlan Sprague-Dawley on postnatal day (PND) 21. Rats were housed in pairs or triples in hanging wire mesh cages. The room in which subjects were both housed and tested was maintained on a reversed dark/light cycle. (Lights on: 2100; lights off: 0730)

On PND25, subjects were randomly assigned to one of three Pb treatment conditions stratified for weight, food intake, and water intake. These three conditions corresponded to the concentration of Pb acetate in the drinking water. (1) High Pb (n=14): Subjects' water contained 250 ppm Pb acetate. (2) Low Pb (n=13): Subjects' water contained 50 ppm Pb acetate. (3) Control (n=15): Subjects' water contained 250-ppm sodium acetate instead of Pb acetate. Subsequent analysis of water revealed unexpectedly low Pb concentrations, particularly in the 50ppm Pb acetate condition. Consequently, in order to produce the desired Pb level, starting at PND132 Pb acetate was dissolved in MilliQ water (Millipore, Bedford, MA) and concentrations were increased to 75 ppm and 300 ppm for the low and high Pb group respectively. The control group received 300-ppm sodium acetate dissolved in MilliQ water (Millipore, Bedford, MA). Drinking water was available ad libitum. Research in this lab has demonstrated that these regimens do not result in differential water intake. (Saelens and Strupp, unpublished data). Rats were maintained on these treatments throughout

behavioral testing. Subjects were maintained on a semipurified diet (AIN76, US Biochemicals Corp) to maximize lead absorption.

**Blood lead analyses**

Following completion of behavioral testing, a blood sample was taken from each subject via tail-nick. Blood lead (BPb) concentration assays were performed by the Wisconsin State Hygiene Laboratory (WSHL) using graphite furnace atomic absorption spectrophotometry. The WSHL, in cooperation with the Centers for Disease Control and the Bureau of Maternal and Child Health, administers the Nationwide Blood Lead Proficiency Testing Program.

**Apparatus**

Testing was conducted in 11 automated Plexiglas chambers enclosed in sound-attenuating wooden boxes, each operated by a Commodore 128 computer. Each chamber consisted of a square waiting area (26.5 cm by 25 cm by 30 cm), adjacent to a testing alcove containing 3 funnel-shaped ports. An LED was mounted above each port. These 2 compartments were separated by a thin metal door, which was raised to initiate each trial. The left and right ports were at an approximate 45-degree angle relative to the center port and the distance between the left and right ports was 8 cm. Each port was connected by tubing to three bottles containing liquid odorants, attached to a board placed outside of the box Solenoid valves controlled the presentation of compressed air, pumped through a specific odorant and through a specific port. The airflow rate was 1.0 L/min and the air in the chamber was cleared via small centrifugal fans mounted on the outside of the chambers, at a rate of four complete exchanges per minute. A set of infrared phototransistors and a light source monitored the entrance to the alcove and each port. A nose-poke of ≥ 1 sec into one of the three ports

constituted a response. Correct responses were reinforced with a 45 mg food pellet (Noyes, Lancaster, NH) delivered into the alcove from a pellet dispenser (Lafayette Instrument Co., Lafayette, IN).

**Prior behavioral testing**

Training/behavioral testing began for all subjects on PND171. During behavioral testing, subjects were maintained on a restricted feeding regimen: 4 hours ad lib access daily, immediately following behavioral testing. Subjects were trained on several attentional tasks as part of a previous experiment (Strupp, et al, 1993): 1) a three-choice visual discrimination task in which the correct port was designated by the illumination of an LED mounted over that port immediately after trial onset; and 2) a vigilance task in which the interval between trial onset and cue illumination varied.

**Distraction Task**

Daily sessions consisted of 100 trials or 90 minutes, whichever came first. The opening of the alcove door signaled the onset of each trial. After the subject entered the testing alcove, one of the three LEDs was illuminated for 1 sec duration. Two parameters were randomly varied across the trials in the session for all subjects: 1) pre-stimulus delay (2 or 3 sec after alcove entry); and 2) presentation and timing of distractors: On 2/3 of the trials in each session, one of nine different odors was delivered through one of the three ports, either one second ("-1 distractor condition") or two seconds ("-2 distractor condition") prior to visual cue onset. No odors were presented on the remaining one third of the daily trials (nondistraction trials). The delay, cue location, and distraction condition for each trial were selected pseudorandomly, balanced for each session. A 1-sec nose-poke into the port under the illuminated light was deemed correct, resulted in the delivery of a reinforcement, and

ended the trial, signaled by the closing of the alcove door. A nose-poke of ≥ 1 sec into any port prior to the light cue onset (premature response) or into an incorrect port following the cue ended the trial with no reinforcement. If the animal failed to enter the alcove within 60 sec or failed to respond within 60 sec of light cue offset, the alcove door closed and a nontrial was recorded. Nontrials did not count towards the maximum 100 trials per day, nor were they averaged into the calculation of percentage correct for that session. An inter-trial interval of 15 sec was imposed between successive trials. Subjects received 14 days of testing on this task.

Behavioral testing was conducted by personnel who were blind to treatment condition of the rats.

**Statistical Analysis**

Behavioral data were analyzed with a mixed models analysis of variance procedure using the SAS version 6.11 PROC MIXED on Cornell's mainframe computer. Percentage correct was calculated for each session and analyzed as a function of lead treatment (termed Pb), day, pre-stimulus delay, distraction condition, and the relevant interactions of these variables. Nontrials and premature responses were analyzed as a function of Pb treatment, day, and the interaction of these variables.

## RESULTS

**Body weight**

The body weights of the three treatment groups did not differ significantly ($F=1.47$, $p=0.48$). Mean body weights prior to start of the distraction task were 266, 275, and 286 g for the control, low, and high Pb groups respectively.

**Blood lead levels**

Median blood Pb (BPb) levels were <5 µg/dl, 19 µg/dl, and 39 µg/dl for the control, low, and high Pb groups respectively. Results of a subsequent study comparing the accuracy of BPb sampling techniques indicated that tail-nick blood samples such as these tend to overestimate BPb levels (See Morgan, et al, 2000). Thus, it is likely that true BPb values for these subjects were lower than reported.

**Percentage Correct**

Percentage correct for a given session was calculated as number of trials on which a correct response was made divided by the total number of response trials in a session, multiplied by 100. Response trials were defined as those trials on which the animal entered the testing alcove and made a response within 60 seconds. There was a main effect of Pb treatment on percent correct [$F(2,39)=8.07$, $p=0.0012$) reflecting the fact that percent correct was lower, relative to controls, in both the high ($p=0.0151$) and low ($p=0.0003$) Pb treatment groups (See figure 2.1) There was a significant overall effect of distraction condition on percent correct [$F(2,2787)=248.86$, $p=0.0001$], reflecting the fact that there was a significant reduction in percent correct under both distraction conditions relative to the nondistraction condition, the "-1" distraction condition in which distractors were presented 1 second before light cue presentation [$p=0.0001$] and the "-2" distraction condition [$p=0.0001$] in which distractors were presented 2 seconds before light cue presentation. In addition, percent correct was significantly lower under the "-2" distraction condition relative to the "-1" distractor condition [$p=0.0001$]. There was a borderline interaction of Pb treatment and distraction condition [$F(4,2787)=2.45$, $p=0.0577$]. However, this borderline interaction did not reflect greater group differences under the distraction conditions relative to the nondistraction condition (which would indicate greater

distractibility in the lead-exposed animals). Both Pb-exposed groups were similarly impaired relative to the control group in all three conditions (i.e., the nondistraction condition and the two distraction conditions), as depicted in Figure 2.2.

Percent correct increased across days of testing [F (13,497)=14.45, p=0.0001] reflecting an increase in performance in the distraction conditions. The interaction of Pb treatment and day [F (26, 497)=0.95, p=0.53] was not significant, nor was the interaction of Pb treatment, day, and distraction condition [F (52,2787)=0.97, p=0.55].

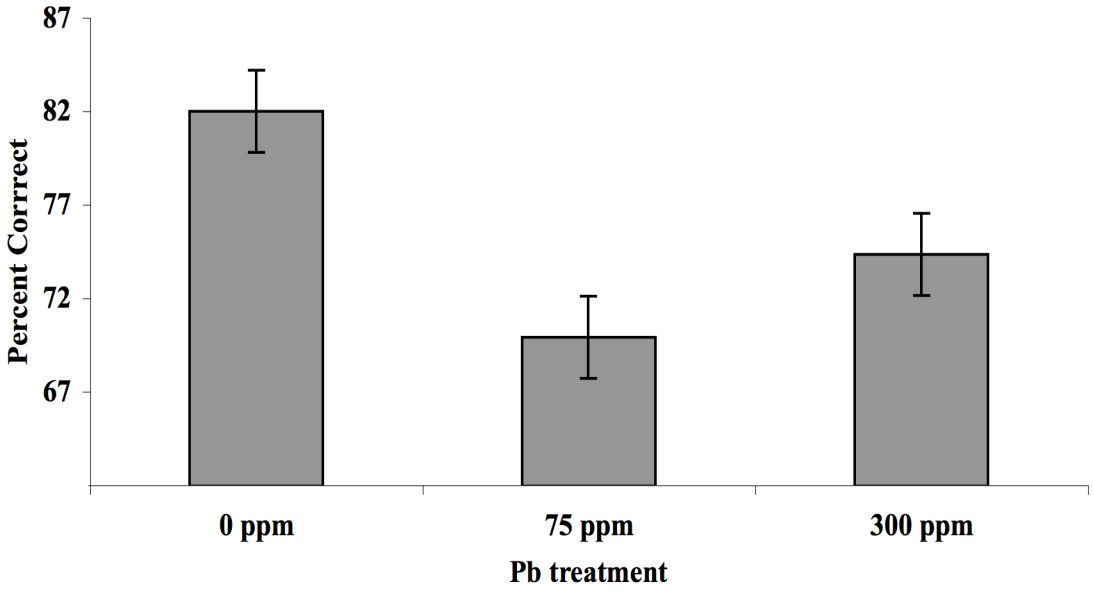


**Figure 2.1**. Pb treatment significantly reduced percent correct (p=0.0012), reflecting the fact that percent correct was lower relative to controls in both the high (p=0.0151) and low (p=0.0003) Pb treatment groups. This reduction in percent correct reflected Pb-induced decreases in accuracy.

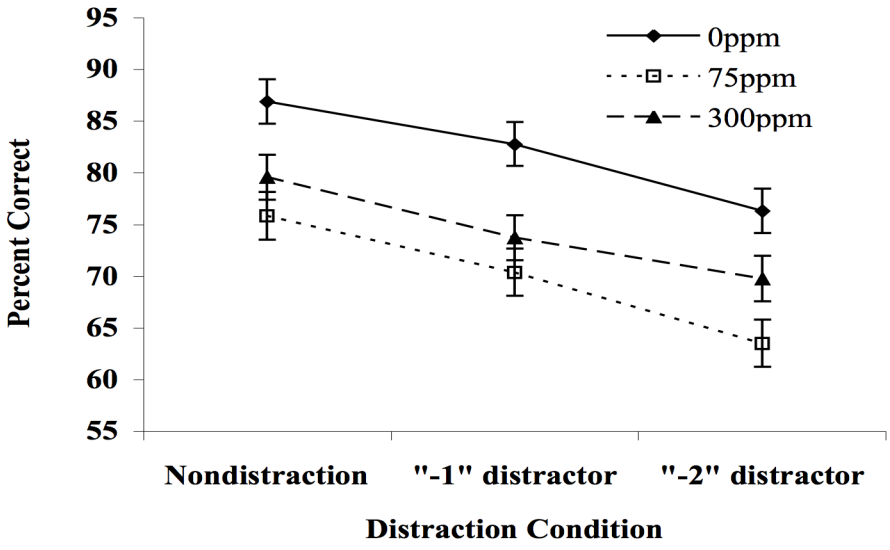


**Figure 2.2**. Both Pb-exposed groups were similarly impaired relative to the control group during all three distraction conditions: the nondistraction trials [low vs. control: p=0.0004; high vs. control: p=0.01], the "-1" distractor condition [low vs. control: p=0.0001; high vs. control: p=0.003], and the "2" distractor condition [low vs. control: p=0.0001; high vs. control: p=0.03].

**Premature Responses**

Premature responses for a given session were calculated as those trials in the session on which the subject made a ≥1 sec nosepoke to any port prior to light cue presentation. This type of response terminated the trial and was considered an index of impulsivity and/or deficient response inhibition. The rate of premature responses was not significantly altered by Pb treatment [F (2, 39)=0.85, p=0.44], testing day [F (13, 491)=1.47, p=0.13], or the interaction of these variables [F (26, 491)=0.96, p=0.53]. Mean percent premature responses were 10.7±2.3, 15.2±2.5, and 12.7±2.4 for the control, low Pb, and high Pb treatment groups respectively.

**Nontrials**

Nontrials for a given session were calculated as those trials in the session on which the subject either failed to enter the testing alcove within 60 seconds, or entered the alcove but failed to make a response within 60 seconds. The rate of nontrials was not significantly altered by Pb treatment [$F(2, 39)=1.15$, $p=0.33$], testing day [$F(13, 494)=1.03$, $p=0.42$], or the interaction of these variables [$F(26, 494)=1.01$, $p=0.46$]. Mean nontrials were 6.9±2.0, 7.1±2.1, and 10.8±2.1 for the control, low Pb, and high Pb treatment groups respectively.

## EXPERIMENT 2

As discussed in the Introduction, Experiment 2 was designed to test the hypothesis that alterations in the NE system contribute to Pb induced attentional dysfunction. Rats exposed to Pb and control rats were administered a range of doses of idazoxan (IDZ), an alpha-2 adrenergic antagonist that increases NE release from LC projections (Aghajanian, et al, 1977; Dennis, et al, 1987), prior to testing on a variant of the distraction task used in study one. IDZ dose-effect curves for Pb-exposed and control subjects were compared; altered sensitivity to the attentional effects of IDZ in Pb-exposed subjects was considered indicative of alterations in the NE system underlying attention.

# MATERIALS AND METHODS

## Subjects

Female Long-Evans rats were obtained from Harlan Sprague-Dawley on postnatal day (PND) 21. Rats were housed in pairs or triples in hanging wire mesh cages. The room in which subjects were both housed and tested was maintained on a reversed dark/light cycle. (Lights on: 2100; lights off: 0730)

On PND25, subjects were randomly assigned to one of two lead treatment conditions, stratified for weight, food intake, and water intake. These conditions corresponded to the concentration of lead acetate in the drinking water. Pb exposed subjects (n=11) had ad lib access to MilliQ water (Millipore, Bedford, MA) adulterated with 300 ppm Pb acetate. The water of the control subjects (n=9) contained 300-ppm sodium acetate instead of Pb acetate. Drinking water was available ad libitum and rats were maintained on their respective treatment throughout behavioral testing. Subjects were maintained on a semipurified diet (AIN76, US Biochemicals Corp.) to maximize lead absorption.

## Blood lead analyses

Following completion of behavioral testing, a blood sample was taken from each subject via tail-nick. Blood lead (BPb) concentration assays were performed by the Wisconsin State Hygiene Laboratory (WSHL) using graphite furnace atomic absorption spectrophotometry. The WSHL, in cooperation with the Centers for Disease Control and the Bureau of Maternal and Child Health, administers the Nationwide Blood Lead Proficiency Testing Program.

**Apparatus**

Testing was conducted in the same 11 automated Plexiglas chamber described in experiment one, each operated by a Commodore 128 or IBM PC XT computer.

**Prior Behavioral Testing**

Initial training/behavioral testing began for all subjects on PND 90. Subjects were trained on the same prior attentional tasks described in experiment 1, followed by 14 days of training on a variant of the distraction task described below. Prior to initiation of the IDZ challenge study, subjects received 12 additional days of testing on the distraction task to achieve a stable baseline performance level for each rat, then participated in a study designed to test the effects of the D1 dopamine agonist SKF81297 (Bayer, et al, 1994).

**Distraction Task**

This distraction task was identical to the task described for experiment 1, with the following exceptions: 1) The duration of illumination of the LED visual cue was randomly varied across subjects; half of the subjects in each treatment group were presented with a 500 msec cue and half were presented with a 1 sec cue. 2) Trials on which olfactory distractors were presented were reduced to 1/3 of daily trials. No odors were presented on the remaining two thirds of the daily trials.

**Idazoxan challenge study**

Subjects were maintained on a restricted feeding schedule to maintain adequate motivation. Food weights were determined for each animal on an individual basis to identify the largest daily intake that would still maintain high response trials and low nontrials. Daily sessions consisted of 100 trials or 90 minutes, whichever came first.

IDZ (0.0, 0.5, 1.0, 1.5 mg/ml/kg; Research Biochemicals, Inc, Natick MA) was dissolved in sterile MilliQ water (Millipore, Bedford, MA) immediately prior to each daily session and was administered subcutaneously 15 minutes prior to testing. Each subject received each of the 4 doses 3 times (in three testing blocks), with dose order determined by a Latin Square design. 48 hrs were imposed between successive injection days to allow drug clearance.

**Response types**

Percentage correct, premature responses and nontrials were calculated for each session. Omission errors, in which the rat entered the testing alcove but failed to respond within 10 seconds, were calculated separately from nontrials in Experiment 2 and counted toward the overall percent correct figure. This calculation of omission errors differed from that used in study 1, in which this response type was counted as a nontrial and did not count towards the overall percent correct figure. This change was made based on evidence that omission error rate reflects distinct cognitive processes from those tapped by nontrials (for discussion see Bayer, et al, 2000, 2002). In addition, as the Pb-induced impairments in Experiment 1 appeared related to treatment differences in accuracy, inaccurate responses were also calculated separately to provide a more in-depth analysis of this response type.

**Statistical Analysis**

Data were analyzed with a mixed models analysis of variance procedure using the SAS version 6.11 PROC MIXED on Cornell's mainframe computer. To achieve a more normal distribution, percentage error-type data were calculated across all three sessions of a given dose for each rat and arcsin square root transformed prior to

analysis. Percentage error-type data (percentage errors of omission, percentage accuracy errors, percentage premature responses) were analyzed as a function of lead treatment (termed Pb), IDZ dose (termed IDZ), pre-stimulus delay, light cue duration, distraction condition, and the relevant interactions of these variables. Similar analyses were conducted on the percentage correct, although testing block was also included in these analyses. Nontrials were analyzed as a function of lead treatment, IDZ dose, and the relevant interactions of these variables.

In all of these analyses, the critical test of differential sensitivity of the treatment groups to IDZ was an interaction of Pb treatment and IDZ dose.

## RESULTS

### Body weight

The body weights of the two treatment groups did not differ significantly ($T(20)=0.625$, $p=0.27$). Mean body weights on day one of the IDZ challenge study were 325 and 331 g for the control and Pb-exposed groups respectively.

### Blood lead levels

Median blood lead (BPb) levels were <5 μg/dl and 56 μg/dl for the control and Pb groups respectively. The results of a subsequent study comparing the accuracy of several BPb sampling techniques indicated that tail-nick blood samples such as these tend to overestimate BPb levels (see Morgan, et al, 2000). Thus, it is likely that the true BPb values for these subjects were somewhat lower than reported.

### Percentage Correct

Percentage correct for a given session was calculated as the percentage of trials in the session on which a correct response was made within 10 seconds of trial onset.

There was no effect of Pb treatment on overall percentage correct [Pb: F (1,1231)=0.32, p=0.57], nor was there an overall effect of IDZ on this measure [IDZ: F (3,1231)= 0.48, p=0.69]. This measure also did not reveal an interaction of Pb treatment and IDZ dose [Pb*IDZ: F (3,1231)=0.93, p=0.42]. Mean overall percentage correct was 84.69$\pm$2.40 and 82.85$\pm$2.20 for the control and Pb-exposed groups respectively.

**Inaccurate Responses**

Inaccurate responses ("timely" responses to the wrong port) were calculated for each condition as follows: The number of trials on which an incorrect port was chosen after light cue presentation but within 10 seconds of trial onset was divided by the total number of response trials in that condition. A trend was seen for IDZ to alter inaccurate response rate, reflecting a progressive decline in this type of error with increasing dose. [IDZ: F (3,45)=2.15, p=0.10]. There was a significant interaction of IDZ dose and cue duration [F (3,45)= 2.66, p=0.05], reflecting the fact that IDZ reduced inaccurate responses in the short [p=0.01] but not the long [p=0.51] cue condition (see figure 2.3). Moreover, the effect of IDZ under the short cue condition tended to be limited to those conditions where distractors were presented, [IDZ*cue duration*DISTRACT: F (6,332)=1.86, p=0.08], specifically the short cue "-1" distractor condition [p=0.001]; and the short cue "-2" distractor condition [p=0.04]. There was no effect of IDZ on the short cue nondistraction trials [p=0.28].

