

**THE EFFECTS OF BICUCULLINE ON COCAINE SELF-ADMINISTRATION
IN MALE RATS DEVELOPMENTALLY EXPOSED TO LEAD**

A Thesis

by

RODRIGO VALLES JR

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2003

Major Subject: Psychology

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August 2003

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ABSTRACT

The Effects of Bicuculline on Cocaine Self-Administration in Male Rats

Developmentally Exposed to Lead. (August 2003)

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Chair of Advisory Committee: Dr. Jack R. Nation

Rationale: Lead-exposure during developmental periods may alter reinforcing patterns of drugs of abuse in adulthood. Anxiety related mechanisms may also influence drug intake. Interactions between the two altering factors may exist. *Objectives:* The present study examined the effects of perinatal lead-exposure on cocaine self-administration after a GABA_A antagonist pre-treatment. *Methods:* Female rats were exposed to a regimen of 16 mg lead daily for 30 days prior to breeding with un-exposed males. This continued throughout gestation and lactation until postnatal day (PND) 21. On PND 63, animals were implanted with indwelling jugular catheters. After a 7 day recovery period, animals were trained to self-administer 0.50 mg/kg cocaine intravenously [IV]. After stable responding had been established, testing procedures began using combinations of 0.03 and 0.06 mg/kg cocaine [IV] and 0.00, 0.50, 1.00 and 2.00 mg/kg bicuculline (a GABA_A antagonist) intraperitoneal [IP]. *Results:* Bicuculline pre-treatment caused directionally opposite effects in both treatment groups (Group 0-Lead and Group 16-Lead) at the 0.06 mg/kg cocaine dose. Group 0-Lead animals showed an increase in self-administration, while Group 16-Lead animals showed a decrease in responding on the active (cocaine) lever. Results at the 0.03 mg/kg cocaine dose showed no discernable pattern. Group 0-Lead animals decreased in active lever responding at the 2.00 mg/kg bicuculline dose.

Group 16-Lead animals showed no differences in responding at any dose of bicuculline.

Conclusions: These data further suggest the influential role of GABA in mediating cocaine reward and the ability of developmental lead-exposure to alter mechanisms mediating drug responsiveness even after exposure has terminated.

To Michelle

ACKNOWLEDGEMENTS

I would like to thank Jack Nation, Paul Wellman, Gerald Bratton, Antonio Cepeda-Benito, Aaron Cardon, Angelica Rocha, Anna Diller, Avanthi Tayi, Clint Tippett, Allison Davidson, Carol McNamara and Dao Ho for their help in completing this thesis.

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INTRODUCTION

Lead (Pb) contamination continues to be one of the major pollution problems in North America despite increased governmental regulations targeted to reduction of selected contaminants. Various routes of exposure to humans exist, of which most seem to be concentrated in inner cities (Harwell et al., 1996; Pirkle et al., 1998). Many of these urban communities have substandard living areas where residents do not possess the resources to protect themselves against toxicity-related health hazards. Lack of proper monitoring of lead safety levels in these areas has resulted in proliferation of exposure vectors. Substandard areas are often near industrial sections of a city or heavy traffic areas. Lead particles in the air, dust, soil, or water supply of these communities can often be a source for lead ingestion or inhalation. Occupational exposure can be directly dangerous for smelters, plumbers, welders, or paint plant workers and indirectly to their families since particles are often brought home on their clothes and shoes. Lead based paints and dust inhalation are a common source of non-occupational hazard.

Of particular concern are issues related to prenatal/postnatal exposure in children, who are especially at risk of being exposed to the effects of lead directly and/or via the mother during critical developmental periods. Inner city reports of lead-exposure state that up to 70% of children may be exposed to lead at clinically unsafe levels (Mielke, 1999). Continuous exposure, even at low levels, via inhalation or ingestion in children may result in cognitive and physiological deficiencies and subsequent behavioral alterations that could last well into adulthood (Godwin, 2001). Moreover, lead

This thesis follows the style and format of Psychopharmacology.

distribution to the fetus or newborn may have serious consequences because of the immaturity of the blood brain barrier which is yet to fully develop. Children exhibit increased brain lead absorption and decreased lead excretion. This is of particular concern based on recent findings from Canfield et al. (2003) confirming that even exposure below current allowable safety limits (10 μ g/dl) produce intellectual impairments in children.

Lead Neurotoxicity

Lead exposure in the living organism has various debilitating effects on the central nervous system. When introduced into an organism, lead ions are primarily drawn to proteins that normally bind to either calcium or to zinc. Sometimes these lead bound proteins become excessively active but in general lead tends to inhibit activity. Known lead/zinc interactions are the inhibition of the zinc enzyme δ -aminolevulinic acid dehydratase [resulting in inhibition of the heme-biosynthetic pathway] (Warren et al., 1998), transcription factor sites on several proteins, the human protamine 2 site affecting spermatogenesis (Quintanilla-Vega et al., 2000), and sites that affect DNA binding activity. Lead interactions with calcium suggest that it targets cells using calcium-mediated signal transduction by competitive interference of depolarization-induced exocytosis of neurotransmitter (Manalis and Cooper, 1973). Lead appears to promote phospholipid binding at lower concentrations than does calcium, particularly at the C2 binding site located on calcium mediated proteins (Markovac and Goldstein, 1988). Lead is believed to affect calcium-dependent activation of protein kinase C (PKC). This could potentially impact numerous endpoints from cell growth to memory (Newton, 1995).

Glutamatergic Systems

Glutamate is the primary excitatory neurotransmitter in the central nervous system. Four types of glutamate receptors are presently thought to exist, the *n*-methyl-*d*-aspartate (NMDA), AMPA, and kainate receptor (all of which are ionotropic), and the Metabotropic Glutamate Receptor. Lead toxicity appears to alter the NMDA receptor complex via noncompetitive blockade of calcium-mediated ion channels (Ujihara and Albuquerque, 1993). This results in lower levels of stimulated glutamate release in animals chronically exposed to inorganic lead (Lasley et al., 1999; Lasley and Gilbert, 2002). Further, statistically significant decreased glutamate levels have been noted in the rat dorsal hippocampus, medial-basal hypothalamus, rostral neostriatum and brain cortex of maternally lead-exposed animals (Leret et al., 2002). Interference with the NMDA receptor complex is also thought to affect thresholds for long-term potentiation (LTP), a cellular substrate of learning and memory (Gilbert et al., 1998). In addition, acute lead-exposure may cause cellular apoptosis in the developing brain by means of NMDA blockade (Dribben et al., 2002).

GABAergic Systems

Gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the central nervous system. The system includes two primary types of receptors, GABA_A (which acts on ionotropic chloride channels) and GABA_B (which acts on metabotropic potassium channels). Lead effects on the GABAergic system are similar to those of the glutamatergic system, acting primarily on voltage-dependent calcium channels.

Decreases in the amounts of evoked GABA release seem to occur in the brains of animals exposed to chronic lead in areas such as the dorsal hippocampus (Lasley et al., 1999;

Lasley and Gilbert, 2002) and brain cortex (Leret et al. 2002). Decreases in the pre-synaptic uptake of GABA have also been noted, possibly due to a decreased number of synaptic vesicles or an altered mitochondrial structure (Jablonska et al. 1994).

Dopaminergic Systems

Dopamine stimulates both inhibitory and excitatory postsynaptic potentials depending on the postsynaptic receptor. Two types of dopamine receptors have been documented, D1-like (includes D5) and D2-like (includes D3 and D4). D1 receptors may be exclusively postsynaptic and D2 are found both post and presynaptically. D1 stimulation increases second messenger cyclic-AMP whereas D2 stimulation decreases it. Insults caused to the dopaminergic system by lead-exposure affect all aspects of neurotransmitter function. Changes are observed in dopamine synthesis (Jadhav and Ramesh, 1997), release (Zuch et al., 1998; Kala and Jadhav, 1995a) and binding (Pokora et al., 1996). However these changes occur differently contingent on the lead-exposure period. Specifically, prenatal exposure results in increased dopamine and dopamine metabolite levels in discrete areas of the brain (Leret et al., 2002). Adult exposure results in directionally opposite effects (Tavakoli-Nehzad et al., 2000) which result in D1 and D2 supersensitivity due to upregulation stemming from decreased synaptic dopamine levels (Cory-Slechta and Widzowski, 1991). Several reports indicate that lead accumulation occurs in higher levels in the nucleus accumbens [NACC] (Kala and Jadhav, 1995b) and hippocampus (Gutowski et al., 1997; Leret et al. 2002; Stoltenburg-Didinger, 1994). This is of particular importance in that the NACC is a known mediator of drug rewarding effects (Roberts and Koob, 1982). Possible determinants include modulation of the calcium

dependent PKC 2 system (Ramin et al., 1993) or synaptic terminal auto-receptors (Lasley, 1992).

