National Institute on Drug Abuse

RESEARCH

MONOGRAPH SERIES

Biological Mechanisms and Perinatal Exposure to Drugs

158

U.S. Department of Health and Human Services • Public Health Service • National Institutes of Health

Biological Mechanisms and Perinatal Exposure to Abused Drugs

Editor:

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NIDA Research Monograph 158
1995

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

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ACKNOWLEDGMENT

This monograph is based on the papers from a technical review on "Biological Mechanisms and Perinatal Exposure to Abused Drugs" held on May 25-26, 1994. The review meeting was sponsored by the National Institute on Drug Abuse.

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National Institute on Drug Abuse
NIH Publication No. 95-4024
Printed 1995

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Preface

Pushpa V. Thadani

On May 25 and 26, 1994, the National Institute on Drug Abuse held a Technical Review entitled "Biological Mechanisms and Perinatal Exposure to Abused Drugs" to review and discuss state-of-the art findings in this important area, to identify needs and future research opportunities, and to promote interdisciplinary communications. Biomedical researchers discussed the recent advances in the development of innovative animal models and their utilization to monitor drug-induced changes in fetal and neonatal physiological systems under controlled conditions. They also discussed the biological mechanisms responsible for the transient or long-term effects of perinatal exposure to abused substance(s) or the lack of these effects and the relevance of these experimental findings in different animal species to clinical reports/observations. The methodological and paradigmatic issues that need to be considered and integrated in future experimental study designs were also discussed.

A conference report summarizing the major findings and the methodological and experimental issues discussed at the meeting (and which lists all conference participants) was published in the March 1995 issue of "Synapse" (Thadani 1995). This monograph consists of the papers presented at the Technical Review meeting.

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Species-, Gender-, and Pregnancy-Related Differences in the Pharmacokinetics and Pharmacodynamics of Cocaine

Hisayo O. Morishima and Robert A. Whittington

INTRODUCTION

A great number of studies in cocaine research have expanded researchers' basic knowledge of the pharmacologic and physiologic effects of cocaine in humans and animals. This chapter will discuss species-, gender-, and pregnancy-related differences in cocaine disposition and how these biological factors may modify the effects of cocaine. The effects of route and mode of drug administration that may likewise influence the pharmacokinetics, pharmacodynamics, and possibly the toxicity of cocaine will also be examined. It is not the authors' purpose to provide a complete review of the effects of cocaine, but rather to illustrate the importance of these factors in cocaine-associated adverse effects. More specific information regarding perinatal cocaine exposure on the fetus and newborn has been reviewed in other chapters of this research monograph.

COCAINE METABOLISM, PHARMACOKINETICS, AND PHARMACODYNAMICS

The metabolism of cocaine involves several different pathways (figure 1). In humans, cocaine has an elimination half-life from 42 to 82 minutes (Barnett et al. 1981) and is metabolized rapidly by three major pathways with the resultant formation of two primary metabolites: ecgonine methyl ester and benzoylecgonine. The metabolite ecgonine methyl ester is thought to be formed by hepatic as well as by plasma esterases, while formation of benzoylecgonine occurs by spontaneous, nonenzymatic hydrolysis particularly at physiological pH (Ambre et al. 1991; Stewart et al. 1979; Van Dette and Comish 1989).

About 1 to 2 percent of cocaine is biotransferred to a pharmacologically active metabolite, norcocaine, by an N-demethylation reaction catalyzed