The timing of distractor presentation in relationship to trial onset (ie. the delay and distraction condition combination) also significantly affected the ability of IDZ to increase accuracy [IDZ*DELAY*DISTRACT: F (6, 332) =2.10, p=0.05]. Under conditions of distraction, IDZ's enhancing effect was greatest when the distractors were presented one second after trial onset, specifically the 2 sec delay "-1" distractor

condition [p=0.01] and the 3 second delay "-2" second distractor condition [p= 0.02]. There was a tendency for IDZ to also alter inaccurate responses in the 2 sec delay "-2" distractor condition [p=0.09] but not in the 3 sec delay "-1" distractor condition [p=0.74]. (See figure 2.4) IDZ did not significantly alter inaccurate responses on nondistraction trials in either of the two delay conditions, the 2 sec delay [p=0.78] or the 3 sec delay [p=0.66].

Pb treatment did not alter inaccurate response rate [Pb: F (1,14)=0.47, p=0.50], nor was there a Pb treatment by IDZ dose interaction on this measure [Pb*IDZ: F (3, 45)=0.62, p=0.60]. In no cases where the IDZ effects were significant did the effect differ between Pb and control groups [all p values > 0.5]. (See Figure 2.5)

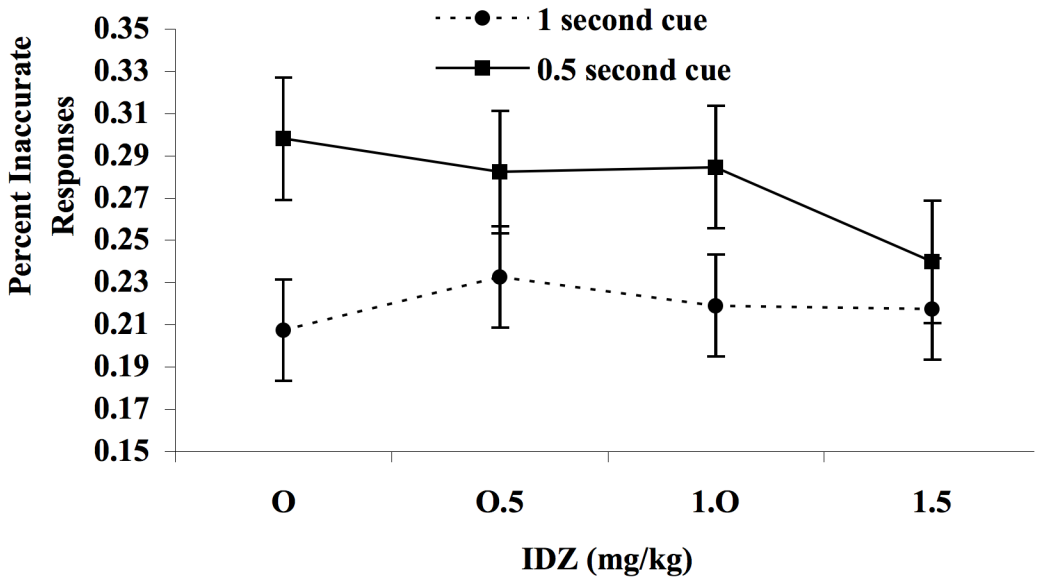


**Figure 2.3**. IDZ significantly reduced percentage inaccurate responses only under the short cue condition (IDZ * cue duration, p = 0.05) (0.15 and 0.35 on this scale correspond to 2.2 and 11.8 % respectively.)

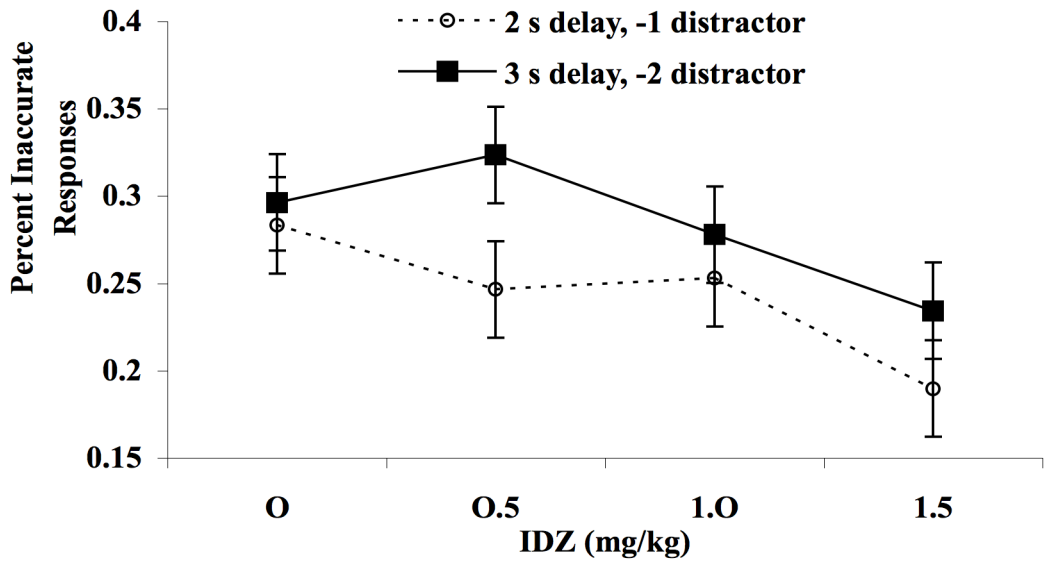


**Figure 2.4**. IDZ significantly reduced inaccurate responses only under conditions of distraction and only when distractors were presented one sec. after trial onset, the 2 sec. delay "-1" sec. distractor (p=0.01) and 3 sec. delay "-2" sec. distractor (p= 0.02) conditions. (0.18 and 0.32 on this scale correspond to 3.2 and 9.8 % respectively.)

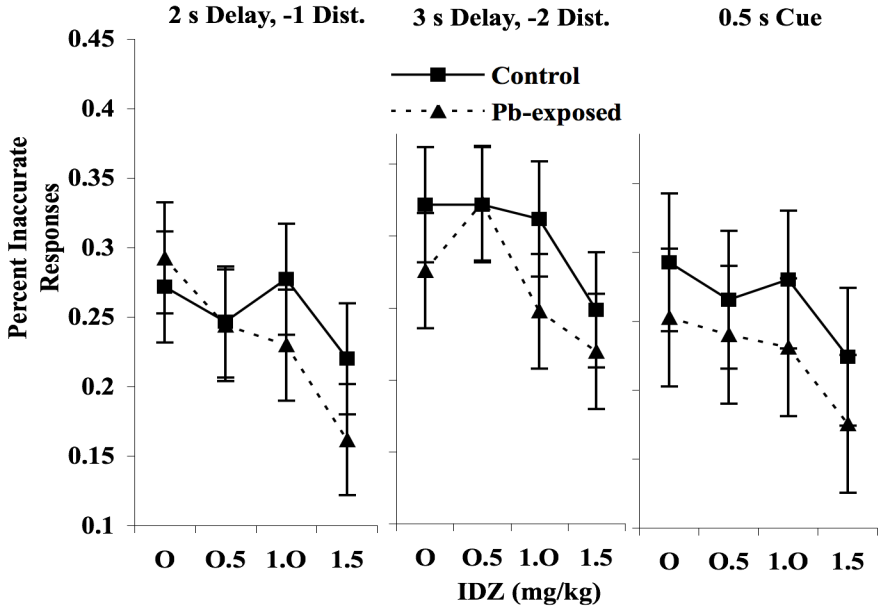


**Figure 2.5**. The effect of IDZ on percentage inaccurate responses did not differ between Pb and control subjects. Pictured here are the effects of IDZ on Pb and Control groups under the three conditions for which an overall IDZ effect was observed: the 2 second delay, -1 distractor condition (averaged across cue durations); the 3 second delay, -2 distractor condition (averaged across cue durations); and the 0.5 second cue duration condition (averaged across delay and distraction conditions).

**Premature Responses**

Premature responses were calculated as the percentage of trials in the session on which the subject made a response prior to light cue presentation. Such a response terminated the trial and was considered an index of impulsivity and/or response inhibition. In order to reduce the proportion of zeros on this measure in the dataset, it was necessary to average data across delay and timing of distractor. There was no effect of Pb treatment on premature responses [Pb: $F(1, 16) = 0.06$ $p=0.81$], nor was there a Pb by IDZ interaction on this measure [Pb*IDZ: $F(3, 48) = 0.55$ $p=0.64$]. IDZ overall did not alter premature responses [IDZ: $F(3, 48) = 0.83$ $p=0.48$]. Mean percent premature responses were $4.03 \pm 5.40$ and $3.84 \pm 5.36$ for the control and Pb-exposed groups respectively.

**Omission errors**

Errors of omission were calculated as the percentage of trials in the session on which the subject entered the testing alcove, but failed to respond within 10 seconds of trial onset. These errors were considered indicative of lapses of attention. There was no effect of Pb treatment on omission errors [Pb: $F(1, 14) = 2.87$, $p=0.11$], nor was there a Pb by IDZ interaction on this measure [Pb*IDZ: $F(3, 42) = 1.34$, $p=0.27$]. IDZ overall did not alter omission errors [IDZ: $F(3, 42) = 0.54$, $p= 0.65$]. Mean percent omission errors were $3.71 \pm 4.04$ and $6.88 \pm 6.48$ for the control and Pb-exposed groups respectively.

**Nontrials**

Pb treatment did not alter nontrials [Pb: $F(1, 16)=1.41$, $p=0.25$]. IDZ tended to decrease nontrials [IDZ: $F(3, 190)=2.26$, $p=0.08$] overall. The effect of IDZ on

nontrials did not differ statistically as a function of Pb treatment [Pb*IDZ: F (3, 190)=1.91, p=0.12].

## DISCUSSION

The results reported here have implications for both the nature and the neurochemical basis of lead-induced attentional deficits. In addition, these results have implications for the role of NE in the modulation of normal attentional function. All three issues will be discussed below.

### Clarifying the nature of lead-induced dysfunction

In study 1, both Pb treatment groups demonstrated a decrease in overall percentage correct on the distraction task. There was no effect of Pb treatment on premature responses, ruling out differences in inhibitory control as the basis of the Pb effect and indicating that this overall difference in percent correct was due to a Pb-induced decrease in accuracy on the task. Pb treatment also did not significantly alter nontrial rate, ruling out differences in nonspecific performance factors such as motivation as the basis of the Pb effect and suggesting that both levels of Pb-exposure produced attentional deficits. The observation that these deficits were not limited to the trials on which distractors were presented suggests that these impairments were not related to Pb-induced increases in distractibility. Rather, the increased error rate demonstrated by Pb-exposed subjects on both distraction and nondistraction trials was likely related to impairments of sustained or focused attention. This interpretation is consistent with prior findings from this same cohort of animals when tested on visual discrimination and vigilance tasks (Strupp, et al, 1993). Specifically, preliminary analyses suggested that Pb-exposure did not alter performance on a 3-choice visual

discrimination task in which the light cue was presented immediately at trial onset and remained illuminated until the rat responded. However, both levels of Pb-exposure caused a decrease in overall performance on a vigilance task with a variable delay preceding presentation of a brief light cue. This deficit appeared to be greater during conditions where delays preceded the cue relative to conditions when the cue was presented immediately at trial onset (Strupp, et al, 1993). The suggestion that Pb-exposure impairs focused attention is also consistent with the lasting vigilance deficits demonstrated in studies of early postnatal Pb-exposure from this laboratory (Morgan, et al, 2001, Stangle, et al, 2007). In addition, the Pb-induced attentional dysfunctions observed in the current study are also consistent with impairments reported on the CPT by some groups (Bellinger, et al, 1994; Harvey, et al, 1988; Walkowiak, et al, 1998) and with impairments on reaction time tasks that place increased demands on sustained attention in human children (Harvey, et al, 1988; Hunter, et al, 1985; Minder, et al, 1994; Needleman, et al, 1979; 1990; Raab, et al, 1990). Moreover, because the present study tested subjects in adulthood and the exposure regimen included a concurrent as well as an early (post-weaning) component, the observation that accuracy was impaired in Pb-exposed subjects on this attention task may also provide support for previous findings that link adult occupational Pb-exposure to deficits in attention (Pfister, et al, 1999; Stewart, et al, 1999; Tang, et al, 1995; Hogstedt, et al, 1983).

It is also possible that the reductions in accuracy demonstrated by the Pb-exposed subjects in the current study are related to alterations in the regulation of negative emotion or arousal. This interpretation is consistent with the finding that the Pb-induced deficits in sustained attention reported by Morgan, et al (2001) and Stangle et al (2007) were most pronounced on trials following an error. In these studies, performance for all animals was disrupted on trials immediately following an error

(relative to trials following a correct response). However, the performance- disrupting effects of prior errors were greater in Pb-exposed rats relative to controls. Although data on the effects of an error on prior trials were not collected in this study, it is possible that the impaired attention observed in the lead exposed rats in the present study were due, at least in part, to altered arousal and/or emotion.

One additional possibility is that Pb-exposure increased sensitivity to the performance-disrupting effects of the distractors on this task. The unpredictable presentation of potent distractors, such as those utilized in the present study, can result in a generalized disruption of performance, such that performance is disrupted on trials without distractors, as well as on trials with distractors (Arnsten and Contant, 1992). Thus, the observation in the current study that chronic post-weaning Pb-exposure decreased accuracy on both distraction and nondistraction trials may be due to a Pb-induced increase in sensitivity to the performance disrupting effects of the distractors, again suggesting impaired arousal regulation or emotional reactivity in the Pb-exposed subjects. One or both of these types of impaired emotional regulation may have contributed to the attentional impairments in the current study. Such increases in emotional regulation may be a contributing factor in the disruptive classroom behavior and increased delinquency rates associated with childhood Pb-exposure (Bellinger, et al, 1994; Davidson, et al, 2000; Dietrich, et al, 2001; Needleman, et al, 1996).

It is notable that premature response rate did not differ among Pb treatment groups in the present study, suggesting that Pb-exposure did not alter inhibitory control, at least within the context of this distraction task. This finding is consistent with other animal model studies of both early (Hastings, et al, 1979; Dietz, et al, 1978; Hopper, et al, 1986; Garavan, et al, 2000; Zenick, et al, 1979; Morgan, et al, 2001) and concurrent (Hilson & Strupp, 1997; Rice, 1988) Pb-exposure in which no effects on inhibitory control were demonstrated, and with some studies of the effects of human

childhood Pb-exposure on indices of inhibitory control (Baloh, et al, 1975; Bellinger, et al, 1994; Hansen, et al, 1989; Harvey, et al, 1988).

**NE involvement in Pb-induced attentional dysfunction**

A comparison of the effects of IDZ in the Pb and control animals sheds light on the hypothesis that NE alterations contribute to alterations in attention produced by Pb-exposure. IDZ decreased inaccurate responses, specifically in response to distractors, a pattern that is likely to reflect IDZ's effect on the coeruleocortical noradrenergic system that modulates selective attention and distractibility (Arnsten, et al, 1992; 1996; 1997; 2005; Aston-Jones, 1988; 1997; Bunsey and Strupp, 1995; Ramos and Arnsten, 2007; Robbins, et al, 1985; 1997; Sara, 1985; Sara, et al, 1988; discussed below). However, the two treatment groups did not differ in their response to IDZ on this measure, suggesting that alterations in the noradrenergic attentional system do not mediate the attentional dysfunction produced by Pb exposure.

As noted in the Introduction, the available literature concerning the effects of Pb-exposure on NE function does not present a clear picture, very likely due to the numerous differences in the developmental timing, duration and intensity of lead-exposure, as well as differences in the specific measure of NE function assessed. Consistent with our lack of differential drug effect in the Pb-exposed subjects, several groups have reported no effect of Pb-exposure on NE measures in brain regions relevant to attention: telencephalon (McIntosh, et al, 1989), midbrain (McIntosh, et al, 1989), forebrain (Grant, et al, 1976; Rossouw, et al, 1987; Silbergeld and Goldberg, 1975), cortex (Grant, et al, 1976; Meredith, et al, 1988; Rossouw, et al, 1987; Sobotka and Cook, 1974; Sobotka, et al, 1975; reviewed in Moore, et al, 1986), frontal cortex (Agrawal, et al, 1986), hippocampus (Collins, et al, 1984; Meredith, et al, 1988; reviewed in Moore, et al, 1986), and striatum (Agrawal, et al, 1986; Bailey and

Kitchen, 1986; Baksi and Hughes, 1982; Grant, et al, 1976; McIntosh, et al, 1988; Sobotka and Cook, 1974; Sobotka, et al, 1975). However, other groups have reported lead-induced increases in measures of NE in forebrain (Rossouw, et al, 1987; Silbergeld and Goldberg, 1975) and frontal, parietal, and occipital cortex (Freedman, et al, 1990); and both increases (Devi, et al, 2005; Dubas, et al, 1978; McIntosh, et al, 1989; Kumar and Desiraju, 1990; Prasanthi, et al, 2005; Rossouw, et al, 1987) and decreases (Collins, et al, 1984; Devi, et al, 2005; Dubas, et al, 1978; Jason and Kellogg, 1981; McIntosh, et al, 1989; Prasanthi, et al, 2005; Rajanna, et al, 1997; Rossouw, et al, 1987) in midbrain (Dubas, et al, 1978; McIntosh, et al, 1989), hippocampus ( Devi, et al, 2005; Collins, et al, 1984; Kumar and Desiraju, 1990; Prasanthi, et al, 2005), cortex (Devi, et al, 2005; Prasanthi, et al, 2005; Rajanna, et al, 1997; Rossouw, et al, 1987), and striatum (Dubas, et al, 1978; Jason and Kellogg, 1981; Kumar and Desiraju, 1990). In addition, in contrast to the lack of Pb-induced differences in IDZ sensitivity reported here, both increased (Carter and Leander, 1980; Rafales, et al, 1981) and decreased (Carter and Leander, 1980; Grover, et al, 1993; Rafales, et al, 1981; Reiter, et al, 1975) sensitivity to NE drugs has been reported. Differences in level, timing, and duration of lead-exposure likely account for these widely discrepant results; utilization of several different exposure regimens within a study have been reported to produce different effects on the same NE parameters. (Collins, et al, 1984; Devi, et al, 2005; McIntosh, et al, 1988; 1989; Meredith, et al, 1988; Prasanthi, et al, 2005; Rossouw, et al, 1987; reviewed in Moore, et al, 1986).

It may be noted that, although Pb-exposure resulted in decreased accuracy on the distraction task in study 1, no attentional effects of Pb alone were seen in the similarly exposed Pb group in study 2 despite testing on a distraction task that was almost identical to that used in the first study. The results of a subsequent study from this lab may shed light on why Pb-induced attentional impairments were not seen in

study 2. Specifically, the toxin-induced impairment revealed by these tasks waned over time, suggesting that exposed animals, despite their attentional impairment, were eventually able to achieve "normal" levels of performance after extensive training on the task (Morgan, et al, 1997). Although data on performance during initial training on the distraction task is unavailable for the cohort of animals used in study 2, it is likely that these animals exhibited the same Pb-induced deficits exhibited by the similarly exposed subjects in study 1, but that this impairment decreased with their increasing experience on the task. It is therefore not surprising that the Pb and control animals did not differ in performance in study 2, after weeks of extensive training on the distraction task. However, the toxin-induced deficits revealed in the Morgan, et al (1997) study were found to re-emerge in the exposed subjects when challenged with IDZ or the selective D1 dopamine agonist SKF81297, drugs specific to neurochemical systems altered by the exposure (Bayer, et al, 2000; 2002). Thus, if NE alterations caused the attentional impairments demonstrated by Pb-exposed subjects in study 1, these differences should have re-emerged in response to the IDZ challenge in study 2. Therefore, the lack of a differential effect of IDZ on accuracy in the Pb-exposed subjects in study 2 suggests that the basis for the accuracy impairments identified in study 1 is not likely to be a Pb-induced alteration in noradrenergic activity. It is particularly important to note that IDZ specifically altered accuracy in study two, the same measure on which Pb-induced deficits were revealed in study 1. Thus, the two studies used the same Pb-exposure regimen and the same outcome measure to both identify the Pb-induced cognitive impairments and to examine the role of NE in these impairments. This convergence allows inferences to be drawn about the neurochemical basis for these Pb-induced attentional deficits, without the problems associated with methodological differences discussed above (eg. differences in timing,

level, and duration of Pb exposure or differences in outcome measures between studies).