Lead and Behavior

Lead effects on central nervous system activity are, predictably, not without behavioral consequences. As previously mentioned, lead toxicity is thought to affect LTP, which is model of learning and memory systems. Disruptions from lead-exposure can lead to altered performance in tests that measure learning and memory (Salinas and Huff 2002a; Salinas and Huff 2002b). While there does not exist a sizable literature regarding lead and anxiety, known components of stress mechanisms are affected by lead toxicity. Specifically, lead effects on neurotransmitter mechanisms are thought to possibly affect stress reactivity or baseline anxiety levels in chronically exposed animals, in addition to locomotor activity and exploratory behavior (Moreira et al., 2001). Lead treated animals also have exhibited impulsive behaviors or an inability to suppress incorrect responding (Brockel and Cory-Slechta 1997). Pertinent to the present study, various alterations in schedule-controlled performance measures have been documented (Cory-Slechta, 1997). This pattern of behaviors prompted the examination of possible anxiety increasing properties of lead-exposure and the interaction with drug self-administration.

Lead/Drug Interactions

The widespread distribution of lead in urban areas coincides with a prevalence of drug use in these same areas and therefore merits closer examination (Ensminger et al., 1997). Animal studies have demonstrated numerous behavioral changes as a result of developmental exposure to lead at levels comparable to that of children who have been chronically exposed to the metal. Mesocorticolimbic (neurons originating in the ventral

tegmental area [VTA] and terminating in the NACC and frontal cortex) systems are known to be integral in modulating the rewarding effects of drugs with abuse liability and also are known, as mentioned above, to be affected by lead toxicity. Therefore it is hypothesized that lead burdens in the organism could possibly be affecting drug sensitivity or intake via altered molecular mechanisms. In regards to this issue, lead aberrations have been determined to have interaction effects with psychomotor stimulants and opiates in various behavioral measures, however the period of exposure is crucial in mediating the resulting behaviors (Miller et al., 2000a; Miller et al., 2000b; Nation et al., 2003a; Nation et al., 2003b; Rocha et al., 2003; Valles et al., 2003). Developmental exposure to lead results in a more dynamic and sometimes directionally opposite pattern of effects when compared to post-weaning lead-exposure.

Opiates

Disruption of opioid ligand and receptor development and function occurs due to developmental and adult lead toxicity (Kitchen, 1993). Adult animals exposed to 16 mg of lead acetate for 30 days resulted in a decreased magnitude of sensitization to the locomotor stimulating properties of repeated morphine administrations, even after blood lead had returned to control levels [$<1\mu\text{g/dl}$] (Miller et al., 2000b). Examination of the conditioned reinforcing properties of morphine has revealed attenuation at doses of 1.25 and 2.50 mg/kg by perinatal lead exposure to 16 mg lead acetate (Valles et al., 2003). These effects are further seen in a self-administration investigation using heroin, where a general suppression of responding was observed at intermediate intravenous [IV] doses (0.0023, 0.0045, 0.009, and 0.018 mg/kg/infusion) and in a progressive ratio preparation where attenuation was seen at all heroin doses [0.0023, 0.0045, 0.009, and 0.018

mg/kg/infusion] (Rocha et al., 2003). Since glutamate and dopamine are thought to aid in behavioral sensitization (Vanderschuren and Kalivas, 2000) and reinforcement (Bardo, 1998) of opiates, lead insults to these systems are likely resulting in changes to neurotransmitter concentrations and efficiency.

Neural Mechanisms of Cocaine

The behavioral effects of cocaine appear to be mediated by blockade of the reuptake transporter in pre-synaptic dopaminergic neurons resulting in an accumulation of dopamine in the synaptic cleft. Mesocorticolimbic neurons originating in the ventral tegmental area and terminating in forebrain areas are implicated in the rewarding effects of cocaine and other drugs of abuse (Bardo, 1998). Particular attention has been given to the NACC dopamine innervations (Roberts and Koob, 1982). Both D1 (Koob et al., 1987) and D2 (Bergman et al., 1990) receptor antagonism has resulted in an attenuation of the reinforcing properties of cocaine in self-administration preparations. Recent investigations have also suggested that D1 and D2 receptors differentially mediate cocaine reinforcement. Selective agonism of each receptor subtype produced downward (D1 agonist SKF 82958) and leftward (D2 agonist quinolorane) shifts in the cocaine dose-response curve (Caine et al., 2000).

In addition to dopaminergic activity, an increasing body of literature exists detailing the modulation of dopamine levels via other neurotransmitter systems, including the GABAergic system (Rahman and McBride, 2002). VTA GABAergic and glutamatergic inputs originating in forebrain regions respond to D1 dopamine receptor blockade by decreasing cocaine reward, lending to the possibility of dendritically modulated dopamine cell activity by indirect stimulation (Ranaldi and Wise, 2001).

Glutamate stimulation in NACC is also thought to augment the hedonic value of cocaine but not be necessary for maintaining self-administration (Cornish et al., 1999; Pulvirenti et al., 1992). Sensitization is known to occur via chronic administration of cocaine into NACC. This is prevented using MK-801 (an NMDA antagonist) in VTA but not induced by NMDA agonism suggesting a vital, but not sole, role for VTA projections modulating dopaminergic terminals in NACC (Vanderschuren and Kalivas, 2000). Both GABA and dopamine also have been shown to modulate cocaine-induced reinstatement, possibly via a dorsal prefrontal cortex-NACC core-ventral pallidum circuit (McFarland and Kalivas, 2001).

Cocaine

Chronic administration of inorganic lead in the adult animal produces an attenuation of the locomotor stimulating effects of repeated administration of 10 mg/kg cocaine IP (Nation et al., 1996), and attenuation of schedule-controlled operant responding at an intermediate dose of 20 mg/kg cocaine intraperitoneal [IP] (Burkey et al., 1997). Direct brain self-stimulation into the medial forebrain bundle is also diminished in animals exposed to lead as adults (Burkey and Nation, 1994). In addition, extracellular dopamine levels in the NACC are attenuated by chronic adult lead-exposure (Nation and Burkey, 1994). This dopamine depletion results is manifested in schedule-controlled drug self-administration as an attenuation to the decrements in responding produced by dopamine agonists (Cory-Slechta et al., 1996). These findings support the position that adult inorganic lead-exposure causes dopamine depletions in brain areas mediating drug reward.

Elsewhere, animals exposed to lead throughout gestation and lactation (perinatal exposure) using 16 mg lead show an attenuation of the reinforcing properties of 1.25 and 2.5 mg/kg cocaine [IP] in a conditioned place preference paradigm [CPP] (Miller et al., 2000a). Similar antagonism has been found in drug discrimination studies where perinatal lead-exposure has resulted in a subsensitivity to SKF-82958 (D1-like dopamine agonist), quinpirole (D2-like dopamine agonist) and apomorphine [D1/D2-like dopamine agonist] (Miller et al., 2001). However, directionally opposite effects are seen in acute versus chronic cocaine exposure in developmentally lead-exposed animals. Animals exposed to an identical lead-exposure protocol showed less responsiveness to the initial administration of cocaine but an augmentation of the stimulatory (locomotor sensitization) effects of repeated administration of 10.00, 20.00, and 40.00 mg/kg cocaine [IP] (Nation et al., 2000). Increased sensitivity has also been shown in a cocaine reinstatement preparation. Animals trained to self-administer 0.50 mg/kg cocaine [IV] were extinguished for 3 hours and then primed before the final test hour using either 0.00, 5.00, 10.00, 20.00 mg/kg cocaine [IP]. Developmentally lead-exposed animals infused greater amounts of saline after receiving 5.00 and 10.00 mg/kg cocaine (Nation et al., 2003b). Most pertinent to the proposed study, animals exposed to 16 mg lead throughout gestation and lactation, demonstrated an elevated sensitivity to repeated cocaine self-administration, manifested as a functional shift left from that of control animals in the dose-effect curve. Lead-exposed animals responded at significantly greater rates for 0.06 mg/kg cocaine, and at significantly lower rates for 1.25 and 2.5 mg/kg cocaine [IV] providing further supporting evidence that reinforcement mechanisms are altered so as to produce increased sensitivity in lead-treated animals (Nation et al., 2003a). Data from

Lynch and Carroll (2001) have sited station factors as possible determinants for decreased responding to increased or functionally higher doses of drug. It is important to note that in the Nation et al. (2003a) study, lead had gained clearance from tissues, yet the altered behavioral effect persisted. These data point to relatively permanent neuronal alterations in cocaine/dopamine related circuitries occurring during a critical period of developmental lead-exposure.