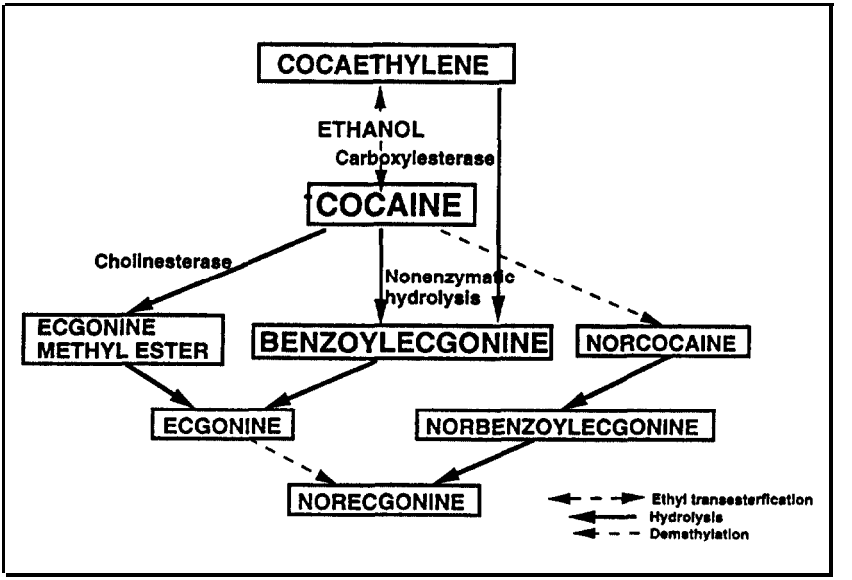

FIGURE 1. *Metabolic pathways of cocaine.*

by hepatic microsomal enzymes. These enzymes play an important role in drug-induced toxicity. It is suggested that the cocaine-induced hepatotoxicity seen in animals may be due to norcocaine and its metabolite N-hydroxy-norcocaine (Hawks et al. 1975; Nayak et al. 1976). The authors' comparative toxicity study of norcocaine and cocaine in rats indicates that the lethal dose of norcocaine is smaller. In addition, massive cerebral and hepatic hemorrhages were commonly observed in animals that died from norcocaine (Morishima et al., unpublished data). Data also show that human plasma norcocaine concentrations measured during concomitant alcohol and cocaine use are 2 times higher than those measured in the presence of cocaine alone (Farre et al. 1993). At present, it is unclear whether these increased norcocaine concentrations are a result of alcohol-related changes in either microsomal enzymes or nonspecific esterases.

In humans, cocaine metabolites are excreted almost entirely in urine with an elimination half-life of 4.2 hours for ecgonine methyl ester and 5.1 hours for benzoylecgonine (Ambre et al. 1984, 1988; Hamilton et al. 1977; Inaba et al. 1978; Jeffcoat et al. 1989; Jindal et al. 1978; Smith 1984).

The pharmacological and toxic effects of cocaine may be potentiated by combined ethanol use. Cocaine in the presence of alcohol abuse is biotransformed into ethyl-benzoylecgonine (cocaethylene) by a nonspecific liver carboxylesterase (Bosron et al. 1991; Boyer and Petersen 1991; Dean et al. 199 1; Elsworth et al. 1990). Concentrations of cocaethylene often exceed the concentration of cocaine in blood, brain, liver, and urine (Hear-n et al. 1991; Jatlow et al. 1991). Moreover, in the brain cocaethylene may be a much more selective dopamine agonist than cocaine (Jatlow 1993). Findings also indicate that cocaine and cocaethylene induce similar psychomotor stimulant effects in mice (Katz et al. 1992). On the other hand, as shown in figure 2, data in rats demonstrate that cocaethylene is more potent than cocaine in producing central nervous system (CNS) and cardiovascular manifestations (Whittington et al. 1995). Furthermore, unlike cocaine, there were no gender- or pregnancy-related differences in the observed cocaethylene toxicity.