**NE modulation of normal attentional function**

The observed pattern of IDZ effects in study 2 is consistent with the posited role of NE activity in normal attentional function. Improved performance was seen only for a specific response type (accuracy) and only under the most attentionally demanding conditions (shorter cue duration, presentation of distractors). This pattern of effects rules out changes in nonspecific performance factors such as motivation or motor behavior as the basis of the drug effect. In this study, IDZ appeared both to enhance attentiveness to target stimuli by increasing accuracy under the short cue condition and to inhibit attention to distractors by enhancing performance under conditions of distraction. Because IDZ increases firing of the LC through its actions at presynaptic alpha-2 receptors, and thus increases coeruleocortical NE activity (Aghajanian, et al, 1977; Dennis, et al, 1987), this pattern of results provides support for the theory that LC activity and cortical NE release modulate selective attention (reviewed in Aston-Jones, 1988; 1997; Foote, et al, 1983; Sara, et al, 1988).

It is notable that IDZ's performance enhancing effect was most prevalent under conditions where distractors were presented 1 second after trial onset. Distractors administered at this time have proven to be most disruptive to rats in several studies in our laboratory (Gendle, et al, 2004). Electrophysiological data suggest a possible mechanism for this effect. Recordings from delay active neurons in the principal sulcus of monkeys during working memory tasks reveal that 80% of these neurons exhibited peak activity in the first few seconds of the delay period (Goldman-Rakic, 1987). Presentation of distractors or electrical stimulation of this region are most disruptive to performance when they occur at this time, suggesting that these early

seconds are the most crucial for processing. Although these data were recorded during performance of delayed response and delayed alternation tasks, there is evidence to suggest that this finding has implications for tasks of attention as well as working memory. Specifically, the anatomical convergence of delay-locked electrophysiological activity in the sulcus principalis in primate studies of both working memory (see Goldman-Rakic, 1987; 1990 for review) and attention (Lecas, 1995) suggests that these functions may be mediated by the same populations of neurons (Lecas, 1995). Thus, the conditions in our study in which distractors were presented 1 second into the trial were most distracting because these conditions occurred during the most physiologically crucial processing period. It is also relevant that the drug showed a tendency to enhance performance when the distractor was presented immediately at trial onset (the 2 second delay, -2 distractor condition), but had no effect on performance during either the nondistraction trials or the condition when the distractor was presented 2 seconds into the trial (the 3 second delay, -1 distractor condition). During this condition, the distractors were presented after the posited most physiologically crucial processing period, and were therefore presumably less distracting. The finding that the beneficial effect of IDZ was limited to the most distracting conditions is consistent with the hypothesized role of the coeruleocortical NE pathway in selective attention and distractibility (Arnsten, et al, 1992; 1996; 1997; Aston-Jones, 1988; 1997; Bunsey and Strupp, 1995; Ramos and Arnsten, 2007; Robbins, et al, 1985; 1997; Sara, 1985; Sara, et al, 1988), suggesting that the IDZ-induced decrease in inaccurate response rate is likely related to the drug- induced increase in LC activity.

**Summary**

In the present series of studies, Pb-exposed subjects demonstrated significant impairments in accuracy relative to controls on a distraction task. IDZ selectively increased accuracy in this task, consistent with the role of coeruleocortical NE in modulating selective attention and distractibility. However, the effect of IDZ on attention on this task did not differ between lead treatment groups. Taken together, these findings suggest that Pb-exposure causes specific deficits in attention and/or arousal regulation, but that Pb-induced alterations in alpha-2 mediated NE activity do not underlie this dysfunction.

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# CHAPTER 3

## PRENATAL COCAINE EXPOSURE ALTERS SENSITIVITY TO THE EFFECTS OF IDAZOXAN IN A DISTRACTION TASK*

## ABSTRACT

The present study was designed to test whether prenatal cocaine (COC) exposure alters sensitivity to the attentional effects of idazoxan (IDZ), an alpha-2 adrenergic antagonist that increases coeruleocortical NE activity (Aghajanian, et al, 1977; Dennis, et al, 1987). The task assessed subjects' ability to selectively attend to an unpredictable light cue and disregard olfactory distractors. IDZ increased commission errors specifically under conditions of distraction, an effect that was similar in the COC and control groups. In contrast, COC animals were significantly more sensitive than controls to the effects of IDZ on omission errors and nontrials. The pattern of effects suggests that the differential treatment response to IDZ on these latter measures resulted from an alteration in norepinephrine (NE)-modulated dopamine release in the COC animals, reflecting lasting changes in dopaminergic and/or noradrenergic systems as a result of the early cocaine exposure. Based on the behavioral measures that showed a differential response to IDZ in the COC animals, it seems likely that these changes may contribute to the alterations in sustained attention and arousal regulation that have been reported in both animals and humans exposed to cocaine *in utero.*

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* Bayer LE, Kakumanu S, Mactutus CF, Booze RM, Strupp BJ (2002) Prenatal cocaine exposure alters sensitivity to the effects of idazoxan in a distraction task. Behavioral Brain Research 133: 185-196.

# INTRODUCTION

Each year, thousands of infants are exposed to cocaine *in utero*. Although initial reports of devastating neurobehavioral consequences of prenatal cocaine exposure were not substantiated, more recent controlled studies provide evidence for subtle, but functionally significant, alterations in cognition and neurochemistry. Several studies suggest that prenatal cocaine exposure causes attentional impairments in children (Azuma and Chasnoff, 1993; Beckwith, et al, 1995; Karmel and Gardner, 1996; Leech, et al, 1999; Mayes, et al, 1998; Richardson, et al, 1996). Although many of these studies are difficult to interpret due to confounding factors such as polydrug abuse and poor postnatal care, a recent study has identified attentional impairments in cocaine-exposed children in a population where such confounding was reduced (Richardson, et al, 1996). In this study, prenatal cocaine exposure caused an increase in omission errors on the Continuous Performance Test (CPT), indicative of impairment in sustained or focused attention. Similarly, studies using a rat model of intravenous (IV) prenatal cocaine exposure revealed deficits in both focused and selective attention in adult animals, long after the period of exposure (Bushnell, et al, 2000; Garavan, et al, 2000; Morgan, et al, 1997; Strupp, et al, 1998). Similarly, behavioral changes indicative of altered selective attention have also been reported in studies using both rabbit (Romano and Harvey, 1996) and mouse (Gabriel and Taylor, 1998) models of prenatal cocaine exposure. Identification of the specific neural changes that underlie these attentional deficits is needed to develop drug therapies that may ameliorate the dysfunction. This goal motivated the present study.

Damage to the coeruleocortical noradrenergic system is a plausible cause of the attentional dysfunction produced by prenatal cocaine exposure. Considerable evidence implicates the coeruleocortical NE system in various aspects of normal

attentional processing, including selective and focused attention (see Arnsten, et al, 1996; Aston-Jones, et al, 1999; Aston-Jones, 1988; Bunsey and Strupp, 1995; Bushnell, 1998; Clark, et al, 1987a; Clark, et al, 1987b; Robbins, 1997; Sara, 1985; Sara, et al, 1988 for review), scanning attentiveness (Aston-Jones, et al, 1999) and alerting (Marrocco and Davidson, 1998). Moreover, previous work in our lab suggests that idazoxan, a drug that increases NE release from the LC (Aghajanian, et al, 1977; Dennis, et al, 1987), specifically alters selective attention (Bayer and Strupp, 1995; Bunsey and Strupp, 1995) in the same distraction task that revealed altered selective attention in rats exposed to cocaine prenatally. In addition, reduced tyrosine hydroxylase immunostaining in the LC (Booze, 2000, personal communication) has recently been reported for the same IV rat cocaine model that was used in the studies demonstrating lasting attentional changes as a result of prenatal cocaine exposure. These findings, taken together, suggest that the lasting attentional changes produced by prenatal cocaine exposure may be due to underlying changes in the coeruleocortical NE system.

Unfortunately, the literature concerning the effects of prenatal cocaine exposure on central noradrenergic function does not present a clear picture, for several reasons. First, previous studies of the effects of prenatal cocaine exposure on various functional measures of central NE activity have yielded results that are inconsistent with regard to both the presence and direction of prenatal cocaine's effects. Such findings have been reported for NE concentration (Vathy, et al, 1993), turnover (Seidler and Slotkin, 1992), receptor number (Booze, et al, 1994; Henderson, et al, 1991; Seidler and Slotkin, 1992; Wallace, et al, 1996), sensitivity to adrenergic drugs (Byrnes, et al, 1993; Cutler, et al, 1996; Kunko, et al, 1996; Meyer, et al, 1992; Peris, et al, 1992; Simansky and Kachelries, 1996), and tyrosine hydroxylase activity (Akbari, et al, 1992; Booze, et al, 1994; Meyer and Dupont, 1993). The lack of

consistency in results may reflect, at least in part, the fact that different routes of administration (intragastric (PO), subcutaneous (SC), or intravenous (IV)) and widely varying cocaine doses were used in these studies. An additional limitation of the existing work is that the significance of the reported neural changes for behavior or cognition has not been assessed in most studies. In summary, the available data on CNS noradrenergic changes in animal models of prenatal cocaine exposure do not provide conclusive evidence for or against the hypothesis that changes in this system underlie the reported attentional dysfunction.

The present study used a drug challenge design to test this hypothesis. Cocaine was administered intravenously (IV), the route that most closely mimics the rapidly peaking pharmacokinetic profile and peak arterial plasma levels that are seen following recreational cocaine inhalation or IV injection in humans (Booze, et al, 1997; Evans, et al, 1996). Adult offspring were administered a range of doses of idazoxan (IDZ), an alpha-2 adrenergic antagonist that increases firing of the LC (Aghajanian, et al, 1977; Dennis, et al, 1987), prior to testing on an attentional task. IDZ dose-effect curves for cocaine-exposed and control subjects were compared. This study was designed to provide information on the lasting effects of prenatal cocaine exposure on the noradrenergic system, as well as information about the functional consequences of these putative changes.

# MATERIALS AND METHODS

## Subjects

Nulliparous Long Evans rats (Harlan Sprague Dawley, Indianapolis, IN), approximately 11 weeks old, were housed in AAALAC-accredited animal facilities maintained at 21 ± 2°C, 50% ± 10% relative humidity with a 12 hour light: 12 hour dark cycle. Food (Pro-Lab Rat, Mouse, Hamster Chow No. 3000) and water were available ad libitum. Subjects were randomly assigned to either receive a surgical catheterization procedure (described below) or to serve as unoperated surrogate controls. Following surgery, subjects in the catheterization group were randomly assigned to receive either cocaine (COC) or saline (SAL; vehicle control). This research protocol was approved by the animal care review boards of the University of Kentucky and Cornell University.

## Catheterization

Subjects in the catheterization group were surgically implanted with a sterile IV catheter (described in detail in Mactutus, et al, 1994). The distal end of the catheter was inserted into the jugular vein and threaded centrally. The proximal end of the catheter, including the injection cap, was left as a small SC pouch on the dorsal surface of the animal through which chronic IV injections were made. Catheter patency was maintained by daily flushing with 0.2 ml of heparinized saline (2.5%).

## Mating

After recovery from surgery (4-8 days) the females were group-housed (n=3) with a male rat. Daily vaginal lavage of each female was performed to keep track of

estrus cycles and to assist in defining conception. Conception (gestational day 0, or GD0) was confirmed by a sperm-positive lavage.

**IV Drug Injection**

IV drug administration procedures were conducted as described in Mactutus, et al. (Mactutus, et al, 1994). Briefly, all dams received daily IV saline injections (1ml/kg) from conception until gestational day (GD) 7. Dams in the saline subgroup (n=8) continued to receive IV saline injections once per day from GD8-14 and twice daily from GD15-21. Dams in the cocaine subgroup (n=8) received cocaine hydrochloride (3.0mg/ 1ml /kg, IV; Research Triangle Institute, NC) once per day from GD8-14 and twice daily from GD15-21. The drug was dissolved immediately prior to injection.

This regimen (route, dose, and rate) produces 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 (e.g., Mactutus, 1999; Mactutus, et al, 1994). Furthermore, an IV injection procedure model by Robinson, et al demonstrated that IV administration of cocaine does not reduce food intake of dams even when utilizing a cocaine dose as high as 6 mg/kg (Robinson, et al, 1994).

**Offspring treatment**

Within 24 hours of birth, pups were weighed, culled to 4 males and 4 females per litter (when possible), and fostered to a surrogate dam that had given birth within the preceding 24 hours. Fostering was conducted for both cocaine (COC) and saline-exposed (SAL) offspring in order to remove potential effects of gestational cocaine treatment on maternal behavior as a source of offspring differences. On postnatal day 21 (PND21), pups were weaned and earpunched for identification. Within 10 days of

weaning, one male and one female offspring from each litter were transported under environmentally-controlled conditions from Lexington, KY to Ithaca, NY (Cornell University). Upon arrival, subjects were housed in same sex pairs on a reversed dark/light cycle and allowed to acclimate to the new environment for 2-3 weeks. Training/ behavioral testing began for all animals on PND48. The animals were approximately 160 days of age at the beginning of the present study. The subjects tested in the current study consisted of 14 cocaine-exposed (7 male, 7 female) and 15 saline-exposed (7 male, 8 female) offspring.

**Behavioral testing**

***Apparatus***

Testing was conducted in 12 automated Plexiglas chambers enclosed in sound-attenuating wooden boxes, each operated by an IBM PC XT computer with an 8088 processor. Each chamber consisted of a square waiting area (26.5 cm by 25 cm by 30 cm), adjacent to a testing alcove containing three funnel-shaped ports. An LED was mounted above each port. A thin metal door, raised at the initiation of each trial, separated these two compartments. The left and right ports were at an approximate 45° angle relative to the center port and the distance between the left and right ports was 8 cm. Each port was connected by tubing to three bottles containing liquid odorants, attached to a board placed outside of the box. Solenoid valves controlled the presentation of compressed air, pumped through a specific odorant and through a specific port. The airflow rate was 1.0 L/min and the air in the chamber was cleared via small centrifugal fans mounted on the outside of the chambers, at a rate of four complete exchanges per minute. A set of infrared phototransistors and a light source monitored the entrance to the alcove and each port. A nose-poke of ≥ 1 sec into one of the three ports constituted a response. Correct responses were

reinforced with a 45 mg food pellet (Noyes, Lancaster, NH) delivered into the alcove from a pellet dispenser (Lafayette Instrument Co., Lafayette, IN).

***Prior behavioral testing***

Subjects had been trained on several attentional tasks as part of a previous experiment (see Morgan, et al, 1997; 2002): 1) a three-choice brightness discrimination task in which the correct port was designated by the illumination of an LED mounted over that port; 2) a series of vigilance tasks in which the interval between trial onset and cue illumination varied, as did the duration of the light cue; and 3) a variant of the distraction task described below.

***Distraction Task***

Prior to initiation of the present IDZ challenge study, subjects received 12 days of testing on the distraction task to achieve a stable baseline performance level for each rat. Testing was conducted by personnel who were blind to prenatal treatment condition. The opening of the alcove door signaled the onset of each trial. After the subject entered the testing alcove, one of the three LEDs was illuminated. Several parameters were randomly varied across the trials in the session: 1) pre-stimulus delay (2 or 3 seconds after alcove entry); 2) stimulus duration (300 or 700 msec); and 3) presentation and timing of distractors: On 1/3 of the trials in each session, one of nine different odors was delivered through one of the three ports, either one second ("-1 distractor condition") or two seconds ("-2 distractor condition") prior to visual cue onset. No odors were presented on the remaining two thirds of the daily trials (nondistraction trials). The delay, duration, cue location, and distraction condition for each trial were selected pseudorandomly, balanced for each session. A 1-sec nose-poke into the port under the illuminated light resulted in

delivery of a 45 mg pellet (Noyes, Lancaster, NH) and ended the trial, signaled by the closing of the alcove door. A nose-poke of ≥ 1 second into any port prior to the light cue onset, into an incorrect port following the cue, or a failure to respond within 15 seconds of light cue offset ended the trial with no reinforcement. If the animal failed to enter the alcove within 30 seconds, the alcove door closed and a nontrial was recorded. An intertrial interval of 5 seconds was imposed between successive trials. A variant of this task had previously revealed enduring effects of prenatal cocaine exposure in this cohort of animals (see Morgan, et al, 1997; 2002).

**Idazoxan challenge study**

IDZ (0.0, 0.5, 1.0, 1.5 mg/ml/kg; Research Biochemicals, Inc, Natick MA) was dissolved in sterile MilliQ water (Millipore, Bedford, MA) immediately prior to each daily session and was administered subcutaneously 15 minutes prior to testing. Each subject received each of the 4 doses 5 times, with dose order determined by a Latin Square design. 48 hrs were imposed between successive injection days to allow clearance. Daily sessions consisted of 100 trials or 60 minutes, whichever came first.

Subjects were maintained on a restricted feeding schedule to maintain adequate motivation. The daily food ration for each rat had been determined in a previous study (Garavan, et al, 2000). Our goal had been to identify the minimal food restriction that would produce a low number of non-trials and a high rate of response trials. Subjects were weighed daily to ensure that body weight was stable or slowly increasing. The COC and control groups did not differ in the daily food allotment (Garavan, et al, 2000).

**Response types**

Percentage correct and nontrials were calculated for each session. In addition, the following error types were calculated (defined in results section): Premature responses, inhibition errors (premature responses specifically to the odor distractor), inaccurate responses, and omission errors.

**Statistical Analysis**

Data were analyzed using the SAS PROC MIXED mixed models procedure for analyzing linear models that contain both fixed and random factors. The MIXED procedure uses maximum likelihood estimation to account appropriately for the correlated structure within repeated measures designs. The factors litter (within treatment) and sex (within litter and treatment) were used to create three levels of variability for this multi-tiered model (within-litter, within-animal, and residual). However, in some cases, either the within-litter or the within-animal variance component was estimated to be zero, indicating that after adjusting for the systematic differences of the fixed effects, there was a redundancy in the sources of variation. In those cases, the within-animal term was included as the sole variance component. The Satterthwaite correction was used in all cases to determine the correct denominator degrees of freedom.

To achieve a more normal distribution, percentage correct and percentage error-type data were calculated across all five sessions of a given dose for each rat and then transformed using an arcsin square-root transformation prior to analysis. Percentage correct was analyzed as a function of prenatal treatment (termed COC), sex, IDZ dose (termed IDZ), pre-stimulus delay, light cue duration, distraction condition, and the relevant interactions of these variables. Similar analyses were conducted on the various types of errors: percentage accuracy errors, percentage

premature responses, percentage inhibition errors, and percentage omission errors. For the analysis of omission error rate, the outcome of the previous trial (correct or incorrect; a variable referred to as "PREV") was also included in the model because previous data suggested that omissions errors most often occur on trials following errors. In addition, nontrials were analyzed as a function of prenatal cocaine treatment, sex, IDZ dose, and the relevant interactions of these variables. In all of these analyses, the critical test of differential sensitivity of the treatment groups to IDZ was an interaction of prenatal treatment and IDZ.

## RESULTS

### Dam and Pup Data

Prenatal cocaine treatment did not alter maternal weight gain during gestation (measured on GD 0, 8, 15, and 21), as evidenced by the absence of a main effect for prenatal treatment [($F(1,18) = 1.01$, $p \leq 0.33$], and the absence of an interaction of day and COC [$F(2,36) < 1$]. Similarly, there was no effect of cocaine treatment on average maternal gestation weight gain ($F(1,18) = 1.15$, $p \leq 0.30$), gestation length ($F(1,18) = 1.00$, $p \leq 0.33$), litter sex ratio ($F(1,18) < 1$), pup birthweight on PND1 [($F(1,18)<1$], or litter size [$F(1,18) < 1$]. Finally, there was no effect of cocaine treatment on offspring growth (body weight) or survival [all F's < 1].

### Percentage Correct

Percentage correct was calculated as the percentage of response trials in the session in which a correct response was made. Response trials were defined as trials on which the animal entered the testing alcove. Percentage correct was lowest under conditions of greatest attentional demand: trials with the shorter cue length (300 ms)

[Duration: F(1, 1329)=250.37, p≤ 0.0001], trials on which a distractor was presented [Distraction condition: F(2, 1329)=13.78, p≤ 0.0001], and trials with a longer pre-stimulus delay [Delay: F(1, 1329)=39.14, p≤ 0.0001]. Prenatal treatment (COC) did not significantly alter percentage correct [F(1,13)<1], nor was there a COC by IDZ interaction for this measure [F(3,1329)<1]. There was a significant main effect of IDZ [F (3, 1329)=5.46, p≤0.001], as well as an interaction of IDZ and Distraction condition [F(6, 1329)=2.84 p≤0.01]. As seen in figure 3.1, this interaction reflected the fact that the impairing effect of the drug was limited to the –2 distractor condition, which refers to trials in which the distractor was presented 2 seconds prior to onset of the light cue [IDZ effect in the –2 distractor condition: F(3,1329)=7.69 p≤0.0001].