Anxiety

Neurochemistry

Stress can be defined as a physiological response to an aversive stimuli presented to an animal. This response prepares the organism for a possibly dangerous situation by placing the body in a catabolic state. While cardiovascular, immune, gastrointestinal and neuroendocrine systems are all affected in the presence of a stressor, for the purposes of this study the focus will be on neurotransmitters that modulate the hypothalamic-pituitary-adrenal (HPA) axis. The neuroendocrine response to stress begins with an increased secretion of epinephrine and norepinephrine from the sympathetic nervous system and adrenal medulla, followed by the release of corticotropin-releasing factors (CRF), vasopressin from parvicellular neurons into portal circulation, increased secretion of oxytocin from the pituitary and finally, 5-10 seconds later, adrenocorticotropin hormone (ACTH) is secreted by the pituitary which then enters general circulation and stimulates the adrenal gland to release adrenocorticosteroids (Sapolsky et al., 2000; Van de Kar and Blair, 1999). Among these are cortisol (in humans) and corticosterone (in rats). CRF mRNA is expressed in various brain regions including cerebral cortex, paraventricular nucleus of the hypothalamus, amygdala and hippocampus (Bittencourt

and Sawchenko, 2000). Receptors for CRF include two types (CRF1 and CRF2) and are widely located throughout the central nervous system. High concentrations of CRF1 receptors are found in the pituitary, cerebellum, brain stem, amygdala and cortex whereas CRF2 receptors are found predominantly in the lateral septum, choroids plexus, olfactory bulb, amygdala and hypothalamus (Chalmers et al., 1995; Perrin et al., 1993; Primus et al., 1997) CRF actions are mediated through G-protein linked receptors and antagonism is known to cause a decrease in ACTH and corticosterone (Gully et al., 2002, Kim et al., 1998).

GABA receptors are also known to be integrally involved in mediation of stress and anxiety (Nutt and Malizia, 2001), possibly due to forebrain pathways directly involved in stress-induced hormone secretion (Van der Kar and Blair, 1999). Stimulation of the GABA_A receptor complex results in inhibition of ACTH secretion to various stressors (Rhoher et al., 1994; Yagi and Onaka, 1996). Agonism of benzodiazepine receptors in the dorsal hippocampus and median raphe nucleus produce anxiolytic effects in social interaction tests (Gonzalez et al., 1998) and elevated plus-maze tests, perhaps due to autoreceptor blockade leading to endogenous GABA mediated stimulation of post-synaptic GABA_A receptors (Zarrindast et al., 2001). Intracranial antagonism of basolateral amygdala GABA_A receptors using bicuculline methiodide results in conditioned place avoidance (Thielen and Shekhar, 2002). Withdrawal of the GABA_A neuroactive steroid allopregnanone has also resulted in increases in anxiety in plus-maze tests in male and female rats alike (Gulinello et al., 2002; Smith, 2002). These studies implicate the HPA axis and GABA receptor complex as critical mediators of anxiety-like behaviors/states.

Cocaine/GABA/Anxiety Interactions

Since GABA is a known inhibitor of mesolimbic dopamine neurons and GABA is, as previously discussed, a modulator of anxiety related mechanisms; it has been proposed that GABA manipulations can alter cocaine reinforcement via changes in endogenous dopamine function. GABA_B receptor agonists have resulted in a decrease in infusions in self-administration protocols using cocaine (Brebner et al., 1999) and direct brain stimulation reward (Dobrovisky et al., 2002). Moreover, this effect is attenuated by the GABA_B antagonist CGP56433A in similar cocaine self-administration studies (Brebner et al., 2001). Inhibitors of GABA transaminase have shown attenuation to both cocaine sensitization (Gardner et al., 2002) and CPP (Ashby et al., 2002) tests respectively.

The use of diazepam (a GABA_A agonist) in BALB/cByJ knockout mice exhibiting high levels of trait anxiety possibly acts as an anxiolytic and thereby decreases the rewarding value of cocaine, which subsequently is manifest as an increase in cocaine self-administration (David et al., 2001). These same animals have also experienced facilitation to cocaine acquisition. Further evidence of GABA_A involvement in anxiety/rewarding interactions are presented in studies where frontal cortex stimulation of GABA_A neurons resulted in attenuation of locomotor stimulating effects of cocaine (Karler et al., 1998). Furthermore, GABA_A α 1 subunit knock-out mice show no hypolocomotor response to cocaine (Reynolds et al., 2003).

Evaluations of neuroendocrine mechanisms and cocaine reward also have yielded evidence to support an interaction between cocaine/GABA/anxiety. Reactivity to low doses of cocaine may be augmented by increasing circulating levels of plasma corticosterone, functionally altering the efficacy of the drug (Goeders, 2002). CRF

receptor subtype 1 antagonists have been proven to block both the induction (Lu et al., 2003) and reactivation of CPP (Lu et al., 2001). Findings from Lu et al. (2003) further indicate that CRF antagonism decreases the locomotor response, stereotype counts, and extracellular dopamine levels in NACC and VTA to cocaine. The effects of adrenalectomies on cocaine related behaviors parallels CRF antagonism as well (Przegalinski et al., 2000). Along these lines, exposure to repeated stressful episodes increased the locomotor effects of cocaine, facilitated acquisition, prolonged responding, and increased the breaking point in a progressive ratio test (Covington and Miczek, 2001). Since anxiogenic factors have been known to alter the rewarding properties of cocaine (Goeders, 2002), the existence of an interaction between developmental lead-exposure, GABA manipulation and cocaine self-administration is plausible. Accordingly, increases in anxiety have been examined as a possible factor in elevating the reward potency of cocaine.

Self-Administration Procedure

The intravenous self-administration procedure is generally accepted as a gauge of the reinforcing value of drugs with abuse liability. The IV self-administration procedure allows the animal to maintain an optimal level of drug by regulating the amount of responding during a given session (Lynch and Carroll, 2001; Wise et al., 1995). Free access to a drug produces an inverted U-shaped curve that visually represents averaged response rates to increasing concentrations of the drug. Maximal levels of responding are present at optimal doses. Further increases in drug concentration result in diminished responding (characterized as the descending limb of the dose-effect curve) perhaps due to a decreasing reinforcement value of the drug. Manipulations to the procedure that change

the reinforcing value of the drug are characterized as changes in the dose-effect curve. Typically, a shift left would indicate an increased potency whereas a shift right would indicate a decreased potency of the drug being tested.

Design and Hypothesis

In order to examine the underlying mechanisms that might be affected by perinatal lead-exposure, this study employed a standard cocaine self-administration preparation in conjunction with a GABA receptor manipulation. This procedure was aimed at expanding the understanding of the possible interactions between cocaine self-administration and lead-induced shifts in anxiety related endocrine factors stemming from GABA modulation.

Dams were developmentally (both during gestation and lactation) exposed to either 0 or 16 mg lead. Offspring behavioral testing commenced testing at about postnatal day 70. Each animal was trained on a baseline dose of cocaine [IV] and tested on alternating sets of two-day intervals using combinations of cocaine [IV] and bicuculline methobromide (a GABA_A antagonist) [IP] until all combinations were tested. The antagonist bicuculline methobromide was administered to control and lead-exposed (Group 0-Lead and Group 16-Lead respectively) animals approximately 10 minutes prior to introduction into an operant conditioning chamber with a predetermined fixed-ratio (FR) schedule for cocaine infusions.

Predictions

The hypothesis of this study was that bicuculline (a selective GABA_A antagonist) would augment the reward value of cocaine for both Group 0-Lead and Group 16-Lead. Because lead-exposed animals are known to respond differentially to low doses of cocaine in

contrast to non-exposed animals (Nation et al., 2003a), two doses (0.03 and 0.06 mg/kg) on the ascending limb of the dose-effect curve were chosen for testing (See Fig. 1).

Depending on their functional position on the dose-effect curve at either cocaine dose, bicuculline pre-treatment would act as an anxiogenic and shift active lever response rates further to the left; i.e., increased reward potency.

It was predicted that GABA antagonism would result in increased cocaine reinforcement efficacy via one/both of two possible mechanisms: dopaminergic disinhibition and activation of the HPA axis. GABA antagonism in the brain, primarily in the VTA, would result in disinhibition of dopaminergic projections to the NACC (a vital location in mediating drug reward [Roberts and Koob, 1982]) resulting in increased dopaminergic activation. Subsequently, increased reward potency would be experienced when cocaine (a dopamine reuptake inhibitor) was presented. Also, GABA antagonism may possibly affect stress via forebrain pathways involved in hormone secretion (Van der Kar and Blair, 1999). CRF circulation is critical in the stimulated release of ACTH which results in stimulation of adrenocorticosteroid release (Sapolsky et al., 2000; Van der Kar and Blair, 1999). Increased CRF is known to increase cocaine reward potency (Goeders, 2002). GABA_A agonism results in inhibition of ACTH secretion (Rhoher et al., 1994; Yagi and Onaka, 1996); therefore GABA_A antagonism by bicuculline would result in increased ACTH secretion and lead to increased reward potency.