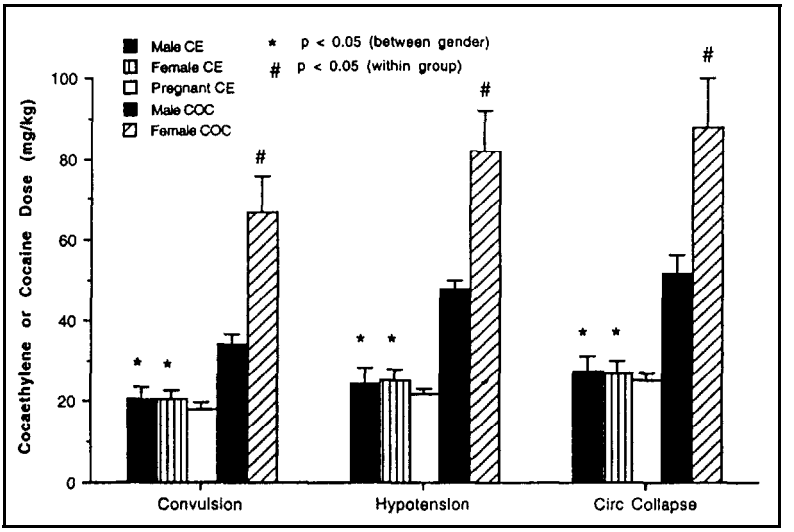

FIGURE 2. *Comparison of the mean (±SE) dose of cocaethylene (CE) and cocaine (COC) necessary to produce major toxic manifestations in awake male, nonpregnant female, and pregnant rats. CE or COC was infused intravenously at a rate of 2 mg/kg/min.*

SPECIES-RELATED DIFFERENCES IN DISPOSITION OF COCAINE

Recent findings show large species-related differences in the pharmacokinetics of cocaine. For example, the volume of distribution of cocaine in humans was approximately 2 liters per kilogram (L/kg) (Barnett et al. 1981), while in guinea pigs, the volume of distribution was found to be dose-dependent (Sandberg and Olsen 1991*a*). For example, at a dose of 2 milligrams per kilogram (mg/kg) the volume of distribution was 2.1 L/kg, while at 6 mg/kg it was 3.4 L/kg. Studies in sheep showed an elimination half-life of cocaine to be 5 minutes following a 5 mg/kg intravenous (IV) dose, confirming previous findings (DeVane et al. 1991). These studies also showed a rapid decline in plasma cocaine concentrations and that ecgonine methyl ester is the major cocaine metabolite in sheep (figure 3). The concentrations of ecgonine methyl ester and benzoylecgonine remained elevated during the first 70 minutes followed by a gradual decrease, while the concentration of norcocaine,

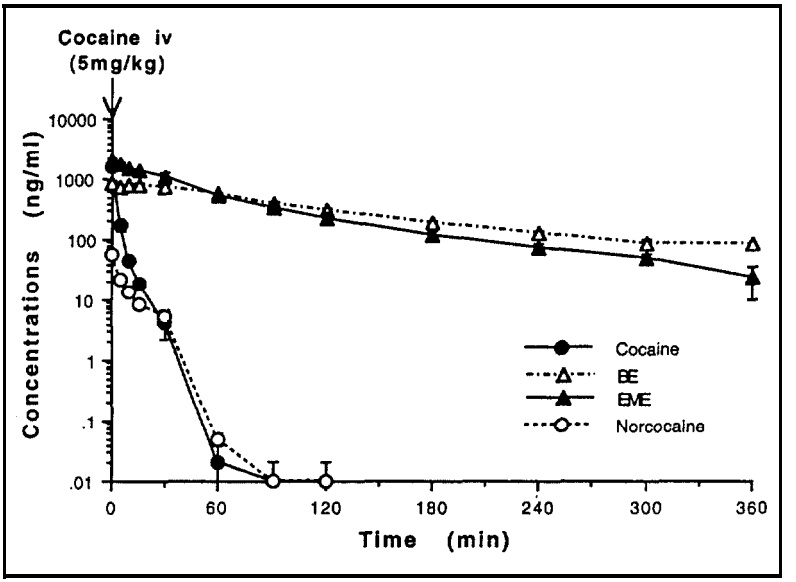

FIGURE 3. *Mean (±SE) values for cocaine, benzoylecgonine, ecognine methyl ester, and norcocaine in nonpregnant sheep following bolus IV administration of cocaine, 5 mg/kg (N = 7).*

KEY: BE = benzoylecgonine, EME = ecognine methyl ester.

a minor metabolite, decreased sharply. The presence of ecgonine methyl ester as a major cocaine metabolite in sheep is interesting because of the extremely low plasma cholinesterase activity (figure 4) that has been observed in sheep compared to several other species (Morishima, unpublished data). This suggests that other esterases (e.g., liver esterases) may be catalyzing the formation of this metabolite in sheep.