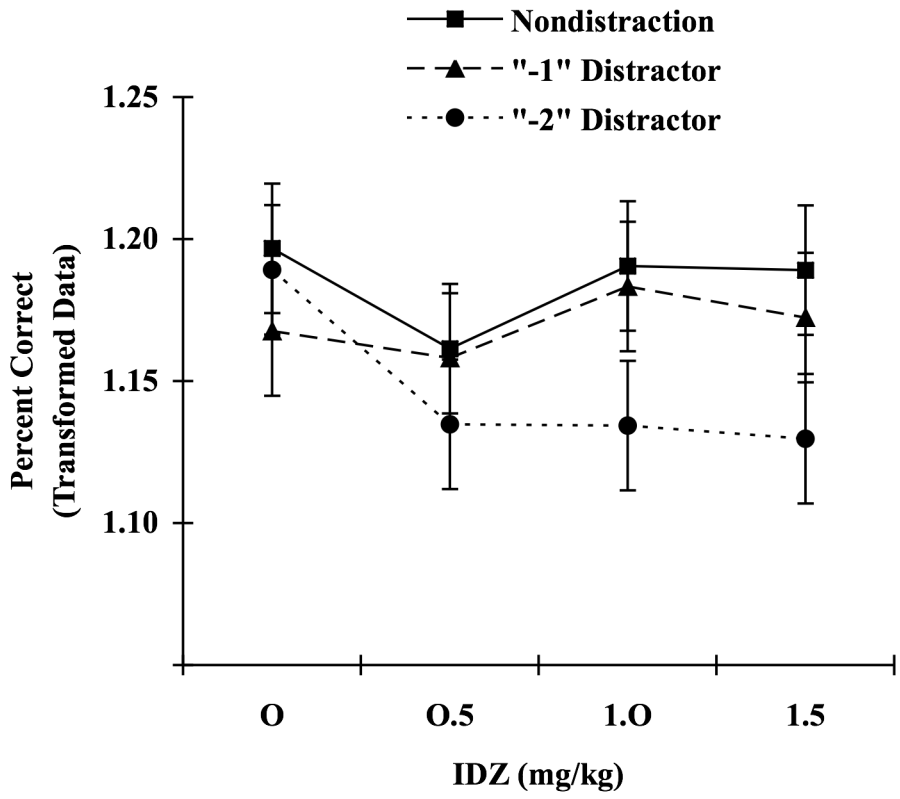


**Figure 3.1.** IDZ significantly decreased percent correct specifically when the distractor was presented 2 s before cue onset (the "-2" distractor condition) (p≤0.0001). The drug did not affect performance on trials without distractors or when the distractor was presented 1 s before cue onset. Points represent mean ± SEM.

**Inaccurate Responses** ("timely" responses to the wrong response port)

An inaccurate response was defined as a response made to an incorrect port after light cue presentation but within 10 seconds of trial onset. For each condition, the percentage of inaccurate responses was calculated by dividing the number of inaccurate responses by the total number of response trials in that condition. The percentage inaccurate responses was highest under conditions of greatest attentional demand; i.e., trials on which the shorter cue was presented [DURATION: $F(1, 1330)=64.43$, $p\leq0.0001$] and trials with distractors [Distraction condition: $F(2, 1330)=3.01$, $p\leq0.05$]. The percentage of inaccurate responses was not significantly affected by Prenatal Treatment [$F(1,16) < 1$]. There was neither a significant COC by IDZ interaction [$F(1,1330)< 1$], nor a significant COC by IDZ by DISTRACTION interaction [$F(2,1330) < 1$]. The main effect of IDZ was not significant [$F(1, 1330) < 1$], but there was a significant interaction of IDZ and DISTRACTION [$F(2,1330)=4.43$, $p\leq0.01$]. As may be seen in Fig. 3.2a, this interaction reflected the fact that IDZ only affected the rate of inaccurate responses in the –2 distractor condition [IDZ effect in the –2 distractor condition: $p\leq0.03$]. A linearly increasing effect of IDZ dose was seen in this condition. IDZ had no effect on inaccurate responses in either the nondistraction [IDZ effect in the nondistraction condition: $p\leq 0.62$] or the –1 distractor [IDZ effect in the –1 distractor condition: $p\leq0.15$] conditions. The main effect of Sex was not significant [$F(1,17) < 1$], but the effect of IDZ differed significantly between the sexes [Sex*IDZ: $F(1,1330)=10.87$, $p\leq0.001$]. As seen in Fig. 3.2b, males were significantly impaired by IDZ [$F(1,638)=6.44$, $p\leq0.01$], whereas females were significantly improved by IDZ [$F(1,685)=4.69$, $p\leq0.03$] The interaction of Sex, IDZ dose, and distraction condition was not significant.

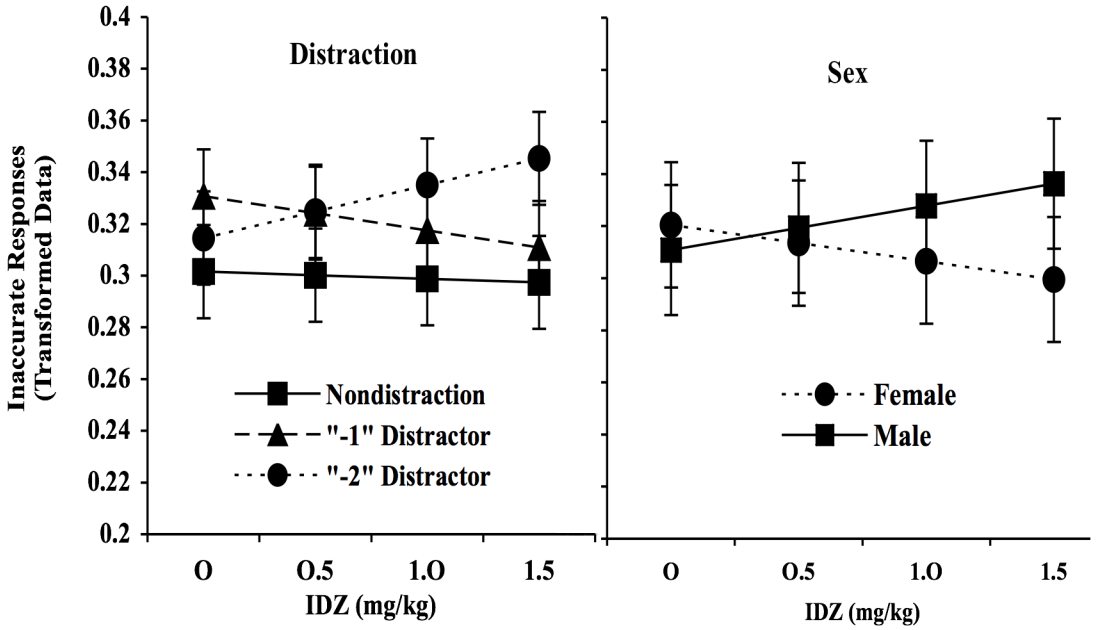


**Figure 3.2**. Effect of IDZ on the percentage of total response trials in which an inaccurate response was made. Points represent mean ± SEM. (0.29 and 0.35 on this scale correspond to 8.2 and 11.8 % respectively). Left panel: IDZ significantly increased the percentage inaccurate responses specifically in the "-2" distractor condition (IDZ * distraction, $p \leq 0.01$). The drug did not affect the rate of inaccurate responses on trials without distractors or when the distractor was presented 1 s before cue onset. Right panel: The effect of IDZ on percentage inaccurate responses differed between male and female rats (Sex * IDZ, $p \leq 0.001$); males were significantly impaired by IDZ [$p \leq 0.01$], whereas females were significantly improved by IDZ [$p \leq 0.03$]. See text for discussion

**Premature Responses**

Premature response rate was calculated as the percentage of trials in the session on which the subject made a response prior to light cue presentation. This type of response terminated the trial and was considered an index of impulsivity and/or deficient response inhibition. In order to reduce the proportion of zeros in this measure in the dataset, it was necessary to average across Cue Duration, Pre-stimulus

Delay, and Distraction Condition. The analyses revealed that premature response rate was not significantly altered by prenatal cocaine exposure [$F(1,13) < 1$]. IDZ also did not affect this measure [$F(3,75)=1.11$, $p \leq 0.35$], nor was there a COC by IDZ interaction [$F(3,75) < 1$].

**Inhibition errors**

An inhibition error was defined as a premature response made in response to the olfactory distracter. This measure was calculated as the total number of premature responses made to any port, prior to the presentation of the light cue but after the presentation of the olfactory distracter, divided by the total number of distraction trials in the session. In order to reduce the proportion of zeros on this measure in the dataset, it was necessary to average data across Cue Duration, Pre-stimulus Delay, and timing of the distracter. Prenatal cocaine treatment did not alter the percentage of inhibition errors [$F(1,13) < 1$], nor was there a COC by IDZ interaction [IDZ: $F(3,75) < 1$]. As depicted in Fig. 3.3, IDZ significantly altered the inhibition error rate overall ($F(3,75)=2.90$, $p \leq 0.04$), reflecting the fact that the lowest dose of IDZ increased this error type. (IDZ: vehicle vs low dose: $F(1,75)=4.79$, $p \leq 0.03$). The effect of IDZ on inhibition errors tended to differ between the two sexes [sex*IDZ: $F(3,75)=2.50$, $p \leq 0.07$]. Although the two sexes did not differ at any dose, the peak of the inverted-U shaped IDZ dose-effect curve appeared shifted to the right in females relative to males (see Fig. 3.4), suggesting that the male rats were more sensitive to the drug effect on this measure than females. This inference is based on the pattern of dose effects in each sex. Specifically, within the males, the overall drug effect [$F(3,75)=2.45$, $p \leq 0.07$] reflected the significant increase in inhibition errors seen at the lowest dose relative to saline [$p \leq 0.01$]. In contrast, within the females, the drug also affected inhibition errors [$F(3,75)=2.96$, $p \leq 0.04$], but the only dose that tended to differ from

the vehicle condition was the medium dose [p≤0.06]; no effect was seen at the lower dose [p≤0.57], in contrast to the males. The highest dose of IDZ did not increase inhibition error rate in either sex.

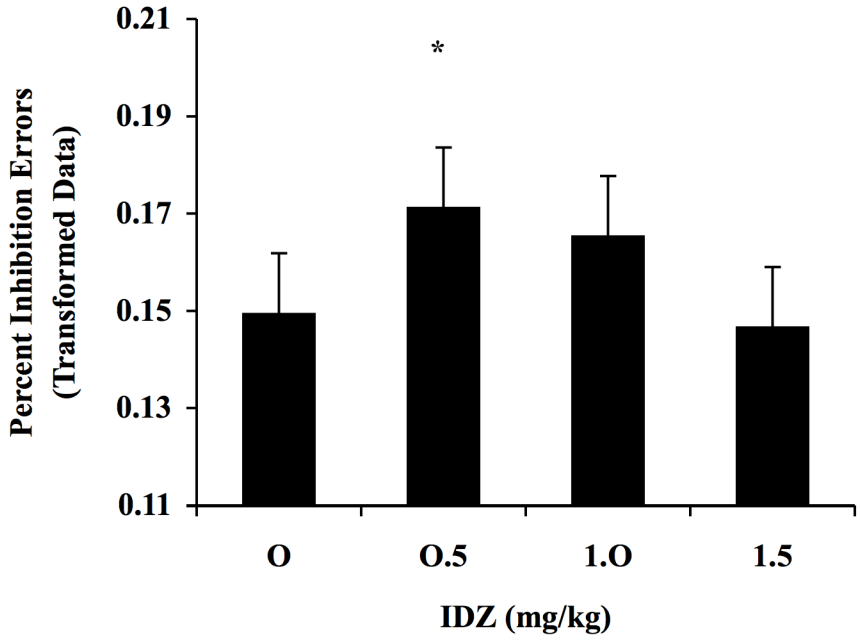


**Figure 3.3.** The lowest dose of IDZ significantly increased inhibition error rate relative to the vehicle condition (p≤0.04). Inhibition errors refer to premature responses made in response to the olfactory distractors. Bars represent mean ± SEM. (0.13 and 0.19 on this scale correspond to 1.7 and 3.6 % respectively.) The * denotes significantly different from the vehicle injection.

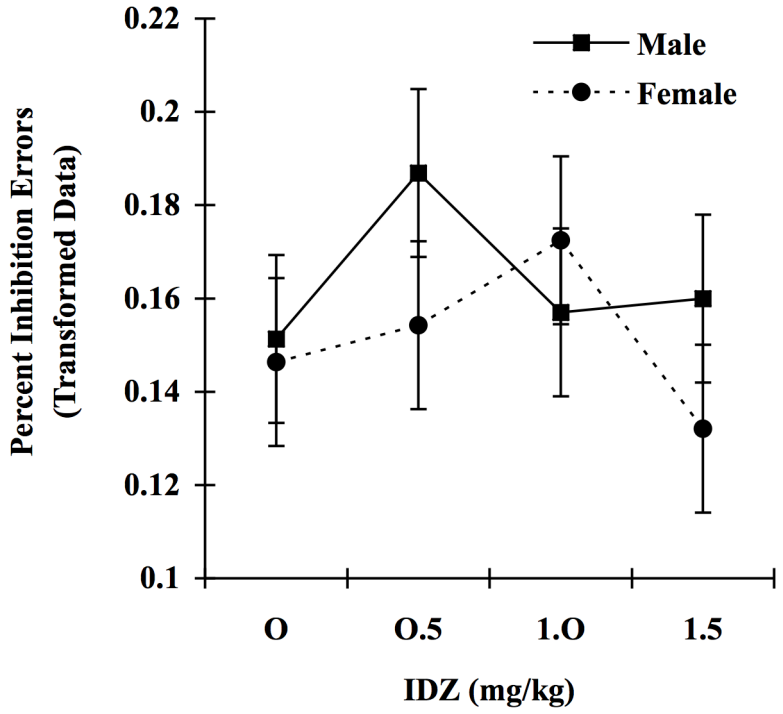


**Figure 3.4.** Male rats tended to be more sensitive than females to IDZ's effect on inhibition errors (sex * IDZ, $p \leq 0.07$). The pattern of effects suggests that the peak in the dose-effect curve is shifted to the right in the males relative to the females. Points represent mean $\pm$ SEM. See text for discussion. (0.13 and 0.19 on this scale correspond to 1.7 and 3.6 % respectively.)

**Omission errors**

Errors of omission were calculated as the percentage of trials in the session on which the subject entered the testing alcove but failed to respond within 10 seconds of trial onset. In order to reduce the proportion of zeros in this measure in the dataset, it was necessary to average across the Delay and Distraction variables. The analysis revealed that, overall, subjects made more omission errors when presented with the shorter light cue relative to the longer cue [$F(1, 391)=8.62$, $p \leq 0.004$]. There was a significant main effect of IDZ [$F(3,391)=4.88$, $p \leq 0.002$], reflecting the fact that each

of the three doses increased omission errors relative to the vehicle (p's for the low, medium, and high doses were 0.0002, 0.04, and 0.02, respectively.) The effect of prenatal treatment was not significant [$F(1,29)<1$], but significant interactions were seen for the interaction of COC and IDZ [$F(3, 391)=2.93$, $p\leq0.03$; see figure 3.5], and the four-way interaction of COC, IDZ, SEX, and outcome of the previous trial [$F(3,391)=3.47$, $p\leq0.02$]. To further elucidate the basis of both interactions, subsequent contrasts were conducted. These contrasts revealed that a significant interaction of COC by IDZ (the test of a treatment difference in IDZ sensitivity) was seen only in female subjects, on trials following errors [COC*IDZ in females after errors: $F(3, 90)= 7.27$, $p\leq0.0002$]. There was no COC by IDZ interaction for females on trials that followed a correct trial [$F(3,91.1)<1$] or in males after either correct [$F(3, 83.9)=1.36$, $p\leq0.26$] or incorrect trials [$F(3, 84.8)<1$]. As depicted in figure 3.6a, the significant treatment difference in IDZ response in the females, on trials that followed an error, reflects an apparent shift to the left of the inverted-U shaped IDZ dose-response curve in the COC group relative to the SAL group. For COC females, the overall effect of IDZ on trials following errors [$p\leq0.0004$] reflected the significant increase in omission error rate produced by the lowest dose (relative to vehicle; $p\leq0.003$]. For SAL females, IDZ also increased omission errors on trials following errors [$p\leq0.02$], but this effect was limited to the highest dose of IDZ [high dose vs vehicle for SAL females after errors: $p\leq0.04$].

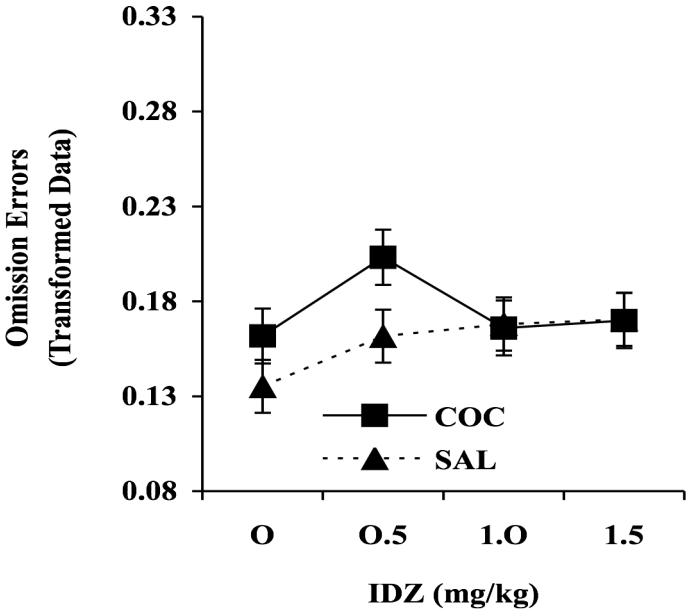


**Figure 3.5.** COC rats were more sensitive than controls to IDZ's effect on omission error rate (COC*IDZ, $p \leq 0.05$). Points represent mean ± SEM. (0.13 and 0.20 on this scale correspond to 1.7 and 3.9 % respectively)

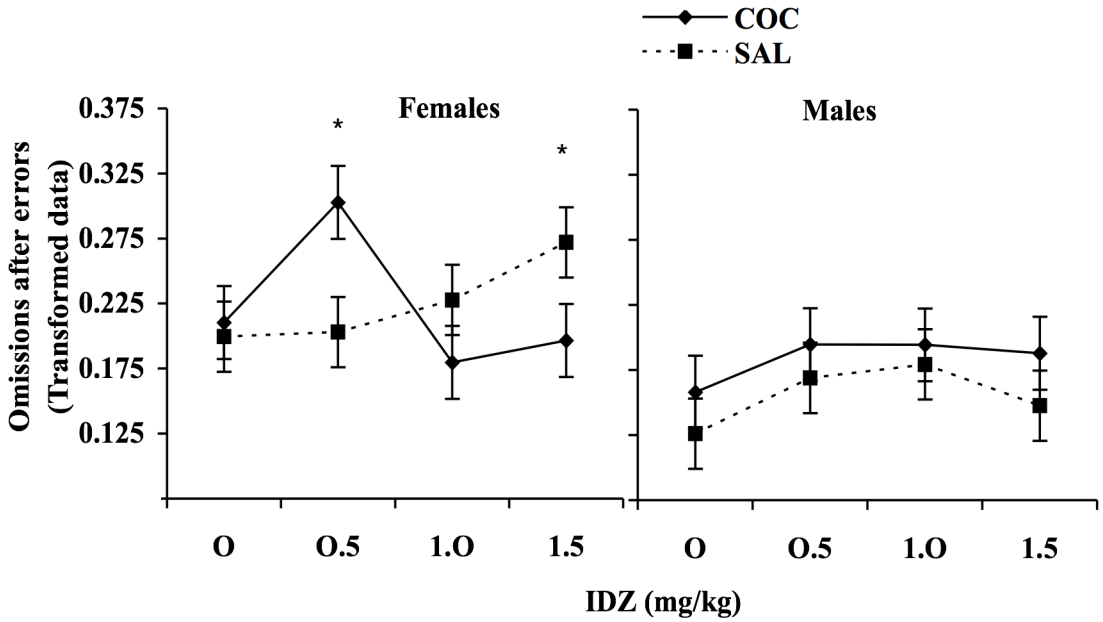


**Figure 3.6.** The increased sensitivity of COC rats to IDZ's effect on omission errors was limited to the females, on trials that occurred after an error. Points represent mean ± SEM. Left panel: On trials that followed an error, COC females were impaired by the lowest dose of IDZ ($p \leq 0.002$), whereas for SAL females, only the highest IDZ dose significantly increased omission errors relative to vehicle ($p \leq 0.04$). Right panel: There was no effect of IDZ in male rats of either treatment group. (0.13 and 0.35 on this scale correspond to 1.7 and 11.8% respectively.)