This increase in the hedonic value of cocaine would manifest itself differently in Group 0-Lead vs. Group 16-Lead animals. As previously mentioned, differential responding (specifically an enhanced sensitivity) at low doses of cocaine is evident in lead-exposed animals. At the 0.03 mg/kg cocaine dose, ascending concentrations of

pretreatment with bicuculline would result in increases in responding by both groups. However, Group 16-Lead was expected to reach optimal levels at a lower concentration of the antagonist. At the 0.06 mg/kg cocaine dose, as concentrations of bicuculline ascended, responding in Group 0-Lead would increase while Group 16-Lead would decrease. Possible simultaneous decreases at the highest dose of bicuculline (2.0 mg/kg) in combination with 0.06 mg/kg cocaine might result in responding that was not statistically different between groups. This would be due to reward potency that had functionally approached or surpassed the 0.50 mg/kg dose of cocaine which is known to produce similar response rates in both non-exposed and lead-exposed rats (Nation et al., 2003a).

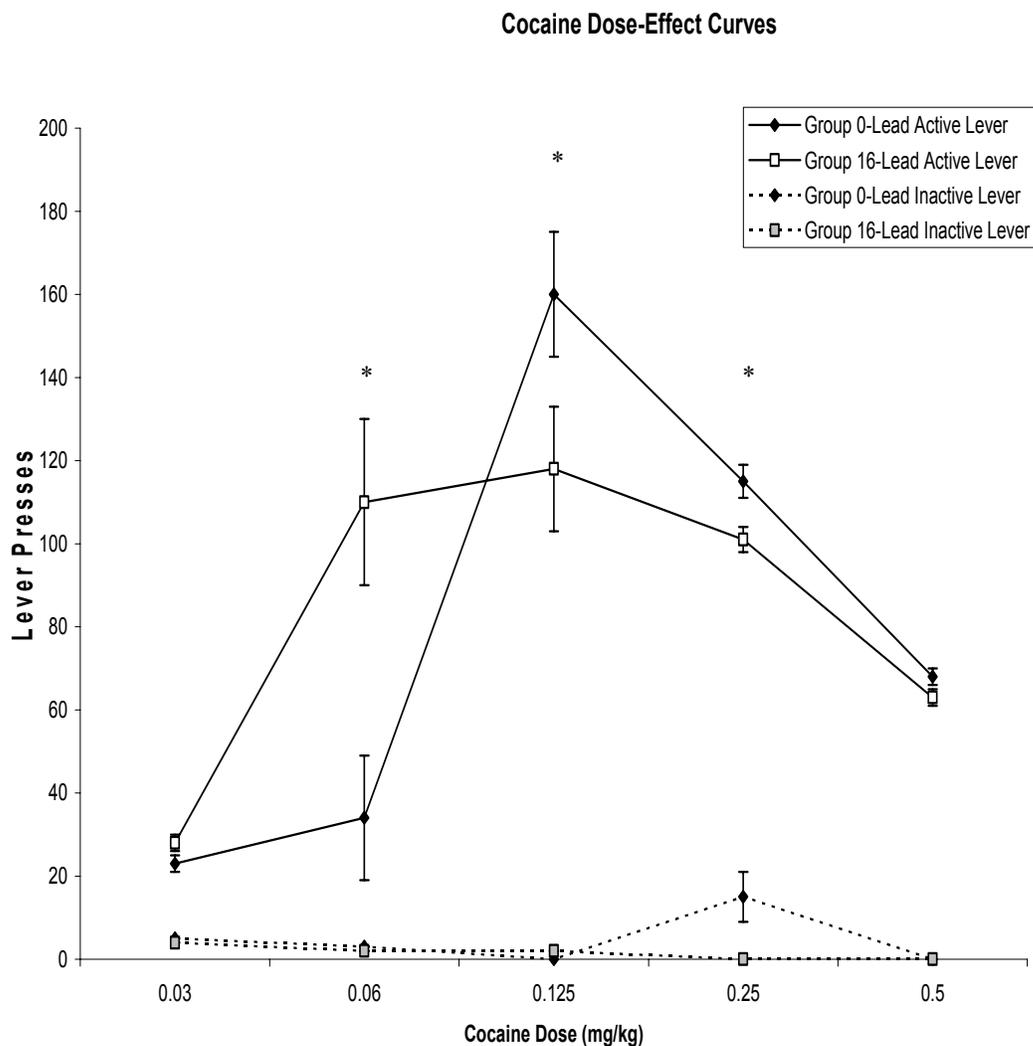


Fig. 1 Perinatal exposure to lead alters self-administration responding to IV cocaine. Mean rates of responding for each group and standard error values. The symbol * denotes statistical significance between groups at that dose (from Nation et al., 2003a).

MATERIALS AND METHODS

Animals

The research design and conduct of the experiment was approved by the Texas A&M University Laboratory Animal Care Committee, and all aspects of the research followed the guidelines outlined in Principles of Laboratory Animal Care (NIH publication No.85-23). All animals were maintained on a 12 hour dark/light cycle consisting of lights on at 8 am and lights off at 8 pm. Adult female Sprague-Dawley (Harlan; Houston, TX) rats (dams) were exposed to 0 (sodium acetate) or 16 mg lead (as lead acetate) daily via gavage, disregarding animal body weight. This approach has been used in our laboratory successfully in order to approximate environmental exposure to humans, because human lead-exposure in the environment does not occur according to bodyweight. A 16 ga gavage needle was used to administer the respective solutions in a volume of 1.0 ml deionized water at a pH of 5.5 for 30 days prior to breeding. Upon completion of the 30-day lead-exposure, non-exposed males were introduced into the home cage of the dam and lead-exposure of dams during breeding was continued as described. Once dams tested positive for copulatory plugs, males were removed from the breeding cage. The lead exposure regimen for dams remained uninterrupted throughout breeding, gestation and lactation. Pups were exposed to lead perinatally, having had transplacental exposure prenatally and no route of lead exposure other than the mother's milk supply postnatally. Dams were maintained on standard rat chow and water *ad libitum* throughout experiment. Daily bodyweights and weekly food intake data were recorded and analyzed.

On postnatal day (PND) 1, litters were culled to eight pups keeping the most males possible and using females if necessary to complete the litter. Only one pup from

each dam was used in the study in order to avoid any litter confounds that could arise (Holson and Pearce, 1992). Pups were weaned from the mother on PND 21 and housed two or three to a cage and placed on *ad libitum* food and water with no further exposure to lead. On PND 50 pups were separated and individually housed for the remainder of the study. Each animal had *ad libitum* access to food and water for the remainder of the study. All animals were kept on a 12hr/12hr dark/light cycle for the entire investigation.

Dams had tail-blood drawn in a volume of 100-150 μ l at the onset of breeding, at 10 days gestation and again at day one parturition (PND 1). At weaning (PND 21), blood was collected via cardiac puncture and brain, liver, kidney, and tibia samples were harvested from dams. Littermates were sacrificed at PND 1 and PND 21, and blood samples were used for later analyses. On completion of the study, all test animals were sacrificed in order to obtain blood and tissue samples as described above.

Surgery

On PND 63, chronic indwelling jugular catheters were implanted in 12 control and 12 lead-exposed male offspring. Rats were anesthetized with separate injections of 50 mg/kg ketamine and 50 mg/kg sodium pentobarbital administered intraperitoneally [IP]. A .01 interior diameter [ID] Silastic tubing (Dow Corning, Midland, MI) catheter was inserted into the right jugular vein and sutured to muscle tissue in the area of the vein. Using an 11 ga stainless steel tube as a guide, the catheter was passed subcutaneously through the body of the animal exiting the back between the scapulae. A backplate consisting of two stainless steel ovals separated by polypropylene mesh (Ethicon, Inc.; Somerville, NJ) provided an anchor for a spring leash, through which the catheter was threaded. Connecting to the backplate at one end, the other end of the leash was connected to a

single channel fluid swivel [22 ga] (Instech Labs, Plymouth Meeting, PA). The swivel design permitted an interlock with separate connecting arms located in the home cage and operant test chambers. The hinged arm allowed for a range of movement in either the home cage or test chamber. A .02 ID catheter continued from the top of the swivel to an infusion pump (Razel Scientific Instruments; Stamford, CT) that controlled the solution delivery. Animals were allowed 7 days to recover from surgery before commencing cocaine self-administration testing on about PND 70. During this recovery period, each animal received automated hourly intravenous [IV] infusions (200 μ l) of a sterile saline solution containing heparin (1.25 U/ml), penicillin g potassium (250,000 U/ml), and streptokinase (8,000 U/ml) in the home cage. After self-administration sessions, each cannulae were flushed with this solution, and then cleared with a subsequent infusion of 500 μ l heparinized saline. Catheter patency was checked throughout the experiment by administering an IV infusion of 7.50 mg/kg sodium pentobarbital. Loss of consciousness confirmed patency of the catheter in the vein.