Species-related differences also exist in the formation of cocaethylene following coadministration of cocaine and ethanol. Virtually no measurable cocaethylene was found in sheep, which is unlike humans (McCance-Katz et al. 1993; Perez-Reyes and Jeffcoat 1992). In humans, a peak cocaethylene concentration of 60 nanograms per milliliter (ng/mL) was observed 2 hours after coadministration of oral ethanol and intranasal (IN) cocaine (Jatlow 1993). The differences in the ability to form cocaethylene may stem from differences in enzyme activity and variations in metabolic pathways present among the different species.

To examine the species-related differences in the excretion of cocaine and its metabolites, urine samples collected over a period of 6 hours were

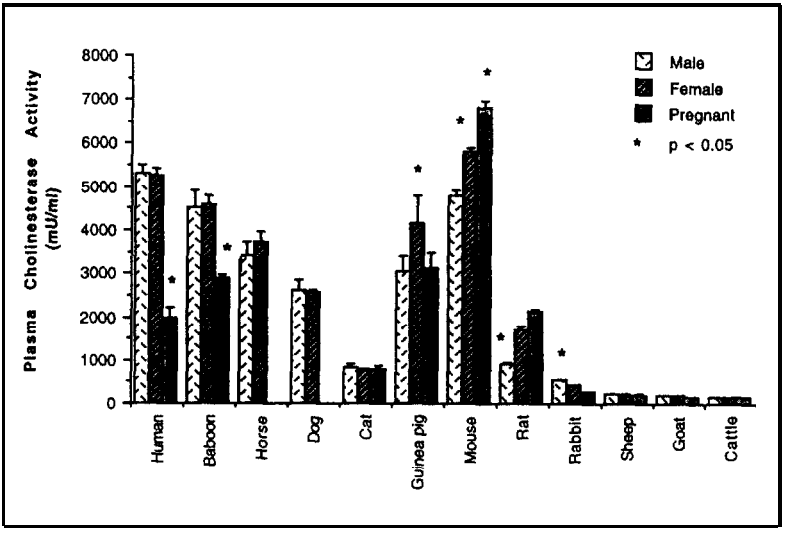

FIGURE 4. *Mean (±SE) values for plasma cholinesterase activity in adult males, nonpregnant females, and pregnant females in 12 species.*

KEY: * = Denotes a significant difference within species.

analyzed following cocaine administration in rats and in sheep. As can be seen in figure 5, both ecgonine methyl ester and benzoylecgonine are excreted in considerable amounts in sheep, whereas in rats benzoylecognine is the major metabolite. The observed species-related differences in cocaine disposition may be due to species-associated variations in enzymatic activities.

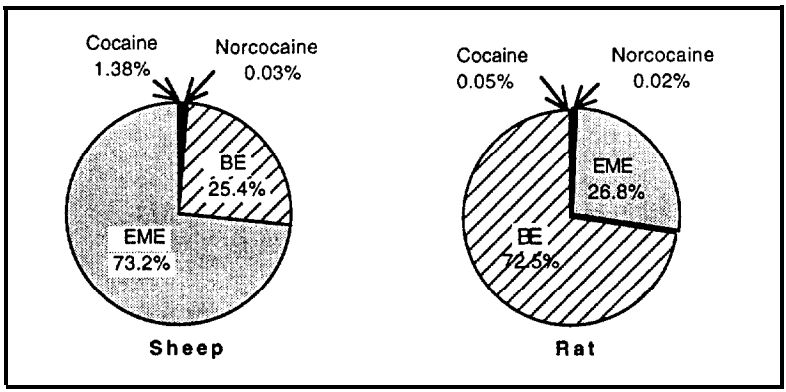

FIGURE 5. *The proportion of cocaine and its metabolites excreted into urine during a 6-hour collection following a bolus IV injection of cocaine (5 mg/kg) to sheep or rat.*

KEY: BE = benzoylecgonine, EME = ecognine methyl ester.