**Nontrials**

Nontrial rate was significantly affected by prenatal cocaine treatment [$F(1,25)=4.70$, $p\leq0.04$], and Sex [$(F(1,25)=8.50$, $p\leq0.007)$], and a borderline overall effect was seen for IDZ [$F(3,75)=2.44$, $p\leq0.07$]. In addition, the two prenatal treatment groups responded differently to IDZ, as demonstrated by a significant interaction of COC and IDZ [$F(3,75)=2.95$, $p\leq0.04$]. As depicted in figure 3.7, this significant interaction reflected the fact that IDZ significantly increased nontrials in the COC subjects [$p\leq0.003$], but not in the SAL subjects [$p\leq0.83$]. A borderline three-way interaction of COC, SEX, and IDZ [$F(3,75)=2.30$, $p\leq0.08$] was also observed, suggesting that the two sexes might be differentially vulnerable to prenatal cocaine exposure's effect on sensitivity to IDZ. This suggestion prompted an assessment of the IDZ effect in each of the four subgroups defined by sex and prenatal treatment (COC female, COC male, SAL female, and SAL male), to avoid making erroneous conclusions about the generality of the effects across gender. Because these four contrasts were performed despite the fact that the interaction term did not achieve classical levels of significance ($p \leq .05$), an alpha of 0.0125 was used to correct for multiple testing. As depicted in figure 3.8, the borderline three-way interaction of COC, SEX, and IDZ reflected the fact that IDZ increased nontrials only in the COC females [$F(3,75)=7.53$, $p\leq0.0002$], an effect that was driven by the lowest dose. IDZ did not increase nontrials in the COC males [$F(3,75)=1.27$, $p\leq0.29$], nor in the SAL animals of either gender [SAL females: [$F(3,75) < 1$; SAL males: $F(3,75) < 1$].

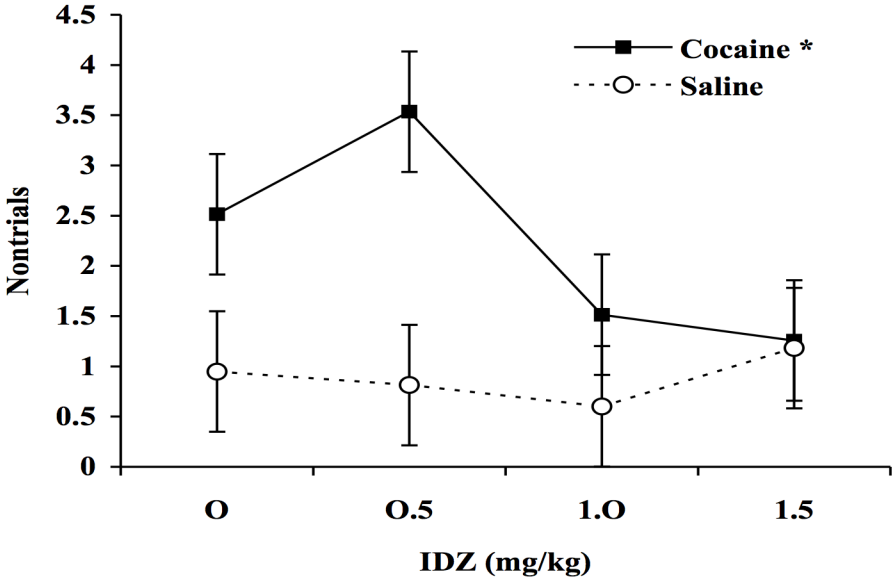


**Figure 3.7.** Cocaine exposure increased sensitivity to the effect of IDZ on nontrials (COC*IDZ, $p \leq 0.04$) IDZ significantly altered nontrials in the COC subjects [$p \leq 0.003$], but not the controls [$p \leq 0.83$]. Points represent mean ± SEM.

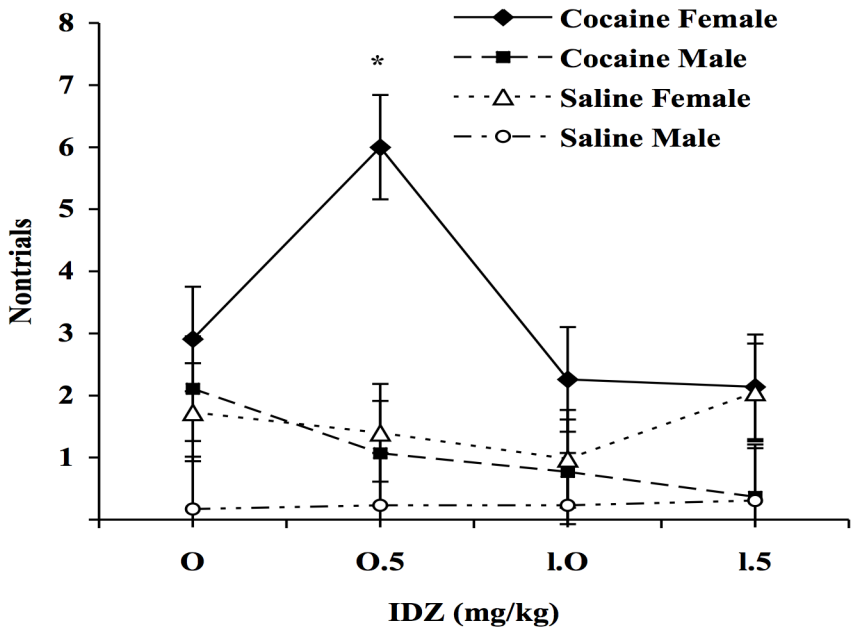


**Figure 3.8.** The treatment group difference in sensitivity to the effect of IDZ on nontrials appeared to be driven by differences in the females. IDZ increased nontrials only in the COC females [$p \leq 0.0002$], not in the SAL females, or either group of male subjects. Points represent mean ± SEM.

## DISCUSSION

A comparison of the effects of IDZ in the COC and control animals sheds light on the hypothesis that catecholaminergic alterations contribute to the lasting cognitive and affective alterations produced by prenatal cocaine exposure. One effect of IDZ was to increase the rate of commission errors (inaccurate responses, inhibition errors) specifically in response to distractors, a pattern that likely reflects the drug's effect on the coeruleocortical noradrenergic system (Arnsten, 1997; Arnsten and Contant, 1992; Arnsten, et al, 1996; Aston-Jones, et al, 1997; Aston-Jones, 1988; Bunsey and Strupp, 1995; Robbins, 1997; Robbins, et al, 1985; Sara, 1985; Sara, et al, 1988; discussed below). Whereas the effects of IDZ on commission errors were similar in the COC and SAL animals, the analyses of two other measures (omission errors and nontrials) revealed a significant interaction of IDZ and Prenatal Treatment, the critical finding considered indicative of an enduring neurochemical effect of prenatal cocaine exposure. Specifically, as discussed below, the pattern of IDZ effects seen for these latter two variables suggests that the COC animals may be ***more*** sensitive than controls to the drug effects on these measures.

The basis of the differential response of the COC and SAL groups to IDZ on these two measures (omission errors and nontrials) is somewhat unclear. In the case of omission error rate, this issue is complicated in part by the fact that this measure appears to reflect multiple influences. Based on numerous studies in our lab using this task or very similar ones, omission error rate reflects, at least in part, sustained attention. This inference is based on the findings that omission error rate increases as the cue duration decreases (Bayer, et al, 2000; Bayer, et al, 1994; Bayer and Strupp,

1995) and as the pre-stimulus delay increases (Bunsey and Strupp, 1995). Thus, a treatment difference in systems that modulate sustained attention is one possible candidate for the differential response of the COC and SAL groups to the IDZ effect on omission errors. Another candidate is suggested by the present finding that omission error rate was significantly greater on trials following an incorrect response than on trials following a correct response. Thus, the increased sensitivity of COC animals to IDZ on this measure may partially reflect prenatal cocaine-induced differences in emotional reactivity, specifically reactivity to errors. This hypothesis is consistent with previous reports that prenatal cocaine exposure alters reactivity to errors in a very similar visual attention task (Morgan, et al, 1997; 2002), and in other situations, alters emotional reactivity, behavioral responses to stress, and arousal regulation (Bilitzke and Church, 1992; Church and Tilak, 1996; Dow-Edwards, et al, 1999; Goldstein, et al, 1993; Johns and Noonan, 1995; Mayes, et al, 1998; Molina, et al, 1994; Murphy, et al, 1995; Wood, et al, 1994; Wood, et al, 1993; Wood, et al, 1995). A final possibility, although less likely, is that the increased sensitivity of the COC animals to the effects of IDZ on nontrials and omission errors reflects prenatal cocaine-induced differences in neural systems underlying motivation and/or response proclivity. Increased omission errors and nontrials have been reported for manipulations that decrease response proclivity such as striatal lesions (Cole and Robbins, 1989), and those that decrease motivation such as increased food intake (Barnabe, unpublished data). However, under the influence of IDZ in this study, subjects did not show a reduction in either premature response rate or in "pre-pokes" (nosepoke responses < 1 sec in duration that did not constitute a choice--data not presented), arguing against a decrease in response proclivity under the drug.

The finding that the statistical interaction of IDZ and Prenatal Treatment was not seen for measures that tap selective attention, but was seen for omission errors and

nontrials, suggests that these latter effects may reflect different processes and underlying neural systems than the selective attention effects. One interpretation that fits the pattern of findings is that the IDZ effect on commission errors is mediated by IDZ-induced increases in LC firing and thus increases in coeruleocortical NE activity (Aghajanian, et al, 1977; Dennis, et al, 1987), whereas the differential effect on omission errors and nontrials reflects a cocaine-induced alteration of norepinephrine-modulated release of DA from the VTA and/or substantia nigra. Alpha-2 adrenergic agents, like IDZ, have been shown to modulate dopamine release (Gresch, et al, 1995) by regulating the activity of the dopaminergic neurons in the ventral tegmental area and substantia nigra (Grenhoff and Svensson, 1988; Grenhoff and Svensson, 1989; Morrow, et al, 1996; Murphy, et al, 1996). In fact, two lines of evidence suggest this interpretation. First, the altered sensitivity to IDZ in cocaine-exposed rats was seen on errors of omission, but not errors of commission. Based on our previous experience with this task, errors of omission in control rats have typically been modulated by drugs that specifically alter DA activity (Bayer, et al, 1994). Second, for both measures on which a differential effect of IDZ was seen in the COC group (omission errors and nontrials) there was a suggestion that female rats were more sensitive than males to IDZ's impairing effects. In view of reports of increased dopaminergic activity in adult female rats relative to adult males (Bazzett and Becker, 1994; Castner and Becker, 1996; Castner, et al, 1993; Rivest, et al, 1995), this differential drug effect in the two sexes also supports a DA mechanism for the instances in which the two treatment groups responded differently to IDZ. It is notable that, in contrast, male rats appeared to be more sensitive than females to IDZ's effect on commission errors (inhibition errors and inaccurate responses). For inhibition errors, this was manifested as a shifting of the dose that produced impairment. For inaccurate responses, IDZ impaired males and improved females, a pattern that also indicates greater sensitivity

of the males, in light of the evidence that (1) NE activity is higher in adult male rats than females (Babstock, et al, 1997; Borsody and Weiss, 1996; Heal, et al, 1989) and (2) NE modulates attention along an inverted U-shaped curve, with either suboptimal or supraoptimal NE levels producing attentional decrements. In sum, the pattern of findings suggest that the commission error measures, on which the IDZ effect was similar in COC and control animals, reflect coeruleocortical NE activity, whereas those measures on which a differential treatment effect of IDZ was seen reflect NE-modulated DA activity. The altered IDZ response in the COC animals on these latter measures could reflect a lasting effect of the early drug exposure on the NE and/or the DA components of this system. Future studies are needed to answer this question.

As noted in the Introduction, the available literature concerning the effects of prenatal cocaine exposure on central catecholaminergic activity does not present a clear picture. This disparity is likely to be due, at least in part, to the wide variation in both doses of cocaine (ranging from 3 to 60 mg/kg), and routes of administration (SC, PO, and IV) used in these studies (Dingell, 1993, Personal communication). For this reason, we felt that it would be useful to ascertain the correspondence of the present findings specifically with those studies that utilized a comparable exposure regimen. Moreover, this IV exposure regimen may arguably provide data that is most relevant to the clinical situation being modeled, for several reasons. First, the IV regimen most closely mimics the rapidly peaking pharmacokinetic profile seen following inhalation or IV injection of cocaine in humans. The 3.0 mg/kg dose utilized in the current study produces peak arterial plasma levels that are similar to those reported for humans administered 32 mg of cocaine IV (Booze, et al, 1997; Evans, et al, 1996). In addition, under experimental conditions, this dose is self-administered by "users" multiple times in a 2.5 hr session (Fischman and Schuster, 1982), and thus represents a low or recreational dose. Finally, cocaine administration via the SC route of

administration, the most common regimen in prior studies, has been shown to cause skin irritation, increased corticosterone levels and adrenal enlargement in the dams (a sign of chronic stress), even with solutions as dilute as 40 mg/3 ml/kg (Dingell, 1993, Personal communication). The fact that PO cocaine administration did not increase corticosterone levels or adrenal size in this same study (Dingell, 1993, Personal communication) suggests that these alterations were due to the dermal effects, not the cocaine per se. Therefore, it is likely that administration of cocaine via the IV route, at a much lower dose (3 mg/kg), also avoids these adverse effects, since dermal irritation is not seen. However, comparable data for the IV route are not available.

The studies that have used the IV route, with comparable cocaine doses, present a remarkably consistent picture, and indeed provide support for the present hypothesis that IDZ causes a greater DA response in COC rats than controls. These studies have shown that prenatal cocaine exposure does not alter basal DA levels or release (Wang, et al, 1995; Wang, et al, 1996), but does increase the DA response to a pharmacological challenge or stressor (Bayer, et al, 2000; Du, et al, 1999; Morrow, et al, 2001; Murphy, et al, 1995). Alpha-2 adrenergic agents such as IDZ modulate stress-induced increases in DA turnover (Murphy, et al, 1996), suggesting that this differential dopaminergic response to stress seen following prenatal cocaine exposure may be due to lasting alterations in NE modulated DA release, consistent with the hypothesis put forward to explain the current results.

This finding that the cocaine-exposed subjects are more sensitive to the effects of IDZ than controls has important functional implications. Based on the evidence that stress and arousal increase endogenous levels of NE and DA (Horger and Roth, 1996; Thierry, et al, 1976), these conditions may exacerbate the cognitive dysfunction seen in individuals exposed to cocaine. This putative effect of prenatal cocaine on stress-induced impairment is consistent with reports of altered stress responses

following prenatal cocaine exposure (Bilitzke and Church, 1992; Campbell et al, 2000; Church and Tilak, 1996; Morrow, et al, 2001; Goldstein, et al, 1993; Goodwin, et al, 1997; Johns and Noonan, 1995; Molina, et al, 1994; Murphy, et al, 1995; Wood, et al, 1993; 1994; 1995). The relatively small magnitude of these observed effects is not unexpected, given that the doses used in this study were selected to model "recreational" cocaine usage. In fact, the magnitude of the group differences seen in this study were similar to those reported for 6-year old children exposed to cocaine *in utero* relative to controls (Richardson, et al, 1996). The relatively small magnitude of the observed effects is reassuring in light of the many children born each year with gestational exposure to this drug. Nonetheless, one cannot rule out the possibility that this dysfunction, albeit selective and subtle, will significantly alter the lives of affected individuals. For example, it has been estimated that a 5-point drop in IQ (comparable in magnitude to the group difference in omission errors observed here), results in a doubling of the number of children in the population diagnosed as mentally retarded (Rice, 1998). Although the population distribution of attentional function is less well-characterized than that for IQ, it is possible that that an attentional deficit of this magnitude might result in a similar increase in the risk of attention deficit disorder or related attentional disorders**.**

It may be noted that, in the current study, effects of prenatal cocaine exposure were only seen under the influence of IDZ; i.e., not in the non-drug state. However, prior findings from this same cohort of animals revealed that the COC animals were significantly impaired relative to controls in terms of both focused and selective attention (Morgan, et al, 1997). A finding from this prior study may explain why attentional impairment was not seen in the COC animals in the present study. Specifically, the magnitude of the selective attention impairment decreased with increasing experience on the task. This finding suggests that the COC animals,

despite their attentional impairment, were eventually able to filter out these distracting cues with extensive training with the same set of distracting cues. There is every reason to believe, however, that the greater distractibility of these COC animals would again become apparent if novel distractors were presented, or when tested in a novel task.

The shape of the IDZ dose-effect curve also deserves comment. For both commission and omission errors, an inverted-U shaped IDZ dose-response relationship was seen, with the greatest behavioral effect generally seen at the lowest dose. This pattern likely reflects the fact that IDZ alters noradrenergic activity via both pre-synaptic and postsynaptic actions, since alpha-2 receptors (the drug's binding site) are localized both pre- and post-synaptically (Aoki, et al, 1994; Aoki, et al, 1998; Venkatesan, et al, 1996). IDZ blockade of presynaptic alpha-2 receptors increases NE release due to a reduction in NE-mediated negative feedback, whereas IDZ's blockade of postsynaptic alpha-2 receptors antagonizes the effects of released NE at synapses with post-junctional alpha-2 receptors. It is possible that the inverted-U-shaped dose effect curve reflects a dose-related change in the ratio of binding to presynaptic vs. postsynaptic receptors. With regard to the commission error effects, it is hypothesized that the lower doses of IDZ impaired attention by blocking presynaptic receptors, thereby increasing NE activity beyond optimal levels; whereas higher doses of IDZ additionally caused blockade of post-synaptic alpha-2 receptors, negating this effect. Similarly, for the measures on which the COC group showed greater sensitivity to IDZ (i.e., the presumed NE-mediated DA effects), it is hypothesized that lowest dose of IDZ disrupted performance by its effect on presynaptic receptors, resulting in increased NE release and, consequently, increased DA release. At higher doses, postsynaptic blockade antagonized the effects of the released NE, thus preventing the increase in DA release seen at lower doses. This

hypothesis assumes that the post-synaptic adrenergic receptor mediating the increased DA release is the alpha-2 sub-type, an assumption that is consistent with the available evidence (Murphy, et al, 1996).

In summary, the results of the current study suggest that prenatal cocaine exposure causes functionally significant changes in NE-modulated DA systems that persist into adulthood. Based on the behavioral measures that showed a differential response to IDZ in the COC animals, it seems likely that these changes may contribute to the alterations in sustained attention and emotional reactivity that have been reported in both animals and humans exposed to cocaine *in utero.*

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# CHAPTER 4

## PRENATAL COCAINE EXPOSURE INCREASES SENSTIVITY TO THE ATTENTIONAL EFFECTS OF THE DOPAMINE D1 AGONIST SKF81297*

## ABSTRACT

Sensitivity to the attentional effects of SKF81297, a selective full agonist at DA $D_1$ receptors, was assessed in adult rats exposed to cocaine prenatally (via IV injections) and controls. The task assessed the ability of the subjects to monitor an unpredictable light cue of either 300 or 700 msec duration, and maintain performance when presented with olfactory distractors. SKF81297 decreased nose-pokes prior to cue presentation, as well as increased latencies and response biases (the tendency to respond to the same port as on the previous trial), suggesting an effect of SKF81297 on DA systems responsible for response initiation and selection. The COC and control animals did not differ in sensitivity to the effects of SKF81297 on these measures. In contrast, the COC animals were significantly more sensitive than controls to the impairing effect of SKF81297 on omission errors, a measure of sustained attention. This pattern of results provides evidence that prenatal cocaine exposure produces lasting changes in the DA system(s) subserving sustained attention but does not alter the DA system(s) underlying response selection and initiation. These findings also provide evidence for the role of $D_1$ receptor activation in attentional functioning.

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* Bayer LE, Brown A, Mactutus CF, Booze RM, and Strupp BJ (2000) Prenatal Cocaine Exposure Increases Sensitivity to the Attentional Effects of the Dopamine D1 Agonist SKF81297. Journal of Neuroscience 20(23): 8902-8908.

# INTRODUCTION

Concern about effects of prenatal cocaine exposure has grown in recent years. Although early media reports of gross neurological sequelae have proven largely unfounded, recent controlled studies have revealed attentional dysfunction in exposed children that persists into school age years (Dow-Edwards, et al, 1999; Leech, et al, 1999; Mayes, et al, 1998; Richardson, et al, 1996). Because maternal cocaine use in these studies generally occurred within the context of multiple risk factors, including polydrug abuse, it is important that animal model studies have demonstrated a clear causal link between prenatal cocaine exposure and attentional dysfunction (Garavan, et al, 2000; Mactutus, 1999; Morgan, et al, 1997; Romano & Harvey, 1996; Wilkins, et al, 1998a; 1998b). Elucidation of the neural bases of these deficits might allow amelioration or reversal of the dysfunction. This goal motivated the present study.