Apparatus

Twelve operant conditioning chambers (Model E10-10, Coulbourn, Allentown, PA) in sound attenuating cubicles served as the test apparatus. Each chamber contained two levers with a stimulus light above each on one side of the enclosure. Drug delivery into the animal was controlled by an infusion pump (Razel Scientific Instruments; Stamford, CT) in each chamber. A 20-ml syringe delivered IV infusions of 160 μ l over a 6.00 sec time frame. Two IBM computers monitored and recorded drug deliveries from the chambers. Animals were run in two squads of 12 each, counterbalanced by group in

terms of squad assignment and chamber. All behavioral testing occurred during the light phase of the dark/light cycle.

Behavioral Testing

Baseline training commenced with infusions of a 0.50 mg/kg concentration of cocaine HCL (administered as the salt). Cocaine was suspended in a heparinized 0.9% saline solution. The schedule began as a fixed ratio (FR)-1 lever press (equaling one infusion) and continued as such until daily responding was steady-state (< 20% fluctuation over 7days) and then was switched to an FR-2 until steady-state responding again (< 20% fluctuation over 7days) was evident. The dose of 0.50 mg/kg/infusion cocaine allowed for a rapid acquisition of the drug-response contingency. Animals were given an initial manual infusion by the experimenter at the onset of the session and were administered another manual infusion if no response was scored for a period of time longer than 15 min thereafter.

All sessions in the operant chambers were 2 hours in duration. Responding at the appropriate FR on the active (right) lever resulted in a cocaine infusion and a simultaneous illumination of the stimulus light positioned directly above the lever. During training and testing, the houselights otherwise remained off. Lever responding at any time on the inactive (left) lever had no consequences but were monitored and recorded. A time out period (6.00 sec), in which further responding had no programmed consequences, was in effect during the duration of each infusion (160 ul over 6.00 sec) and responses during this period are not included in the analyses reported here.

Dose-effect testing commenced after stable baseline training was established (steady-state responding for FR-1 and FR-2). Cocaine test dose, GABA antagonist

(bicuculline) test dose, and squad assignment were counterbalanced across treatment conditions. Squads continued to run in two squads of 12 each. The schedule consisted of two days of testing with a combination of cocaine (0.03 or 0.06 mg/kg) [as indicated these doses were selected based on the previous data of Nation et al., 2003a) and the GABA_A receptor antagonist bicuculline methobromide (0.00, 0.50, 1.00 and 2.00 mg/kg) followed by two days of baseline sessions in order to reestablish stable responding. Bicuculline methobromide was suspended in a 0.9 % saline solution. Sessions continued until all combinations of 0.03 and 0.06 mg/kg cocaine and bicuculline had been completed. Bicuculline injections were administered IP, 10 minutes prior to the onset of testing. Animals were then placed into the operant conditioning chambers. The computers were then programmed to begin monitoring and subjects were given an initial manual lever prime. All doors were then closed and the testing session officially began.

Prior to the self-administration session, the catheter connecting the infusion pump and the swivel was flushed with a 1 ml solution of 95% ethanol and a subsequent 1 ml solution of heparinized saline to clear the line. The catheter was then reconnected to the syringe pump containing a freshly mixed dosing solution and filled completely with the new drug solution. After the jugular catheter was connected to the swivel, it formed a closed drug delivery system. The pump was then manually activated until the new solution was available to the animal for the initial infusion of cocaine by infusing the saline in the line into the animal.

Following the conclusion of the study, all animals were sacrificed using 50.0 mg/kg pentobarbital [IP]. Blood samples were taken via cardiac puncture. Brain, liver, kidney, and tibia were harvested and frozen at a temperature of -90° C. Blood and tissue

were later acid digested and analyzed for lead residues using mass atomic absorption spectrophotometry (Dearth et al., 2002).

Statistical Analyses

Food intake and body weight data were analyzed using separate analysis of variance (ANOVA) tests. Weekly food consumption served as the dependent measures in a 2 Groups (Group 0-Lead, Group 16-Lead) X (10 and 5) Weeks analyses. Average weekly body weight served as the dependent measures in a 2 Groups (Group 0-Lead, Group 16-Lead) X (11, 7 and 5) Weeks analyses

Performance during testing sessions was analyzed using ANOVA repeated measures on 2 Groups (Group 0-Lead, Group 16-Lead) X 2 Cocaine Doses (0.03 and 0.06 mg/kg) X 4 Bicuculline Dose (0.00, 0.50, 1.00, and 2.00 mg/kg) X 2 Levers (Active, Inactive) to determine if any main effects or interaction effects were present. Within subject factors were Cocaine Doses (0.03 and 0.06n mg/kg), Bicuculline Doses (0.00, 0.50, 1.00 and 2.00 mg/kg), and Levers (Active and Inactive). Baseline responding was measured using a 2 Groups (Group 0-Lead, Group 16-Lead) X 8 Scores X 2 Levers (Active, Inactive) ANOVA to determine if any main effects or interaction effects were present. Within subject factors were Scores and Levers (Active and Inactive). Neuman-Keuls post-hoc test procedures were performed when appropriate. Number of total lever responses was used as the dependent measure. The statistical significance level was set at $P < 0.05$ for all cases. Only animals completing all test dose combinations were included in these analyses. Due to animal deaths prior to finishing the entire study, Group 0-Lead retained $N=10$ and Group 16-Lead retained $N=8$ subjects.

Drugs

Cocaine HCL was provided gratis by NIDA (Research Triangle Park, N.C., USA).

Bicuculline methobromide was purchased from Sigma-Aldrich Co. (St. Louis, MO,

USA). Streptokinase and ampicillan sodium were purchased through the Texas A&M

University Large Animal Veterinary Pharmacy.

RESULTS

Body Weights

Dam bodyweights were analyzed using a 2 Groups X 11 Weeks repeated measures analysis of variance (ANOVA). No significant interaction effects were present [F(10, 230)=0.58, P>0.05]. A significant main effect for Week was found [F(10, 230)=184.33, P<0.001]. Post-hoc analyses revealed that bodyweights increased significantly from Weeks 1 through 7 for both control and lead (Group 0-Lead and Group 16-Lead respectively) animals. This was likely due to weight gains from maturation and later pregnancy. In addition, significant effects were also present by Groups [F(1, 23)=14.98, P<0.01]. One-way ANOVAs indicated statistically significant differences from Week 1 through 7. By way of contrast, Weeks 8 through 11 showed no differences. Together these data suggest different rates of body mass increases between groups until the animals reached stable bodyweights.

Pup bodyweights prior to onset of testing were analyzed with a 2 Groups X 7 Week ANOVA. No significant interaction effects [F(6, 96)=1.24, P>0.05] or Groups effects [F(1, 16)=0.96, P>0.05] were present. Main effects were observed for Week [F(6, 96)=278.25, P<0.001]. Post-hoc tests indicated that pup bodyweights steadily increased from, Weeks 2 -6 for Group 0-Lead and Week 4-7 for Group 16-Lead, as they matured. Group 0-Lead and Group 16-Lead animals had mean bodyweights of 378.0 ± 45.8 and 349.3 ± 48.0 g, respectively at onset of testing.

Pup bodyweights throughout testing were analyzed using a 2 Groups X 5 Weeks ANOVA. No significant interaction effects [F(4, 96)=0.54, P>0.05] or Group effects [F(1, 14)=0.13, P>0.05] were present. Main effects were observed for Week [F(4,

96)=75.94, $P < 0.001$]. Post-hoc tests revealed that bodyweights increased during Weeks 3-5 for Group 0-Lead and from Week 1 to Week 2 for Group 16-Lead. Group 0-Lead and Group 16-Lead animals had mean bodyweights of 448.6 ± 33.8 and 442.3 ± 24.1 g, respectively, at termination of testing and showed no significant differences.

Food Intake

Dam food intake was analyzed using a 2 Groups X 10 Weeks ANOVA. No significant interaction effects [$F(9, 207)=0.62$, $P > 0.05$] or Group effects [$F(1, 23)=3.18$, $P > 0.05$] were present. Main effects were observed for Week [$F(9, 207)=75.13$, $P < 0.001$]. Post-hoc tests revealed that food intake increased from Week 5 through 7 and from Week 9 to 10 for Group 0-Lead animals. This occurred during breeding weeks when males were also present in the homecage. Increases during Week 9 through 10 were likely due to the ability of the developing pups to reach and eat from the food bins. Group 16-Lead animals displayed the same pattern of increases in food consumption again indicating the presence of breeders and pups.