GENDER-RELATED DIFFERENCES IN DISPOSITION OF COCAINE

Recent research in drug metabolism has elucidated molecular and genetic features of hepatic microsomal enzymes, which may explain some of the gender-related variations seen with cocaine's adverse effects. As the majority of pharmacokinetic studies in humans have been conducted in males, it is difficult at present to show gender-related differences (Bamett et al. 1981; Jeffcoat et al. 1989; Wilkinson et al. 1980). However, distinct gender-related differences in animal studies have been demonstrated (Morishima et al. 1993*a*). As shown in figure 6, a smaller dose of cocaine was required in adult male rats to produce various

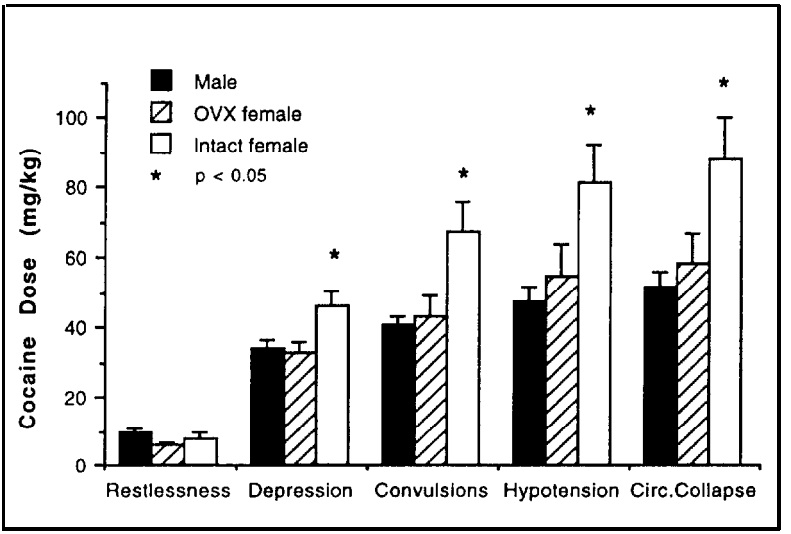

FIGURE 6. *Dose of cocaine required to produce each toxic manifestation in male, ovariectomized (OVX), and intact female rats. Each group contains 12 rats, and each column represents the mean ±SE. Cocaine (2 mg/kg/min) was infused intravenously.*

KEY: * = Denotes a significant difference compared with the other groups.

cocaine-induced toxic manifestations than in intact female rats. On the other hand, a cocaine dose similar to that used for males was needed for ovarectomized females, suggesting that hormonal differences may be the basis for these gender-related differences in toxicity.

Gender-related differences in behavioral responses following acute and chronic cocaine administration in rodents have also been reported recently. Bowman and Kuhn (1994) reported greater cocaine-induced sensitization in female rats than in males following chronic cocaine exposure. In mice, the consumption of cocaine solution was higher in females than in males (Morse et al. 1993).

AGE-RELATED DIFFERENCES IN DISPOSITION OF COCAINE

Another factor that may influence cocaine disposition is the age of subjects. The authors have examined the distribution of cocaine in

plasma and in various tissues with respect to age after a 10 mg/kg dose of cocaine (Morishima et al. 1990). In both adult female rats and in 7-day-old rats, plasma and various tissue concentrations of cocaine reached their peak at 15 minutes (figure 7). Concentrations of cocaine were significantly higher and declined more gradually in neonatal rats, indicating that the elimination rate of cocaine is slower in the neonatal period. At present, it is not known at what age this metabolic pathway matures or the age-related variation disappears.