Altered dopaminergic (DA) activity, particularly in prefrontal and anterior cingulate cortices, is one mechanism that may underlie prenatal cocaine-induced attentional deficits. Moderate DA activity is critical for optimal functioning of frontal cortical regions (reviewed in Arnsten, 1997; Robbins, et al, 1994), thought to subserve attentional functions impaired by cocaine exposure (reviewed in Arnsten, et al, 1994; Arnsten, 1997; Carter, et al, 1997; Coull, 1998; Garavan, et al, 2000; Posner & Rothbart, 1998). Prenatal cocaine exposure has been reported to disrupt CNS DA function, (reviewed in Mayes 1999). However, definitive conclusions about the role of DA alterations in prenatal cocaine-induced attentional dysfunction cannot be drawn for several reasons. First, both the presence and direction of cocaine-induced alterations have been inconsistent across studies. Second, most studies administered cocaine via subcutaneous injections, which often cause necrotic skin lesions in cocaine-exposed dams (Bruckner, et al, 1982). Therefore, the extent to which reported

changes are due to maternal stress rather than, or in addition to, cocaine exposure is unknown. A final limitation is the absence of direct links between DA systems and attentional dysfunction.

The present study was designed to test the hypothesis that altered activity at $D_1$ receptors contributes to lasting attentional dysfunction in prenatal cocaine-exposed animals. Adult IV cocaine-exposed and control rats were administered SKF81297, a selective, full, $D_1$ agonist, prior to testing on an attentional task. Attentional effects of SKF81297 in control subjects were interpreted as supporting a role for $D_1$ receptor mechanisms in attention. Altered sensitivity to the attentional effects of SKF81297 in cocaine-exposed subjects was considered indicative of enduring alterations in DA systems subserving attention.

## METHODS

### Subjects

Nulliparous Long Evans rats (Harlan Sprague Dawley, Indianapolis, IN), approximately 11 weeks old, were housed in AAALAC-accredited animal facilities maintained at 21 ± 2°C, 50% ± 10% relative humidity with a 12 hour light:12 hour dark cycle. Food (Pro-Lab Rat, Mouse, Hamster Chow No. 3000) and water were available ad libitum. Subjects were randomly assigned to either receive a surgical catheterization procedure (described below) or to serve as unoperated surrogate controls. Following surgery, subjects in the catheterization group were randomly assigned to receive either cocaine or saline (vehicle control). This research protocol was approved by the animal care review boards of the University of Kentucky and Cornell University.

**Catheterization**

Subjects in the catheterization group were surgically implanted with a sterile IV catheter (described in detail in Mactutus, et al, 1994). Briefly, one week prior to mating, subjects were anesthetized with a mixture of ketamine hydrochloride (100 mg/kg/ml) and xylazine (3.3 mg/kg/ml) administered IP. A sterile Intercath IV catheter (22 gauge, Becton/Dickson) with a Luer-lock injection cap (Medex) was cut to a length of ~8 cm and implanted subcutaneously (SC). The distal end of the catheter was inserted into the jugular vein and threaded centrally. The proximal end of the catheter, including the injection cap, was left as a small SC pouch on the dorsal surface of the animal through which chronic IV injections were made. Catheter patency was maintained by daily flushing with 0.2 ml of heparinized saline (2.5%).

**Mating**

After recovery from surgery (4-8 days) the females were group-housed (n=3) with a male rat. Daily vaginal lavage of each female was performed to keep track of estrous cycles and to assist in defining conception. Conception (gestational day 0, or GD0) was confirmed by a sperm-positive lavage.

**IV Drug Injection**

IV drug administration procedures were conducted as described in Mactutus, et al. (1994). Briefly, all dams received daily IV saline injections (1ml/kg) from conception until gestational day (GD) 7. Dams in the saline subgroup (n=8) continued to receive IV saline injections once per day from GD8-14 and twice daily from GD15-21. Dams in the cocaine subgroup (n=8) received cocaine hydrochloride (3.0mg/ 1ml /kg, IV; Research Triangle Institute, NC) once per day from GD8-14 and twice daily from GD15-21. The drug was dissolved immediately prior to injection.

This IV injection procedure mimics the rapidly peaking pharmacokinetic profile following inhalation or IV injection of cocaine in humans. The 3.0 mg/kg dose produces peak arterial plasma levels that are similar to those reported for humans administered 32 mg of cocaine IV (Booze, et al, 1997; Evans, et al, 1996). Under experimental conditions, this dose is self-administered by "users" multiple times in a 2.5 hr session (Fischman & Schuster, 1982), and thus represents a low or recreational dose, highly relevant to the clinical situation being modeled. This regimen (route, dose, and rate) produces 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 (e.g., Mactutus, 1999; Mactutus, et al, 1994). Furthermore, this IV injection procedure does not reduce food intake of dams even when utilizing a cocaine dose as high as 6 mg/kg (S. Robinson, unpublished observations), precluding the need for pair-fed controls.

**Offspring treatment**

All cocaine-exposed and saline-exposed offspring used during this study were generated simultaneously. Within 24 hours of birth, pups were weighed, culled to 4 males and 4 females per litter (when possible), and fostered to a surrogate dam who had given birth within the preceding 24 hours. Fostering was conducted for both cocaine (COC) and saline-exposed (SAL) offspring in order to limit the effects of the maternal drug treatment to the prenatal period; i.e., potential effects of gestational cocaine treatment on maternal behavior were removed as a source of offspring differences.

On postnatal day 21 (PND21), pups were weaned and earpunched for identification. Within 10 days of weaning, one male and one female offspring from each litter were shipped under environmentally-controlled conditions from

Lexington, KY to Ithaca, NY (Cornell University). Upon arrival, subjects were housed in same sex pairs on a reversed dark/light cycle and allowed to acclimate to the new environment for 2-3 weeks. Training/ behavioral testing began for all animals on PND48. All personnel handling and testing the animals were blind to their treatment conditions. Offspring tested in the current study included 15 COC (8 male, 7 female) and 15 SAL (7 male, 8 female) animals. The animals were approximately 160 days of age at the beginning of the present study.

**Behavioral testing**

***Apparatus***

Testing was conducted in 10 automated Plexiglas chambers enclosed in sound-attenuating wooden boxes, each operated by an IBM PC XT. Each chamber consisted of a square waiting area (26.5 cm by 25 cm by 30 cm), adjacent to a testing alcove containing 3 funnel-shaped ports. An LED was mounted above each port. A thin metal door, raised at the initiation of each trial, separated these two compartments. The left and right ports were 8 cm apart, each at an approximate 45º angle relative to the center port. Each port was connected by tubing to three bottles containing liquid odorants, attached to a board placed outside of the wooden enclosure. Solenoid valves controlled the presentation of compressed air, pumped through a specific odorant and through a specific port. The airflow rate was 1.0 L/min and the air in the chamber was cleared via small centrifugal fans mounted on the outside of the chambers, at a rate of four complete exchanges per minute. A set of infrared phototransistors and a light source monitored the entrance to the alcove and each port. A one second nose-poke into one of the three ports indicated a response. Correct responses were reinforced with a 45 mg food pellet (Noyes, Lancaster, NH)

delivered into the alcove from a pellet dispenser (Lafayette Instrument Co., Lafayette, IN).

***Training procedure***

As part of a previous experiment (Morgan, et al, 1997), subjects were first trained to make a 1-sec nose-poke into a port to receive a reward pellet. Subjects were then trained on a series of consecutive attention tasks: 1) a three-choice visual discrimination in which subjects were rewarded for responding to the port under the illuminated LED; 2) a series of vigilance tasks in which the delay before cue onset varied as did the duration of the light cue; and 3) a variant of the distraction task described below. Subjects were maintained on a restricted feeding schedule (approximately 18 g/day for females, 21 g/day for males) to motivate the animals to perform the task.

***Distraction Task***

Prior to initiation of the SKF81297 challenge study, subjects received an additional 12 days of testing on the distraction task to achieve a stable baseline performance level for each rat. In this task, the opening of the alcove door signaled the onset of each trial. After the subject entered the testing alcove, one of the three LEDs was briefly illuminated (the discriminative stimulus). A 1-sec nose-poke into the port under the illuminated light was deemed correct and resulted in delivery of a 45 mg Noyes pellet. Several parameters were randomly varied across the trials in each session: 1) the pre-stimulus delay (2 or 3 sec after alcove entry); 2) duration of the light cue (300 or 700 msec); 3) presentation of an olfactory distractor. On 1/3 of the trials in each session, one of nine different odors was delivered randomly through one of the ports (i.e., it could emanate from the correct or incorrect ports), either one

or two sec-prior to visual cue onset. No odors were presented on the remaining third of the daily trials (nondistraction trials). The pre-stimulus delay, cue duration, cue location, and distraction condition for each trial were selected pseudorandomly, balanced for each session. A correct response (defined above) resulted in delivery of a food pellet and ended the trial, signaled by the closing of the alcove door. Incorrect responses included: (1) a 1-sec nose-poke into any port prior to the light cue onset (premature response), (2) a 1-sec nose-poke into an incorrect port following cue presentation (an inaccurate response), and (3) entering the alcove but failing to respond within 15 sec of trial onset (an omission error). Each of these incorrect responses also terminated the trial, but with no reinforcement. If the animal failed to enter the alcove within 30 sec after the door was raised, the alcove door closed and a nontrial was recorded. An intertrial interval of 5 sec was imposed between successive trials. A variant of this task had previously revealed enduring effects of prenatal cocaine exposure on selective attention in this cohort of animals (Morgan, et al, 1997).

**Response types**

Means for percentage correct, alcove and response latencies, and nontrials were calculated for each session. In addition, means for the following error types were calculated: premature responses, inaccurate responses, and omission errors. Also analyzed were (1) response bias, the tendency to respond to the same port as on the previous trial; and (2) prepokes, defined as nose-pokes of less than one second that did not constitute a choice.

**SKF81297 challenge study**

Daily sessions consisted of 100 trials or 60 minutes, whichever came first. Each animal received three test sessions per week (Monday, Wednesday, and Friday). All 30 subjects were tested on each testing day, using three sequential 1-hr shifts (12:30, 1:30, and 2:30 PM), each containing 10 subjects. Prenatal treatment was balanced across shift and testing chamber (n=10). SKF81297 (0.03, 0.1, 0.3 mg/kg; Research Biochemicals, Inc, Natick MA) or vehicle control was administered subcutaneously 15 minutes prior to testing. SC administration was utilized to minimize injection stress. The time-course of the effects of SKF81297 on behavior following this route of administration has been well established (Gerlach and Hansen, 1993; Lublin, et al, 1992; 1993; 1994). Each subject received each of the 4 doses 5 times, with dose order determined by a Latin Square design. Successive injection days were separated by a minimum of 48 hrs to allow drug clearance.

**Statistical Analysis**

Data were analyzed using the SAS version 6.11 PROC MIXED mixed models analysis of variance procedure which uses maximum likelihood estimation to account appropriately for the correlated structure within repeated measures designs. To normalize the distributions, an arcsin square-root transformation was applied to the percentage correct, percentage premature errors, percentage omission errors, and percentage accuracy data. Percentage correct was analyzed as a function of Prenatal Treatment, Sex, SKF81297 Dose, Pre-stimulus Delay, Cue Duration, Distraction condition (no distractor, distractor 1 sec before cue, distractor 2 sec before cue), and the relevant interactions of these variables. Prenatal treatment and Sex were included as between-subjects variables, SKF81297 dose was included as a within-subject variable, and Delay, Duration, and Distraction were included as within-subject,

within-session variables. Similar analyses were conducted on the specific error types: percentage omission errors, percentage accuracy errors, and percentage premature responses.

Similar analyses were conducted on prepokes. Two types of prepokes were distinguished: those prepokes that occurred before the light cue (early) and those that occurred after the cue (late). The former were considered indicative of impulsivity whereas the latter were thought to reflect decision-making processes.

Effects on motivation level were assessed by analyses of nontrials and alcove latency. To normalize the distribution, alcove latencies were natural log transformed prior to analysis, and then analyzed as a function of Prenatal Treatment, Sex, SKF81297 dose, correctness on the previous trial and the relevant interactions of these variables. Nontrials were analyzed as a function of Prenatal Treatment, Sex, SKF81297 dose, and the relevant interactions of these variables.

Similar analyses were conducted on mean response latency. Separate analyses were conducted on those response latencies that occurred on correct trials vs. those that occurred on incorrect trials. The former were considered to reflect information processing speed whereas the latter provide insight into the types of errors committed.

Response bias was analyzed as a function of Prenatal Treatment, Sex, SKF81297 dose, Pre-stimulus Delay, Light Cue Duration, Distraction Condition, correctness of the previous trial, and the relevant interactions of these variables.

## RESULTS

For all figures of percentage correct and percentage error-type data, least squares means (and associated standard errors) of transformed data are presented

rather than raw geometric means because they more accurately reflect the results of the statistical analyses.

**Maternal/litter effects**

Prenatal cocaine treatment did not alter maternal weight gain during gestation, measured on GD 0, 8, 15, and 21 [$F(1,16) = 0.00$, $p \leq 0.98$]; gestation length [$F(1,15) = 0.01$, $p \leq 0.93$]; pup birthweight [$F(1,16) = 0.44$, $p \leq 0.52$]; litter sex ratio [$F(1,14) = 0.66$, $p \leq 0.43$], or litter size [$F(1,16) = 0.01$, $p \leq 0.92$].

**Percentage Correct**

Percentage correct for a given session was calculated as the percentage of trials in the session on which the correct response was made within 10 seconds of trial onset. Percentage correct was lowest under conditions of greatest attentional demand: 300 ms light cue condition [Duration: $F(1,1285)=300.81$, $p\leq0.0001$], trials with distractors [Distraction condition: $F(2,1285)=24.78$, $p\leq0.0001$], and 3 sec pre-stimulus delay [Delay: $F(1,1285)=14.69$, $p\leq0.0001$]. Prenatal cocaine exposure did not alter overall percentage correct [$F(1,27)=0.00$, $p\leq0.9916$], nor was there a Prenatal Treatment by SKF81297 interaction on this measure [$F(3,81)=0.86$, $p\leq0.4634$]. SKF81297 decreased percentage correct [$F(3,81)=24.58$, $p\leq0.0001$], specifically at the highest dose (vehicle vs. high dose: $p\leq0.0001$); the two lower doses of SKF81297 did not significantly alter this response type. As seen in Fig. 4.1, the magnitude of this drug-induced impairment was greatest on trials when a distractor was presented 1 sec prior to presentation of the shorter cue [SKF*cue duration*distraction: $F(6,1285)=2.42$, $p\leq0.0249$].

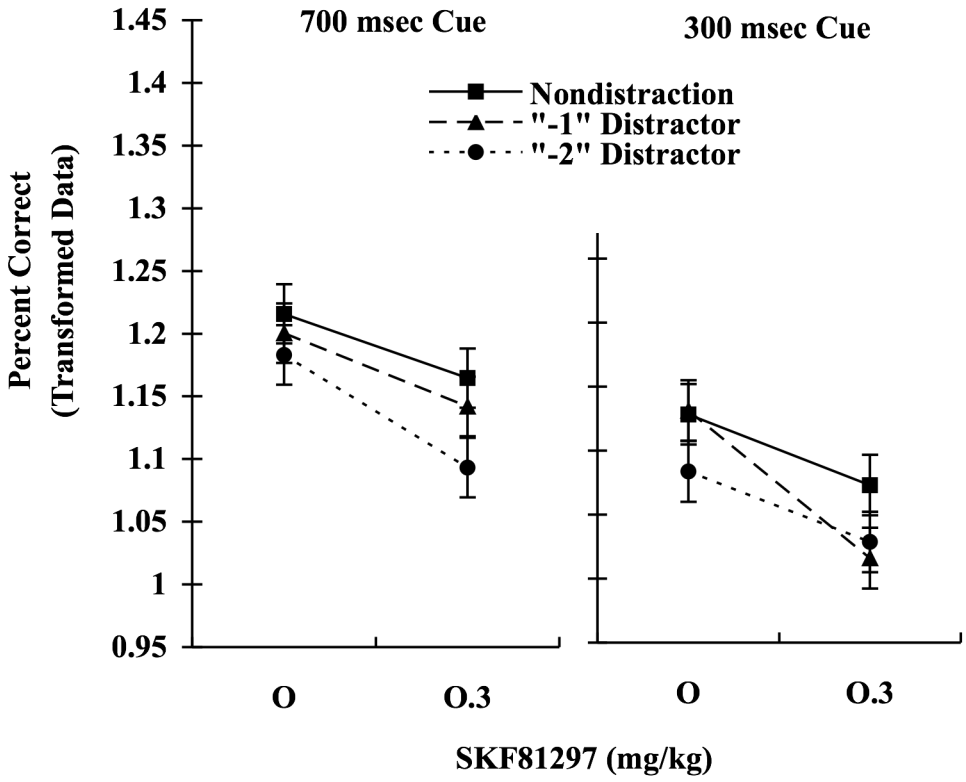


**Figure 4.1.** Effect of SKF81297 on overall percent correct. The highest dose of SKF81297 decreased percentage correct (high dose vs vehicle: $p \leq 0.0001$). To simplify the figure, only the vehicle and 0.3 mg/kg dose are included, as this highest dose is the only one at which a significant drug effect was seen. The magnitude of this impairment was greatest under the 300 msec cue and "-1" distractor condition (SKF*cue duration*distraction, $p \leq 0.0249$). See results for details. (1.01 and 1.24 on this scale correspond to 72 and 89% respectively.)

**Inaccurate Responses**

Inaccurate responses were calculated as the percentage of trials in the session in which an incorrect port was chosen after light cue offset, within 10 sec of trial onset. The rate of inaccurate responses was highest under conditions of greatest attentional demand: trials with the 300 ms light cue [Duration: $F(1,1294)= 347.24$, $p \leq 0.0001$], and trials with distractors [Distraction condition: $F(2,1294)=13.90$,

p≤0.0001]. This measure was not affected by Prenatal cocaine exposure [F(1,26)=0.08, p≤0.7851], SKF81297 dose [F(3,81)=0.65, p≤0.5821], nor the interaction of these variables [F(3,81)=0.46, p≤0.7129].

**Premature Responses**

Premature responses were calculated as the percentage of trials in the session on which the subject made a response prior to light cue presentation. To reduce the proportion of zeros in the dataset, it was necessary to average data across cue duration and timing of distractor onset ("-1" vs "-2" distractor conditions).

Prenatal cocaine treatment did not alter premature response rate [F(1,27)=0.10, p≤0.7498], nor was there an interaction of Prenatal Treatment and SKF81297 dose [F(3,84=1.41, p≤0.2450]. There was a significant effect of SKF81297 on premature response rate [F(3,84)=3.35 0.0228], reflecting the fact that the highest dose of SKF81297 significantly decreased premature responses (vehicle vs high: p≤0.0255), a tendency also seen at the medium dose (vehicle vs medium: p≤0.0894) (see figure 3.2). In addition, females made more premature responses than males [F(1,27)=6.36, p≤0.0179].

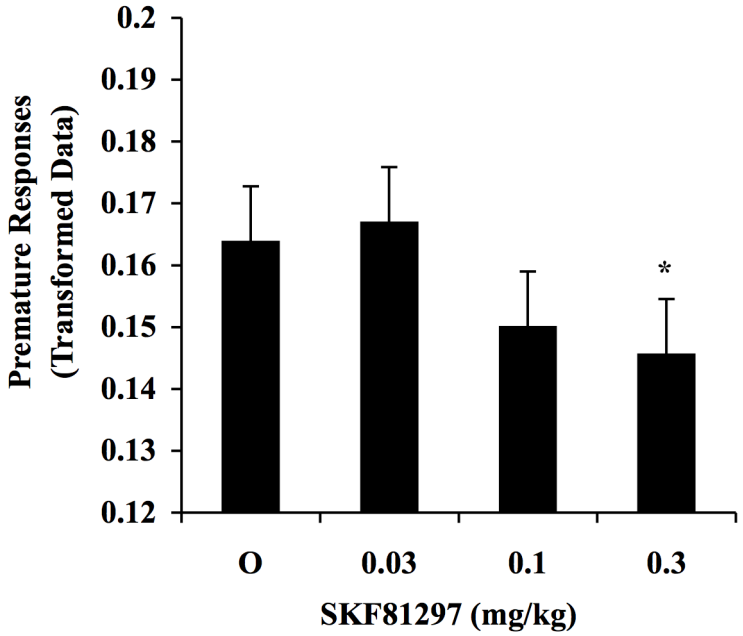


**Figure 4.2.** Effect of SKF81297 on premature responses. SKF81297 decreased the overall premature response rate ($p \leq 0.0228$), specifically at the highest dose (vehicle vs high: dose $p \leq 0.0255$). See results for details. (0.14 and 0.17 on this scale correspond to 1.9 and 2.9 % respectively.)