Pup food intake was analyzed using a 2 Groups X 5 Weeks ANOVA. No significant interaction effects [$F(4, 64)=0.61$, $P > 0.05$] or Group effects [$F(1, 16)=0.842$, $P > 0.05$] were present. Main effects were observed for Week [$F(4, 64)=25.49$, $P < 0.001$]. Post-hoc tests revealed that animals steadily increased in food consumption from Week 1 through 5. All animals were kept on a diet of 20 g of rat chow per day throughout testing therefore food intake was not monitored.

Behavioral Data

A preliminary four-way 2 Groups (Group 0-Lead and Group 16-Lead) X 2 Cocaine Doses (0.03 and 0.6 mg/kg) X 4 Bicuculline Doses (0.00, 0.50, 1.00 and 2.00 mg/kg) X 2

Levers (Active and Inactive) was used to analyze the behavioral data (not depicted). Main effects for Bicuculline Doses were not present [$F(3, 48)=1.70, P>0.05$]. Interaction effects were present for Bicuculline Doses X Groups [$F(3, 48)=3.25, P<0.05$]. This interaction is particularly significant in that it shows bicuculline differential rate-altering effects on active lever responding between Groups.

No interaction effects were observed for Cocaine Doses X Groups [$F(1, 16)=1.34, P>0.05$]. Cocaine Doses did show significant main effects [$F(1, 16)=8.66, P<0.05$]. Overall, less responding occurred at the lower dose of 0.03 mg/kg cocaine. This is congruent with previous studies in which 0.03 mg/kg cocaine maintained the lowest average rates of responding across all groups (Nation et al., 2003a).

Interaction effects for Levers X Groups were not evident [$F(1, 16)=0.99, P>0.05$]. However, significant main effects did exist for Levers [$F(1, 16)=127.73, P<0.001$]. These data illustrate that Inactive Lever was significantly less frequent than Active (cocaine) lever responding and in fact almost non-existent.

Effects for Bicuculline Doses Cocaine Doses approached but did not reach an acceptable level of significance [$F(3, 48)=2.67, P>0.05$]. Interaction for Bicuculline Doses Cocaine Doses X Groups were present [$F(3, 48)=11.43, P<0.001$] indicating alterations in response rates due to bicuculline, cocaine, and lead-exposure. Interaction effects were also present for Bicuculline Doses Cocaine Doses Levers X Groups [$F(3, 48)=9.76, P<0.05$], further showing the that responding was maintained on the active lever. These data prompted additional, more specific, analyses to be performed by Cocaine Doses (either 0.03 or 0.06 mg/kg) and without the inclusion of Inactive Lever

responses. All other pertinent interactions did not reach acceptable levels of significance and are not reported here.

0.03 mg/kg Cocaine

Behavioral data for 0.03 mg/kg cocaine was analyzed using a 2 Groups (Group 0-Lead and Group 16-Lead) X 4 Bicuculline (0.00, 0.50, 1.00 and 2.00 mg/kg) Dose repeated measures ANOVA. No significant effects were observed for Bicuculline Dose [$F(3, 48)=1.85, P>0.05$]. Effects for Group did not reach statistical significance [$F(1, 16)=2.182, P>0.05$]. Bicuculline Dose x Groups interaction effects were present [$F(3, 48)=4.36, P<0.05$]. One-way ANOVAs revealed significant differences between Group 0- and 16-Lead only at the 0.50 mg/kg dose of bicuculline [$F(1,17)=5.74, P<0.05$]. Group 0-Lead animals showed statistically and systemically significant decreases in response rates from doses of 0.50 to 2.00 mg/kg bicuculline (16.22 ± 2.54 and 10.50 ± 1.73 respectively). Group 16-Lead animals showed no changes in response rates for any dose of bicuculline when tested at a cocaine dose of 0.03 mg/kg.

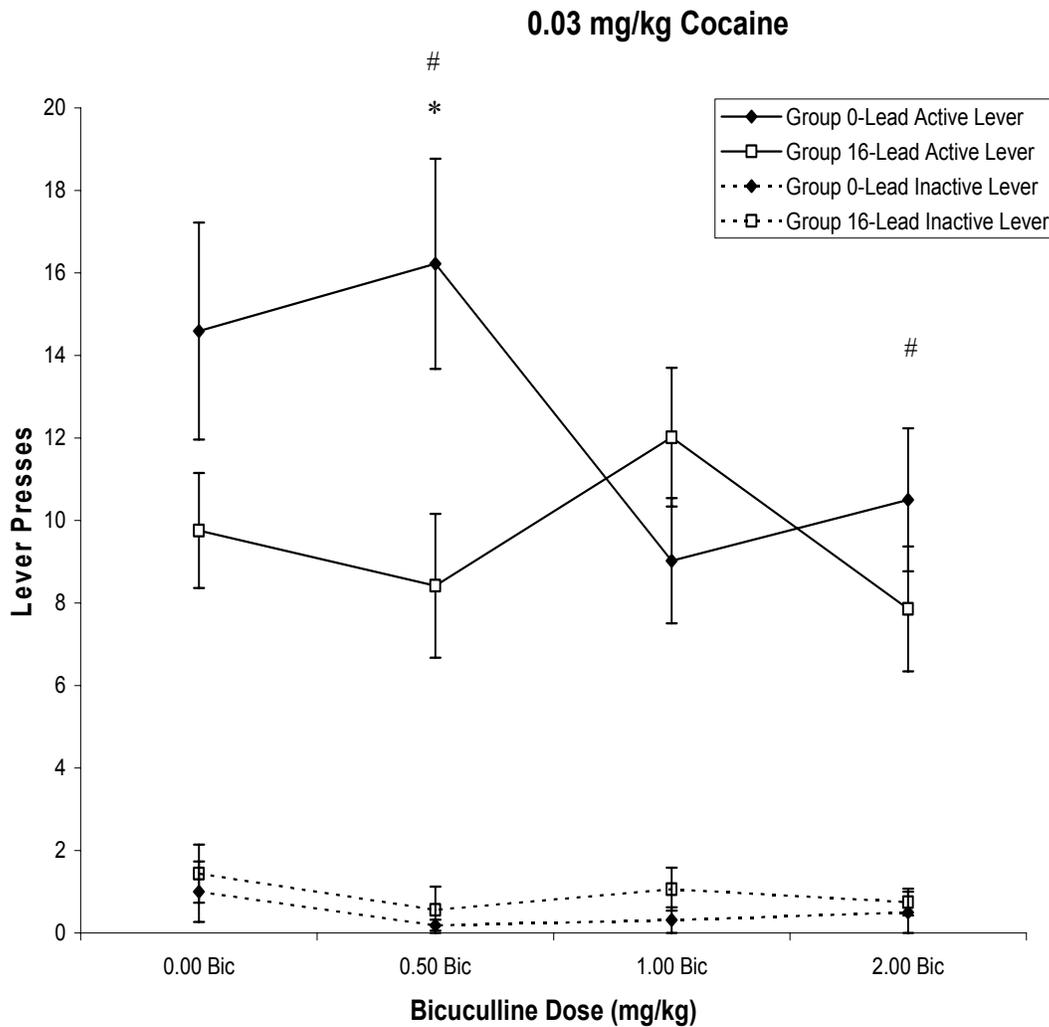


Fig. 2 Mean response rates and standard error values for Group 0- and 16-Lead at 0.03 mg/kg/infusion cocaine and bicuculline. The symbol * denotes statistical difference between groups. The symbol # denotes difference within Group 0-Lead at bicuculline doses of 0.50 and 2.0 mg/kg IP. Group 16-Lead did not produce altered response rates at any dose of bicuculline.

0.06 mg/kg Cocaine

Repeated measures ANOVAs were performed using 2 Groups (Group 0-Lead and Group 16-Lead) X 4 Bicuculline (0.00, 0.50, 1.00 and 2.00 mg/kg) Dose. Tests revealed no statistically significant main effects for Bicuculline Dose [$F(3, 48)=1.92, P>0.05$] or Group effects [$F(1, 16)=0.00, P>0.05$]. Interaction effects were apparent [$F(3, 48)=6.92, P<0.05$] however.

One-way ANOVAs indicated significant separation between Group 0- and 16-Lead at the 0.0 mg/kg dose of bicuculline [$F(1, 17)=10.42, P<0.01$] and 1.00 mg/kg dose of bicuculline [$F(1, 17)=4.66, P<0.05$]. Group 0-Lead post-hoc analysis showed statistically relevant increases from Bicuculline Dose 0.00 to 1.00 mg/kg (10.84±1.77 and 21.48±4.60 respectively). Post-hoc tests performed on Group 16-Lead show significant decreases in active lever responding from Bicuculline Dose 0.00 (23.78±3.91) to both 0.50 (10.11±1.31) and 1.00 mg/kg (9.41±2.35).