**Omission errors**

Percentage of omission errors was calculated as the percentage of trials in the session on which the subject entered the testing alcove but failed to respond within 15 sec of trial onset. These errors were considered indicative of lapses of attention. In order to reduce the proportion of zeros on this measure in the dataset, it was necessary to average data across Pre-stimulus Delay and Timing of distractor onset ("-1" vs "-2" distractor conditions). The rate of omission errors was higher on trials with the 300 ms cue than on those with the 700 msec cue [Duration: $F(1,348) = 49.32$, $p \leq 0.0001$], consistent with the interpretation that omission errors reflect lapses in attention.

There was no main effect of Prenatal Treatment on omission error rate [$F(1, 26)= 0.36$, $p\leq0.5534$]. In contrast, SKF81297 increased omission errors [SKF: $F(3, 81)=57.49$, $p\leq 0.0001$], specifically at the two highest doses (vehicle vs medium: $p\leq0.0456$; vehicle vs high: $p\leq0.0001$). In addition, COC rats were more sensitive than controls to SKF81297's impairing effect on this measure [COC*SKF: $F(3,81)=2.75$, $p\leq0.0481$], exhibiting impairment at a lower dose of SKF81297 than SAL rats. COC rats were impaired by the 0.1 mg/kg dose of SKF81297 (vehicle vs 0.1mg/kg for COC: $p\leq0.0035$) whereas SAL rats were not impaired by this dose (vehicle vs 0.1mg/kg for SAL: $p\leq0.8889$). There was a tendency for the two groups to differ in omission error rate at this dose but the contrast did not achieve statistical significance ($p \leq0.1116$). The 0.3 mg/kg dose of SKF81297 impaired both the COC (vehicle vs 0.3mg/kg for COC: $p\leq0.0001$) and the SAL groups (vehicle vs 0.3 mg/kg for SAL: $p\leq0.0001$), and the two groups did not differ in omission error rate at this dose ($p \leq0.4144$). The groups did not differ in the vehicle condition ($p\leq0.9981$; see figure 4.3).

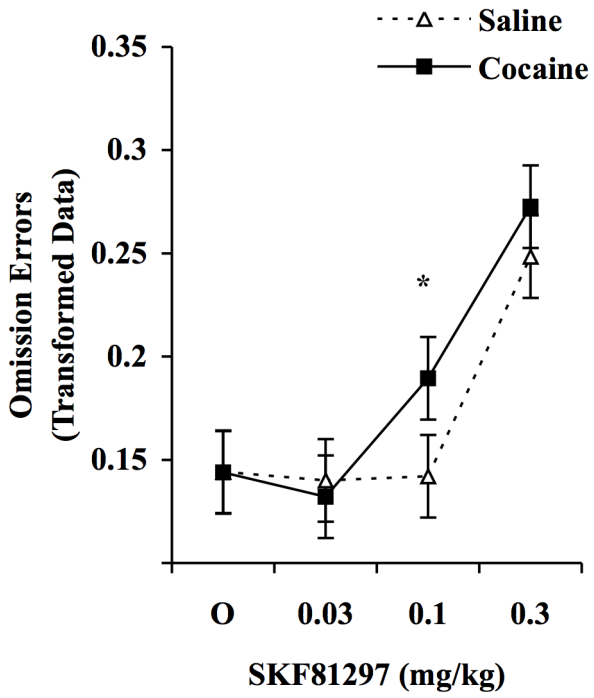


**Figure 4.3.** COC rats showed increased sensitivity to SKF81297s impairing effect on omission errors. COC rats were more sensitive than SAL rats to SKF81297's effect on omission errors (COC*SKF: $p \leq 0.0481$), showing impairment at a lower dose of SKF81297 than SAL rats (vehicle vs 0.1mg/kg for COC, $p \leq 0.0035$; vehicle vs 0.1mg/kg for SAL, $p \leq 0.8889$). See results for details. (0.13 and 0.27 on this scale correspond to 1.7 and 7.1 % respectively.)

**Response Bias**

Response bias, the tendency to respond to the same port as on the previous trial, was also analyzed. The dependent measure was the percentage of trials in a session on which the rat responded to the same port as the previous trial, corrected for the baseline response bias score for that session. This correction was needed because (a) the magnitude of response bias that would be expected by chance is slightly affected by the pattern of correct ports in a given session; and (b) the raw response bias score is correlated with performance level due to the constraint imposed on the number of times that a given port is correct on successive trials.

There was a significant effect of SKF81297 dose on response bias [F(3,2679)=2.88, p≤0.0345], an effect apparently driven by an increased bias seen at the two highest doses relative to the vehicle (vehicle vs. medium: p≤0.0703; vehicle vs. high: p≤0.0763)(see Figure 4.4). In contrast, prenatal cocaine exposure did not affect response bias [F(1,26)=1.33, p≤0.2586], nor did this measure exhibit a significant Prenatal Treatment by SKF81297 interaction [F(3,2679)=1.99, p≤0.1138].

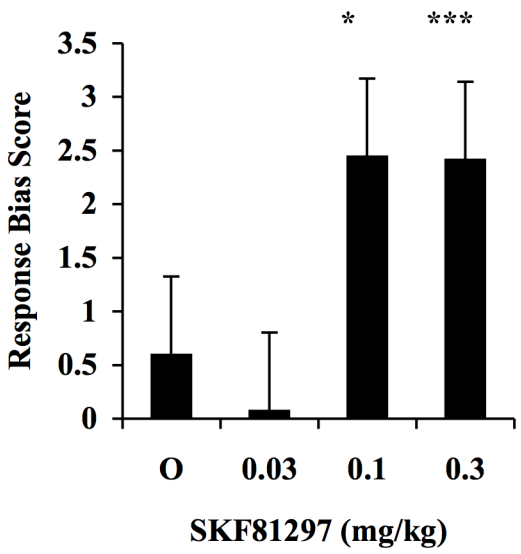


**Figure 4.4.** Effect of SKF81297 on response bias. Response bias was significantly affected by SKF81297 dose (p≤0.0345), an effect that was driven, in part, by a borderline increase in bias produced by the two highest doses relative to the vehicle dose (vehicle vs. medium, p≤0.0703; vehicle vs. high; p≤0.0763). See Results for details.

**Pre-pokes**

Also analyzed were pre-pokes, nosepokes of < 1 sec that did not constitute a choice. Two categories of pre-pokes were analyzed: early pre-pokes, those occurring

before illumination of the light cue and late pre-pokes, those occurring after cue onset. The former were considered indicative of impulsivity whereas the latter were considered to reflect decision-making processes. Data for pre-pokes were averaged across cue duration, delay, and timing of distractor onset ("-1" vs "-2" distractor conditions) to reduce the proportion of zero values in the data set.

Prenatal cocaine exposure did not alter the incidence of early pre-pokes [$F(1,26)=0.04$, $p\leq0.8394$], nor was there a Prenatal treatment by SKF81297 interaction on this measure [$F(3,79)=0.42$, $p\leq0.7390$]. In contrast, SKF81297 markedly decreased the rate of this type of response [$F(3,79)=11.47$, $p\leq0.0001$], specifically at the two higher doses (vehicle vs medium: $p\leq0.035$; vehicle vs high: $p\leq0.0001$)

SKF81297 increased the rate of late pre-pokes [$F(3,549)=33.83$, $p\leq0.0001$], specifically at the two higher doses (vehicle vs medium: $p\leq0.0088$; vehicle vs high: $p\leq0.0001$). However, prenatal cocaine exposure did not alter this measure [$F(1,26)=0.26$, $p\leq0.6158$], nor was there an interaction of Prenatal Treatment and SKF81297 dose [$F(3,549)=1.44$, $p\leq0.2304$].

**Response Latencies**

Prenatal cocaine exposure did not alter response latency on correct trials [$F(1,26)= 0.21$, $p\leq0.6541$], nor was there an interaction of Prenatal Treatment and SKF81297 dose on this measure [$F(3, 78)=1.36$, $p\leq0.2603$]. SKF81297 increased average response latency on correct trials [$F(3, 78)=8.54$, $p\leq0.0001$], an effect limited to the highest dose (vehicle vs high: $p\leq0.0001$).

Prenatal cocaine exposure did not alter response latency on incorrect trials [$F(1,26)= 0.62$, $p\leq0.4398$], nor was there an interaction of Prenatal Treatment and SKF81297 dose on this measure [$F(3, 78)=0.36$, $p\leq0.7797$]. SKF81297 increased

average response latency on incorrect trials [F(3, 73)=9.97, p≤0.0001]. This increase in response latency was observed at each of the three SKF81297 doses relative to controls: low (p≤0.0134), medium (p≤0.0002), and high (p≤0.0001).

**Alcove Latency**

SKF81297 increased mean alcove latency [F(3, 108)=16.34, p≤0.0001], specifically at the highest dose (vehicle vs high: p≤0.0001). However, Prenatal cocaine exposure did not alter mean alcove latency [F(1, 26)=0.03, p≤0.8689], nor was there an interaction of prenatal treatment and SKF81297 dose on this measure [COC*SKF: F(3, 108)=0.11, p≤0.9554].

**Nontrials**

Prenatal cocaine exposure did not alter nontrial rate [F(1,26)=0.16, p≤0.6944]. In addition, there was no effect of SKF81297 on this measure [F(3,485)=1.24, p≤0.2957], indicating that neither the drug nor the prenatal treatment affected motivation level on this task. Finally, the interaction of Prenatal treatment and SKF81297 interaction was not significant [F(3,485)=0.16, p≤0.9216].

## DISCUSSION

The results of the current study have important implications for the role of DA alterations in the attentional dysfunction produced by prenatal cocaine exposure, and for the role of $D_1$ DA activity in normal behavior and cognition.

SKF81297 affected numerous dependent measures in this attention task. The drug decreased early prepokes and premature responses, as well as increased latencies and response bias, a pattern that reflects an effect of SKF81297 on DA systems

responsible for response initiation and selection (discussed below). The effects of SKF81297 on these variables did not differ between treatment groups, suggesting that the DA systems responsible for these functions are unaltered by prenatal cocaine exposure.

In addition, the analysis of omission errors revealed a significant interaction of SKF81297 and Prenatal Treatment, the critical finding considered indicative of an enduring effect of prenatal cocaine exposure on DA systems. The finding that the statistical interaction of SKF81297 and Prenatal Treatment was only seen for omission errors, not for measures that appear to tap DA systems subserving response initiation and selection suggests that the omission error effect was mediated by DA activity in a different brain region(s) than these former effects. One interpretation that fits the pattern of findings is that this differential effect on omission errors reflects a cocaine-induced alteration in mesocortical DA projections underlying attention (reviewed in Arnsten, 1997; Pennington, 1994; Robbins, et al, 1994; Williams & Goldman-Rakic, 1995), whereas the drug effects on response selection and initiation are likely mediated by effects on striatal DA neurons (Brown & Robbins, 1989a; 1989b; 1991; Carli, et al, 1989; Kermadi & Boussaoud, 1995; Montgomery & Buchholz, 1991; Robbins, 1997).

Thus, the pattern of results, overall, suggests that the differential effect of SKF81297 on omission errors in the present study reflects a lasting effect of prenatal cocaine exposure on the DA modulation of attention. However, it should be noted that because this differential sensitivity to SKF81297 was seen on a measure tapping sustained attention, but not on measures which tap selective attention and distractibility (eg premature responses, inaccurate responses), these inferred DA changes are not likely to have relevance for the selective attention dysfunction that has been reported for cocaine-exposed subjects (Gabriel and Taylor, 1998; Garavan, et al,

2000; Romano and Harvey, 1996; Wilkens, et al, 1998a; 1998b). However, they are relevant for the sustained attention deficits that have also been reported in studies of cocaine-exposed children (Richardson, et al, 1996) and animal models (Morgan, et al, 1997). For example, Richardson, et al. (1996) reported that prenatal cocaine-exposed children committed more omission errors than controls on the CPT, a visual attention task. Omission errors in this task, as in the present study, are indicative of lapses in attention. Attentional lapses also seem to contribute to the impaired performance of COC animals in a sustained attention task in rats (Morgan, et al, 1997). By identifying the neural system likely to underlie this type of attentional dysfunction in cocaine-exposed subjects, the present results may aid in identifying possible candidates for therapeutic intervention.

It may be noted that, in the current study, attentional effects of prenatal cocaine exposure were only seen under the influence of SKF81297; i.e., not in the non-drug state. However, prior findings from this same cohort of animals revealed that the COC animals were significantly impaired relative to controls in terms of both sustained and selective attention. Notably the impairment in selective attention was observed in a distraction task that is almost identical to that used in the present study (Morgan, et al, 1997). Another finding from this prior study may explain why attentional impairment was not seen in the COC animals in the present study. Specifically, the magnitude of the selective attention impairment decreased with increasing experience on the task. This finding suggests that the COC animals, despite their attentional impairment, were eventually able to filter out these distracting cues with extensive training with the same set of distracting cues. There is every reason to believe, however, that the greater distractibility of these COC animals would again become apparent if novel distractors were presented, or when tested in a novel task.

The pattern of drug effects on omission errors suggests an increased sensitivity to SKF81297 in the cocaine-exposed animals. Although the basis of this effect is not known, the most likely underlying mechanism is a lasting effect of prenatal cocaine exposure on DA release. The available evidence from studies using similar IV cocaine exposure regimens (cocaine dose and route of administration) support the hypothesis that prenatal cocaine exposure increases the amount of DA released in response to a stressor or a pharmacological challenge, but not does alter basal DA levels or release. Prenatal cocaine-exposed rabbits exhibited enhanced release of DA in caudate nucleus following an amphetamine challenge, relative to controls, whereas basal DA levels were unaffected (Du, et al, 1999). Similarly, cocaine-exposed rats exhibited increased DA metabolic activity (as measured by DOPAC/DA ratio) relative to controls in ventral prefrontal cortex (PFC) following a footshock, whereas basal DA metabolic activity levels were unaffected (Elsworth, et al, l999; personal communication). Further support that prenatal cocaine exposure does not affect basal DA activity is provided by additional findings from the rabbit model showing no change in basal tyrosine hydroxylase immunoreactivity in cingulate cortex (Wang, et al, 1996), nor *in vitro* DA levels in cingulate cortex, frontal cortex, or striatum (Wang, et al, 1995a). Finally, in an in vitro slice system, prenatal cocaine enhanced responsiveness of the presynaptic DA autoreceptor in cingulate and frontal cortices, but not striatum (Wang, et al, 1995a). Enhanced autoreceptor sensitivity may represent a compensatory response to this putative stress-induced increase in DA release (or vice versa), as these receptors provide a negative-feedback system. In light of recent data suggesting that testing on PFC-dependent tasks increases extracellular DA in PFC (Watanabe, et al, 1997), it is possible that the COC animals in the present study experienced excessive DA activity

in PFC when tested on our attentional tasks, contributing to the attentional impairment observed in prior tasks (Morgan, et al, 1997), discussed above.
An alternate hypothesis is that the increased sensitivity of the COC animals to SKF81297 reflects an increased number or efficacy of $D_1$ receptors. However, this mechanism is unlikely based on previous findings that prenatal exposure to cocaine: i) did not affect $D_1$ mRNA's (DeBartolomeis, 1994) or receptor binding in striatum, nucleus accumbens, or VTA (Leslie, et al, 1994), and ii) resulted in decreased $D_1$ receptor G-protein coupling in striatum, anterior cingulate and frontal cortex (Friedman, et al, 1996; Levitt, et al, 1997; Wang, et al, 1995b). In fact, this latter finding of reduced $D_1$ coupling in IV cocaine-exposed rabbits appears inconsistent with the present findings. Future research is needed to determine whether reduced $D_1$ receptor G-protein coupling is also seen following the particular cocaine exposure regimen used in the present study (route, dose, and developmental period), and if so, how this effect can be reconciled with the present findings.

In light of the inconsistent findings concerning prenatal cocaine effects on dopamine function, additional studies are needed to clarify how the increased sensitivity to SKF81297's attentional effects arises at the receptor/intra-cellular level. DA interactions with other neurotransmitter systems may well be involved, consistent with the suggestion of another study from our lab that prenatal cocaine exposure increases sensitivity to the behavioral effects of $\alpha_2$ adrenergic receptor-modulated DA release (Bayer, et al, 1996).

The finding that COC animals are more sensitive than controls to the attentional effects of SKF81297 has functional implications. As stress increases endogenous DA activity (Horger & Roth, 1996; Thierry, et al, 1976), individuals exposed to cocaine *in utero* may be more vulnerable to the adverse effects of stress on cognition. This hypothesis is consistent with the finding that cocaine-exposed rats

showed increased sensitivity to the impairing effects of the pharmacological stressor FG-7142 on a PFC-dependent cognitive task (Murphy, et al, 1995). This hypothesized effect of prenatal cocaine exposure on stress-induced attentional impairment is also consistent with reports of altered behavioral and neurochemical response to stress in cocaine-exposed animals (Bilitzke & Church, 1992; Church & Tilak, 1996; Elsworth, et al, l999; personal communication; Goldstein, et al, 1993; Goodwin, et al, 1997; Johns & Noonan, 1995; Molina, et al, 1994; Wood, et al, 1993; 1994; 1995).

**Implications for the cognitive roles of dopaminergic systems and $D_1$ receptors**

In addition to delineating the mechanism for prenatal cocaine-induced attentional dysfunction, several observed effects of SKF81297 clarify the role of $D_1$ DA activity in specific cognitive functions. The observed pattern of results supports the hypothesized role of DA in response initiation and selection. SKF81297 decreased premature responses and early prepokes, and increased response and alcove latencies, suggesting that the drug altered response initiation, perhaps via activation of $D_1$ receptors in the striatum. This finding corresponds with the hypothesized role of the mesostriatal DA system in behavioral activation and response preparation (Brown & Robbins, 1989a; 1989b; 1991; Carli, et al, 1985; 1989; Pullman, et al, 1988; see Robbins, 1997 for review). SKF81297 also increased response bias, suggesting that the drug altered response selection. This finding is consistent with the theory that striatal DA neurons participate in programming target acquisition and the selection of responses from the repertoire of available responses (Brown & Robbins, 1989a; 1989b; 1991; Carli, et al, 1989; Kermadi & Boussaoud, 1995; Montgomery & Buchholz, 1991).

The attentional impairment produced by SKF81297, manifested as an increase in omission errors, provides new evidence for the hypothesized role of mesocortical DA in attention. This finding corroborates the existing evidence that moderate DA activity in prefrontal cortex is commensurate with optimal functioning of this region, whereas, both suboptimal and supraoptimal DA activity impairs PFC-dependent functions such as working memory (Arnsten, 1997; Murphy, et al, 1996; Zahrt, et al, 1997). Granon and colleagues (2000) proposed that an inverted U-shaped relationship also exists between PFC DA activity and attention, and provided support for the ascending portion of the curve (i.e., attentional enhancement), using PFC microinfusions of the partial $D_1$ agonist SKF38393. The present findings, using the full $D_1$ agonist SKF81297, extend these data by providing evidence for the downward side of the inverted U shaped curve; i.e. attentional impairment. Finally, the present study, along with the report by Granon and colleagues (2000), provides important new evidence for an attentional role for DA activity specifically at the $D_1$ receptor subtype. Although prior investigations have suggested that the $D_1$ receptor subtype is most essential for PFC-dependent cognitive functions (Arnsten, 1997; Arnsten, et al, 1994; Sawaguchi and Goldman-Rakic, 1991; 1994; Williams and Goldman-Rakic, 1995), nearly all evidence comes from working memory, rather than attentional, tasks.

In summary, the current study demonstrated that prenatal cocaine exposure produces enduring effects on the DA system underlying attention, but does not alter DA systems underlying response initiation and selection. These lasting effects on DA activity may contribute to the changes in attention and arousal regulation reported in both animals and children exposed to cocaine *in utero*.

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# CHAPTER 5

# CONCLUSIONS AND FUTURE DIRECTIONS

The studies presented in this volume have important implications for the role of catecholamines in the modulation of normal attentional function. In addition, the results of these studies shed light on the role played by catecholaminergic alterations in the attentional dysfunctions induced by environmental lead (Pb) exposure and prenatal cocaine exposure. All three of these issues will be discussed below.