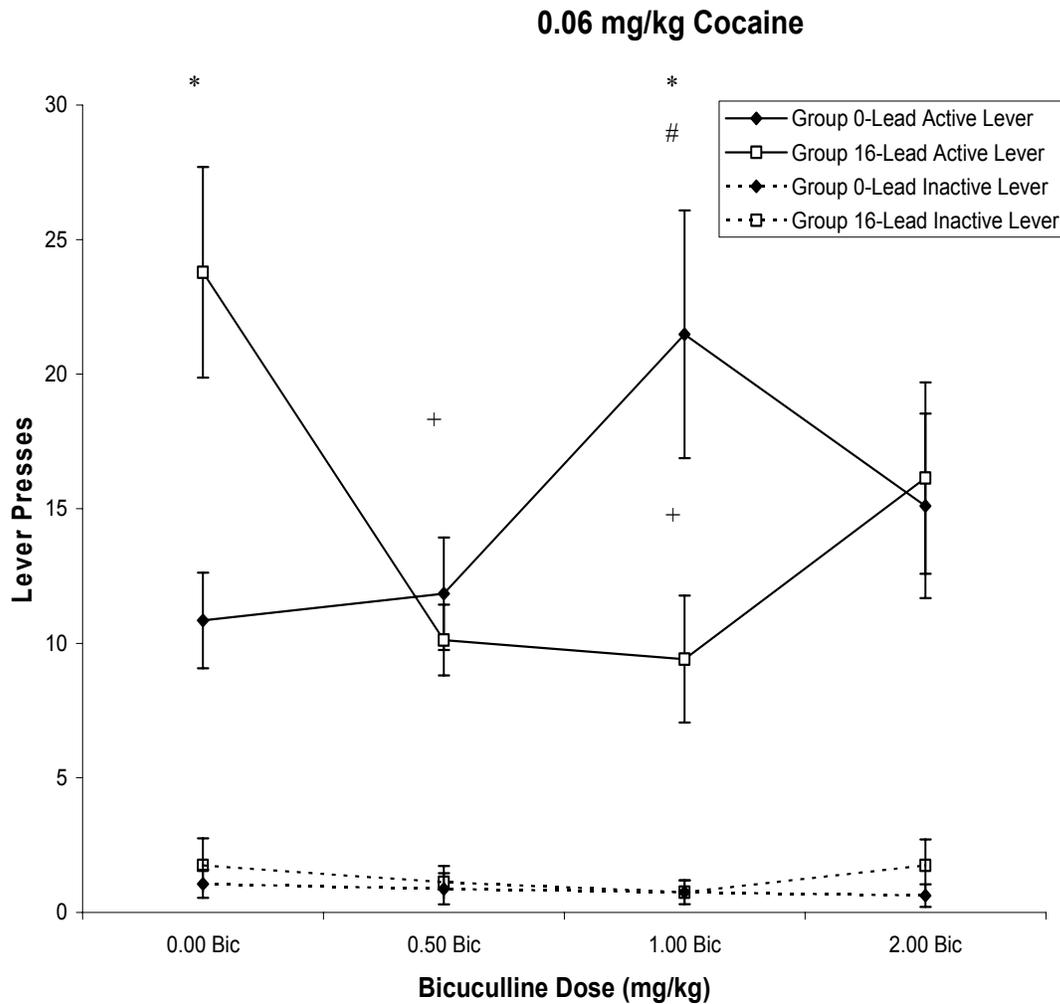


Fig. 3 Mean response rates and standard error values for Group 0- and 16-Lead at 0.06 mg/kg/infusion cocaine and bicuculline. The symbol * denotes statistical differences between groups. The symbol # indicates statistical increases in response rates from 0.00 mg/kg bicuculline IP in Group 0-Lead animals. The symbol + indicates statistical decreases in responding from 0.00 mg/kg bicuculline IP in Group 16-Lead.

0.50 mg/kg Cocaine

Baseline (0.50 mg/kg cocaine) data collected between the respective tests were analyzed using a 2 Groups (Group 0-Lead and Group 16-Lead) X 8 Scores X 2 Levers (Active and Inactive) repeated measures ANOVA. No effects for Scores [$F(7, 112)=1.81, P>0.05$] or Group effects [$F(1, 16)=0.09, P>0.05$] were evident. Main effects were present for Lever [$F(1, 16)=116.53, P<0.05$] indicating that inactive lever responding was insignificant.

These results parallel findings from Nation et al. (2003a), in which 0.50 mg/kg cocaine showed no differences in response rates between groups. All other results were statistically insignificant and were not reported here.

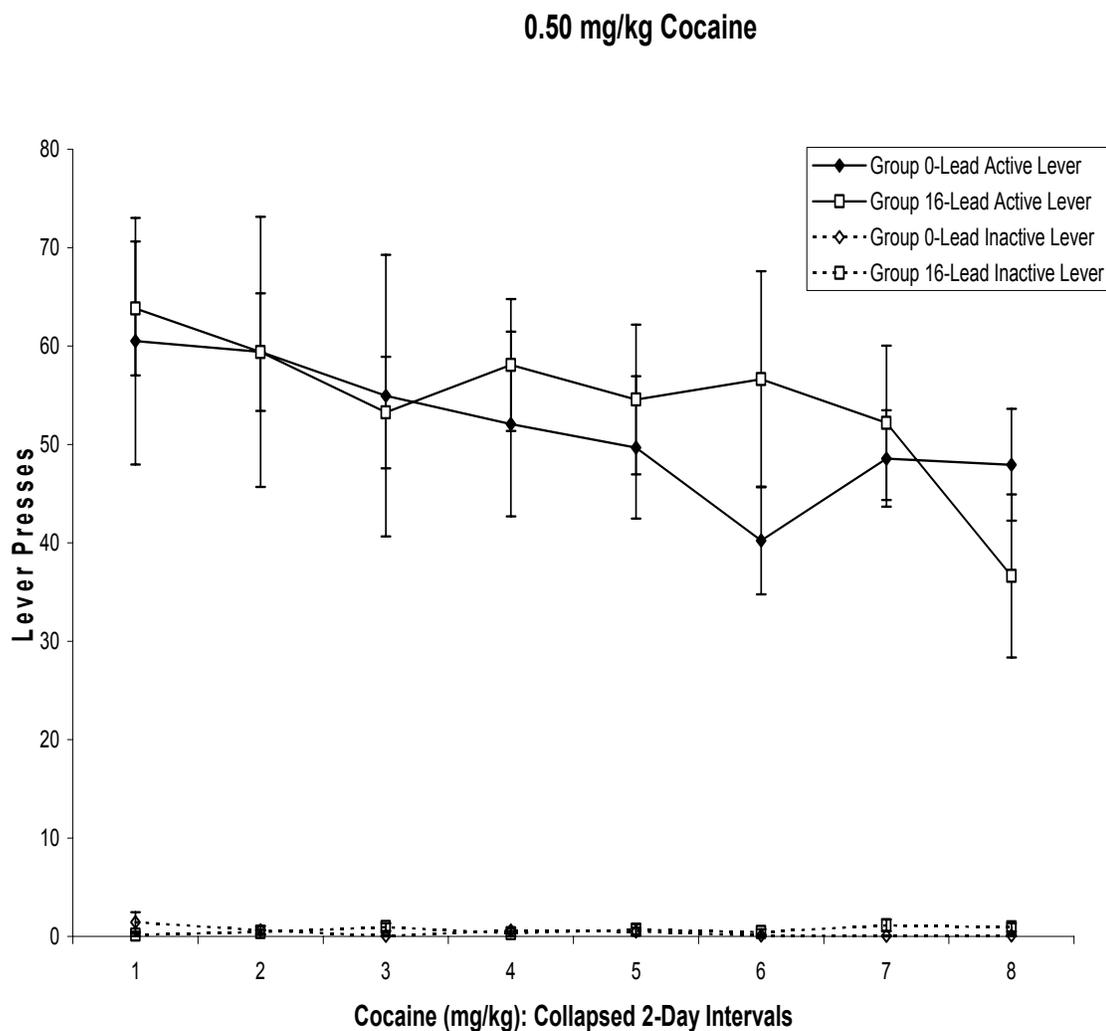


Fig. 4 Mean baseline responding rates and standard error values for Groups 0- and 16-Lead at 0.50 mg/kg/infusion cocaine. Collapsed baseline responding between test sessions showed no statistical differences at any point.

Blood and Tissue

It is apparent from Tables 1 and 2 that dams and pups showed greater concentrations of lead accumulation in blood in Group 16-Lead. Due to a sampling error, termination blood and tissue in subjects is not presented. However, numerous studies using this procedure have shown no difference in blood, brain and liver lead levels upon completion of test procedures (Nation et al., 2003a; Nation et al., 2003b, Rocha et al., 2003; Valles et al., 2003) even though behavioral effects persisted well into the adult life cycle (Nation et al., 2000).

Table 1 Mean blood lead levels for Group 0- and 16-Lead dams at breeding, gestation 10, PND 1 and PND 21.

Dams	Group 0-Lead	Group 16-Lead
Breeding	2.5±0.9 µg/dl	54.3±3.7 µg/dl *
Gestation Day 10	2.0±0.3 µg/dl	44.6±3.0 µg/dl *
PND 1	2.4±0.6 µg/dl	62.0±5.6 µg/dl *
PND 21	3.0±1.3 µg/dl	23.6±1.4 µg/dl *

* Indicates statistical significance from Group 0-Lead at same sampling day.

Table 2 Blood lead levels for Group 0- and 16-Lead littermates at PND 1 and 21.

Littermates	Group 0-Lead	Group 16-Lead
PND 1	1.6±0.3 µg/dl	46.3±8.4 µg/dl *
PND 21	1.2±0.2 µg/dl	11.2±1.6 µg/dl *

* Indicates statistical difference from Group 0-Lead on that sampling day.

DISCUSSION

Intravenous [IV] cocaine deliveries at both the 0.03 and 0.06 mg/kg dose in combination with 0.00 mg/kg bicuculline intraperitoneal [IP] replicated findings from Nation et al. (2003a) inasmuch as lead-exposed (Group 16-Lead) animals maintained responding at higher rates at these low doses of cocaine. A functional shift left of the dose-effect curve was evident in that Group 16-Lead animals lever pressed significantly more at 0.06 mg/kg cocaine and 0.00 mg/kg bicuculline combination than control (Group 0-Lead) animals. Increasing concentrations of bicuculline in combination with 0.06 mg/kg cocaine produced directionally opposite effects for both groups as illustrated in Fig. 2 by the systemically increasing rates of responding by Group 0-Lead and decreasing rates of responding by Group 16-Lead animals.

Brain regions such as the nucleus accumbens (NACC) have long been implicated in cocaine reward (Wise and Bozarth, 1987), however increasing data point to other systems as modulators of cocaine neurochemical effects. Modulation of the GABA receptor complex is known to alter anxiety and stress related behaviors. Stimulation of GABA_A and GABA_B receptors act as anxiolytics (Gonzalez et al., 1998) and are thought to decrease cocaine reward (Brebner et al., 1999; David et al., 2001; Zarrindast et al., 2001). Bicuculline anxiety promoting properties, via antagonism of GABA_A receptors, have been documented (Thielen and Shekhar, 2001) and are the cornerstone of this study. GABA modulation of mesocorticolimbic dopaminergic pathways has been shown to alter cocaine reinforcement by stimulating dopamine neurons in areas known to be integral in drug efficacy (Ranaldi and Wise, 2001). Mechanisms altering corticotropin-releasing factors (CRF) affect adrenocorticotropin hormone (ACTH) release, which result in

secretion of adrenocorticosteroids from the adrenal gland (Gully et al., 2002; Kim et al., 1998). Modulation of this system, the hypothalamic pituitary-adrenal-cortical (HPA) axis, is known to produce altered behaviors in cocaine testing preparations (Goeders, 2002). Since anxiety and stress behaviors are possibly influenced by GABA activity (Nutt and Malizia, 2001; Smith, 2002; Thielen and Shekhar, 2001), it is possible that the GABAergic system is involved in the neuroendocrine cascade resulting from a stress response.

Differential sensitivity to cocaine at the 0.06 mg/kg dose was present prior to the administration of bicuculline due the developmental effects of lead on brain neurotransmitter systems. Lead ions bind to calcium-mediated proteins including GABA receptors in the central nervous system (Godwin, 2001). Lead toxicity causes deficits in GABA availability (Lasley et al., 1999; Lasley and Gilbert, 2002) and possibly increases the reward value for psychomotor stimulants by changing anxiogenic properties. Lead increases dopamine binding in NACC (Pokora et al., 1996) and numbers of dopamine neurons in the ventral tegmental area [VTA] (Tavakoli-Nezhad et al., 2001) adding to the potential for increasing the reward value of drugs of abuse. Behavioral manifestations, as they relate to cocaine self-administration, of such a pattern of neuronal alterations can be seen in Nation et al. (2003a). Therefore, functionally, either treatment group was operating at different levels of the dose-effect curve, which can account for the pattern of responding that resulted from cocaine/bicuculline/lead interactions seen in this investigation. Group 0-Lead animals at the 0.06 mg/kg cocaine dose were on the ascending limb of the curve, 1.25 mg/kg being their optimal dose (See Fig. 1). Group 16-Lead animals, due to the shift left, were experiencing 0.06 mg/kg cocaine as their peak

responding dose (see Fig. 1). Peak responding and the descending portion of the dose-effect are thought to represent satiation effects by the animal to increasing doses of drug (Lynch and Carroll, 2001). This would further suggest that any increase in the rewarding properties of the drug would drive Group 0-Lead animals up and possibly over the ascending limb of the dose-effect curve. Group 16-Lead animals would only show a decrease in responding as they are already responding at peak levels. Therefore the resulting behavioral data for 0.06 mg/kg cocaine are in line with our predictions that Group 0-Lead animals would show increasing, while Group 16-Lead animals would show decreasing response rates due to functionally higher reward value of 0.06 mg/kg cocaine and increasing concentrations of bicuculline. Since bicuculline is thought to cause a potentiation of cocaine reward value either directly via dopamine disinhibition or indirectly via neuroendocrine stimulation of the HPA axis, it is possible that GABA/lead interactions were synergistically increasing cocaine reward. The data from this study parallel this explanation as shown in Fig. 2.

Findings from the 0.03 mg/kg cocaine and bicuculline combinations are less compelling. While the combination with 0.00 mg/kg bicuculline showed no differences between groups as hypothesized, 0.50, 1.00, and 2.00 mg/kg combinations did not produce the predicted trends. In other words, no apparent increases in responding were evident for Group 0-Lead animals. Specifically, only a decrease was shown at the highest dose of bicuculline (See Fig 3.). On the whole, it did not appear that the rewarding properties of cocaine were increased for Group 16-Lead animals, since there were no changes in response rates at any bicuculline dose. Perhaps the 0.03 mg/kg dose of cocaine is just too low to be affected by GABA antagonism.

The results from this study lend to the burgeoning literature that implicates the interaction of various neurotransmitter and neuroendocrine systems in the mechanisms underlying the reward value of drugs of abuse. Increases in GABA availability (Gerasimov et al., 2000) and GABA_B receptor agonism (Brebner et al., 2000) have been known to diminish the reward value of cocaine by decreasing dopamine stimulation. As a result, GABA agonists have the potential for functioning as possible treatments for drug addictions (Brebner et al., 2002; Cousins et al., 2002). The present behavioral data further explicate the anxiety altering properties of the GABA complex and its interaction with cocaine and developmental lead-exposure. Specifically, antagonism may result in indirect increases in rewarding value of drugs with abuse liability. Data from the 0.06 mg/kg cocaine dose illustrate the response rate-altering effects of bicuculline in two groups of animals experiencing functionally different reward values for low doses of a psychomotor stimulant.

These data may be relevant to the human population. Lead contamination continues to be a problem in industrialized nations and particularly in urban areas (Harwell et al., 1996; Pirkle et al., 1998). The coincidence of drug abuse problems with elevated lead-exposure vectors in the inner city are cause for further investigation into possible interactions. Children in particular are susceptible to the debilitating effects of lead-exposure and have been known to show cognitive deficits with blood lead levels below current allowable safety limits (Canfield et al., 2003). Along these lines lead-exposure both developmentally and in adulthood are showing increasing evidence of modulating brain areas associated with drug reward and anxiety mechanisms.

In conclusion, further research is necessary in developing a complete understanding of cocaine/anxiety/lead interactions. Tests measuring anxiety levels in perinatally lead-treated animals receiving GABA agonist or antagonist pretreatments are needed in order to determine anxiety/cocaine interactions in protocols similar to the one used here. Additional measures of reward value using intra-cranial administration could implicate specific brain areas or systems in this complex set of interactions.

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ARTICLES

Cardon AL, Rocha A, Valles R, Bratton GR, Nation JR (2003) Exposure to cadmium during gestation and lactation decreases cocaine self-administration in rats. manuscript submitted.

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