## Significance of results for understanding catecholaminergic modulation of attention

In Chapters 2 and 3, the effects of the alpha-2 adrenergic antagonist idazoxan (IDZ) provide support for the hypothesized role of NE in the modulation of attention, particularly selective attention and distractibility. IDZ altered the rate of commission errors (inaccurate responses, inhibition errors) specifically in response to distractors. In chapter 2, the effect of IDZ was only observed under the 500 msec cue duration condition, the condition of highest attentional demand. These effects were likely mediated by IDZ-induced increases in LC firing, and thus increases in coeruleocortical NE activity (Aghajanian, et al, 1977; Dennis, et al, 1987). These results are consistent with evidence that activity of LC neuron was closely correlated with performance on visual attention tasks in monkeys (Aston-Jones, et al, 1999), and provide support for the theory that LC activity and cortical NE release modulate selective attention by reducing signal–to-noise ratio (reviewed in Aston-Jones, 1988; 1997; Foote, et al, 1983; Sara, et al, 1988), enhancing cortical cue and delay related firing relative to spontaneous firing (Sawaguchi, et al, 1990). In these studies, IDZ appeared both to alter attentiveness to target stimuli by altering accuracy under the short cue condition

and to modulate attention to distractors by altering performance under conditions of distraction. This finding is consistent with evidence that lesions to ascending noradrenergic neurons in rats increase distractibility in maze tasks (Roberts, et al, 1976) and impair performance on tasks of visual attention, specifically under conditions of highest attentional demand such as unpredictable target stimulus onset and presentation of distractors (Carli, et al, 1983; Cole and Robbins, 1992). These studies also provide support for prior evidence that alpha-2 selective agents alter performance on visual attention tasks in rats (Bunsey and Strupp, 1995; Milstein, et al, 2007; Sagvolden, 2006; Sirvio, et al, 1993; 1994), particularly under conditions of greatest attentional demand such as presentation of distractors (Bunsey and Strupp; 1995), reduced cue brightness (Sirvio, et al, 1993; 1994), and unpredictable cue onset (Sirvio, et al, 1993; 1994). In addition, these studies are consistent with evidence that alpha-2 selective agents affect distractibility in monkeys (Arnsten and Contant, 1992) and alter attentional performance in human subjects on the continuous performance task (CPT) (Coull, 1995; McClure, et al, 2007; Scahill, et al, 2001), visuo-spatial and visual target detection tasks of selective attention (Coull, et al, 2004; Smith, et al, 1992; Smith and Nutt, 1996), and the Stroop task (Mair and McEntree, 1986; Taylor and Russo, 2001).

The studies in Chapters 2 and 3 also provide support for the hypothesis that the relationship between NE activity and attention follows an inverted U-shaped curve, with optimal levels of NE producing optimal levels of attention and both suboptimal and supra-optimal levels impairing attention. IDZ increased inaccurate responses and impaired accuracy in male subjects as described in Chapter 3, but reduced inaccurate responses and improved accuracy in female subjects as shown in Chapters 2 and 3. This difference in the effects of IDZ is likely due to sex-related differences in endogenous NE activity, consistent with evidence that NE activity is higher in adult

male rats than females (Babstock, et al, 1997; Borsody and Weiss, 1996; Heal, et al, 1989). The female subjects in chapters 2 and 3 likely had lower levels of endogenous NE activity; IDZ increased NE activity in these subjects, resulting in enhancement of accuracy. In contrast, levels of endogenous NE activity were likely higher in the male subjects in chapter 3; in these subjects, IDZ increased NE activity beyond optimal levels, resulting in reduced accuracy. These findings are consistent with reports that the level of LC activity produces an inverted U-shaped effect on attention, with low levels of firing resulting in insufficient arousal and attention, moderate levels of tonic firing increasing attentional task performance, and high levels of tonic activity of LC neurons impairing vigilance and selective attention (Aston-Jones, et al, 1994).

In Chapter 4, the observed effects of the selective D1 agonist SKF81297 have important implications for the role of DA activity in attentional function. Treatment with SKF81297 decreased subjects' overall percent correct on a distraction task and the magnitude of this impairment was greatest under conditions of high attentional demand, the short light cue condition and the condition in which distractors were presented 1 sec prior to cue onset. The impairment of performance induced by SKF81297 appeared to be due to a dose dependent increase in errors of omission. Omission error rate reflects, at least in part, lapses in focused attention. This inference is based on the findings from the current studies and prior studies that omission error rate increases as the cue duration decreases (Bayer, et al, 1994; Morgan, et al, 2001) and as the pre-stimulus delay increases (Bunsey and Strupp, 1995; Morgan, et al, 2001). The effects of SKF81297 reported in chapter 4 are consistent with the results from a prior study using a similar distraction task in which SKF81297 selectively increased omission errors equally during both distraction and nondistraction trials, but only under the shorter (500 msec) cue duration condition, the condition requiring the greatest vigilance (Bayer, et al, 1994). It should be noted that SKF81297 did not

increase commission errors, which tap selective attention and distractibility in this distraction task (e.g. premature responses, inaccurate responses). These findings suggest that DA activity, specifically at D1 receptors, appears to modulate the focused attention or vigilance component of performance on this visual attention task. This interpretation is consistent with findings from a 5-choice serial reaction time task of sustained attention in rats on which systemic administration of the selective D1 antagonist SCH23390 impaired performance (Passetti, et al, 2003). Similarly extracellular PFC DA was elevated during task performance (Dalley, et al, 2002), and DOPAC/DA ratios in right frontal cortex were significantly correlated with accuracy on this same task (Puumala and Sirvio, 1998).

The effects of SKF81297 on attention observed in Chapter 4 also provides new evidence to support the hypothesis that, like NE, DA modulation of attention also follows an inverted U-shaped curve, with moderate DA activity associated with optimal attentional processing. In Chapter 4, SKF81297 was shown to cause decrements in attentional performance, suggesting that the D1 agonist increased subjects' DA activity at D1 receptors beyond optimal levels, and providing support for the descending portion of the curve. Support for the ascending portion of the curve (ie. attentional enhancement) is provided by evidence from previous studies using visual attention tasks in rats in which prefrontal microinfusions of SKF81297 enhanced sustained attention (Chudasama and Robbins, 2004) and prefrontal microinfusions of the DA D1 receptor partial agonist SKF38393 selectively improved accuracy in subjects with low baseline levels of performance. Similarly, systemic administration of the selective D1 antagonist SCH23390 impaired accuracy and increased errors of omission (Passetti, et al, 2003), and prefrontal microinfusions of SCH23390 selectively impaired accuracy in subjects with high baseline levels of performance (Granon, et al, 2000) on this same task.

The study in Chapter 4 examined the affects of a D1 selective agonist on attention, so conclusions drawn from this study about the role of DA in attention are limited to its effects at the D1 family of receptors, the most prevalent DA receptor subtype in the PFC (Lidow, et al, 1991), a critical brain region for attentional processing (reviewed in Arnsten, 1998; Arnsten and Li, 2005; Chudasama and Robbins, 2006; Robbins, 2000; 2005; Robbins and Roberts, 2007). However, recent evidence suggests that one of the D2 family of receptors, the D4 subtype, may also play an important role in the modulation of attention. D4 receptors have been localized in human prefrontal cortex (Primus, et al, 1997), predominantly on interneurons (Mrzjak, et al, 1996). These receptors have a high affinity for NE as well as DA (Van Tol, et al, 1991), suggesting that this receptor subtype could contribute to noradrenergic-, as well as dopaminergic-modulated attentional processing. A number of studies have shown an association between a specific variant of the gene that codes for this receptor subtype, the DRD4 7-repeat allele, and attention deficit hyperactivity disorder (Faraone, et al, 2001; Shaw, et al, 2007; Swanson, et al, 2000). Studies with D-4 selective agents have provided support for a role for this receptor subtype in PFC dependent cognitive processes such as working memory, (Arnsten, et al, 2000; Zhang, et al, 2004) and attentional set-shifting (Floresco, et al, 2006). Additional studies are needed to determine the role of this specific DA receptor subtype in the sustained and selective attentional processes investigated in this volume.

**Cognitive effects of Pb exposure**

Pb-exposure reduced accuracy in the attention task as described in Chapter 2. These impairments were not limited to the trials on which distractors were presented, suggesting that the deficits were not related to Pb-induced increases in distractibility. Rather, these impairments were likely due to deficient sustained attention. Preliminary

analysis of data from a prior study suggested that these same Pb-exposed subjects showed delay-dependent impairment during the performance of a vigilance task (Strupp, et al, 1993), and sustained attention deficits have also been observed in similar vigilance tasks following some Pb exposure regimens. (Driscoll, 2003; Morgan, et al, 2001; Stangle, et al, 2003). Pb-induced impairments in sustained attention on this task are consistent with the impairments reported on the CPT (Harvey, et al, 1988; Walkowiak, et al 1998) and on reaction time tasks that place increased demands on sustained attention in human children (Harvey, et al, 1988; Hunter, et al, 1985; Minder, et al, 1994; Needleman, et al, 1979; 1990; Raab, et al, 1990). Alternately, the attentional deficits observed in the Pb-exposed subjects may have been related to either an increased reactivity to prior errors, consistent with the results of studies of early Pb-exposure from this laboratory (Morgan, et al, 2001; Stangle, et al, 2007), and/or to an increased sensitivity to the performance disrupting effects of the distractors. This suggestion indicates that Pb-exposure may cause deficits in arousal regulation or the ability to deal with frustration. Such deficits in emotional regulation may contribute to the disruptive classroom behavior and increased delinquency rates associated with childhood Pb-exposure (Bellinger, et al, 1994; Dietrich, et al, 2001; Needleman, et al, 1996). Additional studies are needed to determine which of these possible cognitive mechanisms underlie the observed attentional dysfunctions.

Understanding the specific cognitive deficits that result in these Pb-induced reductions in accuracy on this task is relevant to clinical treatment of Pb-exposed individuals. Chelating agents are administered to children when BPb levels exceed 40 µg/dl, in order to rapidly remove Pb from the body. Recent studies from our laboratory suggest that early treatment with the chelating agent succimer eliminates Pb-induced increases in emotional reactivity, specifically to errors, but is only

moderately successful at alleviating Pb-induced deficits in sustained attention (Stangle, et al, 2007), suggesting that some forms of attentional impairment may be unaffected by such treatment in children. Further studies in children are needed to evaluate the efficacy of these drugs in ameliorating specific Pb-induced cognitive dysfunctions. It is likely that additional pharmacotherapy may be needed to improve cognitive function in affected children, as well as cognitive-behavioral interventions to address the specific cognitive processes that remain affected.

**Neurochemical basis for Pb-induced attentional impairments**

In Chapter 2, Pb-exposure caused attentional dysfunction but Pb-exposed subjects did not demonstrate differential sensitivity to the attentional effects of IDZ on this task. This finding fails to provide evidence for the hypothesis that Pb-induced alterations in NE systems underlie the attentional impairment, suggesting that alterations in other attention modulating neurochemical systems likely mediate these deficits. One plausible alternative candidate for the neural basis for these Pb-induced attentional impairments is the cholinergic system underlying attention; namely the projections from the nucleus basalis to the frontal cortex. Pb-exposure has previously been shown to alter cholinergic functioning (Anwyl, et al, 1982; Bielarczyk, et al, 1996; Bourjeily and Suszkiew, 1997; Carroll, et al, 1977; Manalis and Cooper, 1973; Minnema, et al, 1988; Sun, et al, 1997; Zhou, et al, 2000), and there is considerable evidence for cholinergic modulation of both sustained attention and selection of attentional targets, particularly under conditions of high attentional demand (Robbins, 1997; 2002; 2005; Sarter, et al, 2001; 2005). Consistent with this hypothesis, recent studies have demonstrated differential sensitivity to the attentional effects of the cholinergic antagonist scopolamine and the anti-cholinesterase tacrine in early Pb-exposed rats, using sustained attention tasks similar to the selective attention tasks

described in this volume. Specifically, Pb-exposed subjects were less sensitive to both the attention enhancing effects of tacrine and to scopolamine-induced reductions in accuracy on trials following errors, which were considered a measure of emotional reactivity (Driscoll, 2003). In addition, Pb-exposed rats made more of this error type in the non-drug state following both early and chronic Pb-exposure regimens, and this increased reactivity to errors in Pb-exposed rats was ameliorated by treatment with the drug chlordiazepoxide, suggesting a possible additional role for GABA-ergic mechanisms in these emotional reactivity related attentional deficits (Driscoll, 2003). The Pb-induced accuracy deficits observed in the post-weaning Pb-exposed rats in Chapter 2 may have been related to increased emotional reactivity of the Pb-exposed rats to prior errors or to the presence of distractors on the task, suggesting that these deficits may be related to similar cholinergic and/or GABA-ergic mechanisms. The amelioration of Pb-induced attentional dysfunction observed in the chlordiazepoxide study also suggests the potential of benzodiazepines as possible pharmacotherapeutic agents to alleviate these Pb-induced deficits, although further animal model studies are still needed to determine the safety and efficacy of these drugs for enhancing cognitive function in Pb-exposed subjects.

An additional candidate for the basis of these Pb-induced attentional impairments is the DA system underlying attentional function. As detailed above, the role of the DA system in attentional processes is well established, and Pb-exposure has been demonstrated to alter a variety of measures of central dopaminergic function. Specifically, studies have demonstrated amelioration of Pb-induced cognitive deficits in monkeys by chronic L-dopa treatment (Levin, et al, 1987), and Pb-induced alterations in tyrosine hydroxylase activity (Ramesh, et al, 1998), DA release (Struzynska, et al, 1994), DA metabolism (Lasley, et al, 1988), DA synthesis (Govoni, et al, 1979), and DA turnover (Lasley, et al, 1984). Following Pb exposure,

striatal DA levels decrease (Minnema, et al, 1986), which may cause compensatory supersensitivity of DA receptors (Cory-Slechta, et al, 1992). Alterations in sensitivity to peripherally administered dopaminergic compounds have also been demonstrated in rats using operant tasks (Cory-Slechta, et al, 1991; 1992). Although these findings suggest that the DA system is altered following Pb-exposure, additional studies from our lab shed light on the hypothesis that these DA alterations mediate Pb-induced attentional deficits. Specifically, subjects exposed to the same chronic post-weaning regimen utilized in chapter 2 or to a model of developmental Pb-exposure did not appear to be differentially sensitive to the impairing effects of either the selective D1 agonist SKF81297 or the selective D1 antagonist SCH23390 on the same attentional tasks used in this volume (Bayer, et al, 1994; Bayer and Strupp, unpublished data). These findings fail to provide evidence that alterations in DA activity, specifically at D1 receptors, underlie the Pb-induced deficits demonstrated on our task of visual attention. Further studies are needed to investigate the role of DA activity at D2 receptor subtypes in this dysfunction, as there is some evidence for D2 receptor modulation of some aspects of attentional function (Harrison, et al, 1997; Mehta, et al, 1999; Passetti, et al, 2003). The selective D2 antagonist sulpiride is a good candidate for pharmacological challenge studies investigating this hypothesis, because, unlike other D2 selective agents, it has demonstrated effects on performance on a similar visual attention task in rats (Harrison, et al, 1997; Passetti, et al, 2003) and in human tasks of attentional set-shifting (Mehta, et al, 1999). In light of recent evidence for the role of the D4 subtype of D2-type receptors in attentional dysfunctions (Faraone, et al, 2001; Shaw, et al, 2007; Swanson, et al, 2000), studies investigating the effects of selective D4 agents on attention in Pb-exposed rats would also be warranted. These additional drug challenge studies may help identify additional potential pharmacotherapeutic agents for the treatment of affected children.

**Lasting effects of prenatal cocaine exposure on catecholaminergic modulation of attention**

The pharmacological challenge studies in Chapters 3 and 4 provide evidence that prenatal cocaine exposure produces lasting changes in the catecholaminergic modulation of sustained attention. In Chapter 4, prenatal cocaine exposed rats were more sensitive to the impairing effects of the D1 DA receptor agonist SKF81297 on omission errors, an error type indicative of lapses in attention. In chapter 3, prenatal cocaine exposure also increased sensitivity to the effects of IDZ on this same error type, an effect that likely reflected IDZ-induced increases in NE-modulated dopamine activity. Taken together, these results suggest that prenatal cocaine-induced deficits in sustained attention may be due to increased sensitivity to DA activity, an effect which is likely mediated by prenatal cocaine-induced increases in stimulated DA release. This hypothesis is consistent with evidence from previous studies using similar IV cocaine exposure regimens that suggest that prenatal cocaine exposure does not alter basal DA levels or release (Du, et al., 1999; Elsworth, et al., l999; Wang et al, 1995; 1996), but does increase the amount of DA released in response to a stressor or a pharmacological challenge (Du, et al., 1999; Elsworth, et al., l999; Morrow, et al., 2001; Murphy, et al., 1995). There is evidence that testing on PFC-dependent tasks increases extracellular DA in PFC (Watanabe, et al., 1997), suggesting that the prenatal cocaine-exposed subjects in the present studies may have experienced increased DA release in PFC relative to controls when tested on our attentional tasks. This increased DA activity may have contributed to both the deficits in sustained attention observed in prior tasks (Morgan, et al., 1997), and to the increased sensitivity to the attentional effects of SKF81297 demonstrated in chapter 4. Systemic injections of alpha-2 adrenergic agents modulate stress-induced

increases in DA turnover (Morrow, et al., 2004) by regulating activity in the ventral tegmental area (Grenhoff and Svensson, 1988; Grenhoff and Svensson, 1989; Morrow, et al., 2004), suggesting that this differential dopaminergic response to stress or pharmacological challenge seen following prenatal cocaine exposure may be due to lasting alterations in NE-modulated DA release. Additional studies are needed to differentiate between lasting effects of the early drug exposure on the NE and/or the DA components of this system and to clarify how the increased sensitivity to the attentional effects of these agents arises at the receptor/intra-cellular level.

The increased sensitivity to DA activity demonstrated by prenatal cocaine-exposed subjects in Chapters 3 and 4 has important implications for the effects of stress on children exposed to cocaine *in utero*. Stress increases endogenous levels of NE and DA activity (Horger & Roth, 1996; Thierry, et al., 1976) and impairs prefrontal cognitive functions such as working memory (Arnsten and Goldman-Rakic, 1998) and attention (Hartley and Adams, 1974; Hockey, 1970). These stress-induced cognitive impairments have been shown to correlate with the amount of DA turnover in PFC (Murphy, et al, 1996a; 1996b). Individuals exposed to cocaine *in utero* may be more vulnerable to these adverse effects of stress on cognition. This hypothesis is consistent with the finding that prenatal cocaine-exposed rats showed increased sensitivity to the impairing effects of the pharmacological stressor FG-7142 on a PFC-dependent working memory task (Murphy, et al., 1995). This hypothesized effect of prenatal cocaine exposure on stress-induced attentional impairment is also consistent with reports of altered behavioral and neurochemical response to stress in cocaine-exposed animals (Bilitzke & Church, 1992; Church & Tilak, 1996; Elsworth, et al., l999; 2007; Goldstein, et al., 1993; Goodwin, et al., 1997; Johns & Noonan, 1995; Molina, et al., 1994; Morrow, et al, 2001; Wood, et al., 1993; 1994; 1995). Future research is needed to confirm whether the particular cocaine exposure regimen used in

the present studies increases sensitivity to stress-induced attentional impairment, and whether this increased sensitivity can be ameliorated by treatment with selective D1 or D4 antagonists, or with neuroleptic drugs such as clozapine or haloperidol that block DA and alpha-1 adrenergic receptors. These drugs have demonstrated success in reversing the deficits in PFC cognitive functions caused by stress-induced increases in DA activity in animal subjects (Arnsten and Goldman-Rakic, 1998; Arnsten, et al, 2000; Murphy, et al, 1996), and may have potential for the development of pharmacotherapeutic agents for the treatment of similar dysfunction in children exposed to cocaine in utero. In addition, further behavioral pharmacology studies with the drug guanfacine may provide further information about the basis and potential treatment of the observed prenatal cocaine-induced attentional impairments. Guanfacine, an alpha-2 adrenergic agonist used in the treatment of ADHD, has been demonstrated to improve ADHD patient performance on both standard ADHD symptom rating scales (Hunt, et al, 1995; Scahill, et al, 2001; Taylor and Russo, 2001) and on more specific tests of attention such as the CPT (Scahill, et al, 2001) and the Stroop task (Taylor and Russo, 2001). Additional drug challenge studies could determine whether guanfacine can ameliorate the types of attentional dysfunctions demonstrated by this same cohort of subjects in prior studies (Morgan, et al, 2002), shedding light on the potential use of this drug as a possible treatment for affected children. Finally, the suggestion that prenatal cocaine exposure may increase the adverse effects of stress on attentional function suggests that cognitive and behavioral interventions that focus on stress reduction may be beneficial to affected children.

In conclusion, the current studies demonstrated that dopaminergic and noradrenergic systems modulate specific and different aspects of attentional processing. The studies with Pb-exposed rats revealed that chronic post-weaning Pb

exposure causes impairments in sustained attention and/or emotional regulation, but these deficits are not related to changes in the noradrenergic modulation of attention. In contrast, the lasting impairments in sustained attention caused by prenatal cocaine exposure appear to be related to lasting alterations in NE-modulated DA release. These data provide a basic science contribution to the study of the pharmacology of sustained and selective attention and distractibility. In addition, they have applied value in the development of potential pharmacotherapies for children affected by these developmental toxins.

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