FACTORS INFLUENCING DISPOSITION OF COCAINE DURING PREGNANCY

A considerable number of pregnant women expose themselves and their fetuses to illicit and licit substances such as cocaine and alcohol throughout pregnancy, thereby endangering the well-being and lives of their unborn children as well as themselves. Some of the medical complications associated with cocaine and polydrug abuse include miscarriage, spontaneous abortion, or premature labor due to increased

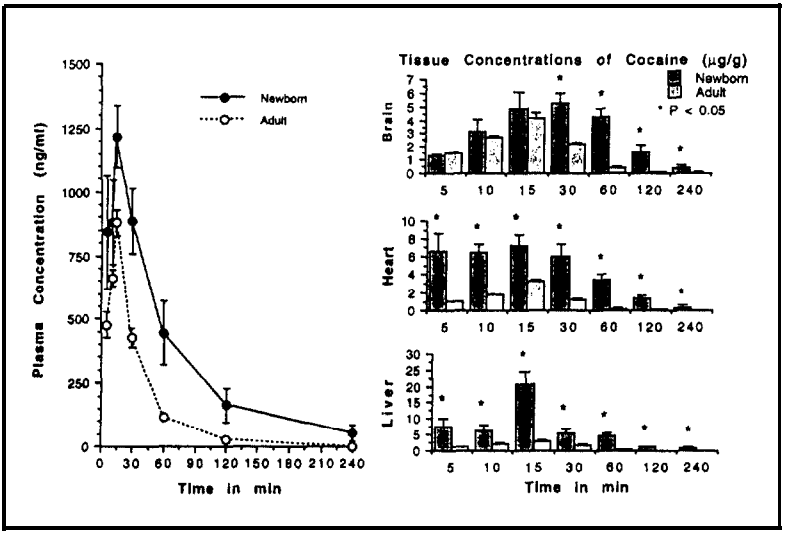

FIGURE 7. *Mean (±SE) values forplasma and tissue concentrations of cocaine in adult and 7-day-old rats following an SC injection of the drug, 10 mg/kg.*

uterine contractions and hypertension. Furthermore, these cocaine-related complications are no longer uncommon occurrences in large cities in the United States.

Pregnancy-associated physiological changes can exert influence on cocaine disposition as well. The progressive increase in uterine blood flow, alterations in the volume of distribution, and changes in protein binding during gestation can alter cocaine kinetics. In vivo and in vitro observations suggest that pregnancy enhances the toxicity of cocaine in sheep (Moore et al. 1986; Plessinger and Woods 1990; Sharma et al. 1992, 1993; Woods and Plessinger 1990). For example, alterations in maternal blood flow and heart rate are dose dependent. Although recent reports indicate that pharmacokinetic profiles may be similar in pregnant and nonpregnant animals depending on the species used, the CNS and hemodynamic effects of cocaine are greater in pregnant versus nonpregnant rats. When pregnant and nonpregnant rats were IV infused with 2 mg/kg/min cocaine, a smaller dose of cocaine was required to produce toxic signs and symptoms in pregnant rats as shown in table 1 (Morishima et al. 1993*b*). Progesterone, a major placental hormone during pregnancy, may be a contributing factor in the augmentation of cocaine-induced adverse effects seen during pregnancy.

TABLE 1. *Mean (±SE) dose of cocaine (mg/kg) required to produce toxic manifestations in pregnant and nonpregnant rats during IV infusion of cocaine,* 2 *mg/kg/min.*

	Pregnant (N=13)	Nonpregnant (N=10)
Restlessness	6.22 ± 1.74	7.70 ± 1.75
Depression	28.35 ± 3.27	45.96 ± 4.73*
Convulsions	41.68 ± 4.86	66.35 ± 9.05*
Hypotension	54.40 ± 4.39	80.74 ± 11.61*
Circulatory collapse	57.86 ± 4.46	87.99 ± 13.01*

KEY: * = Significantly different from pregnant animals.

Another factor that may modify the cocaine disposition during pregnancy is the placenta. Cocaine, like many commonly used local anesthestics, crosses the placenta readily by simple diffusion due to its physico-chemical properties such as low molecular weight and high lipid solubility (Morishima et al. 1975, 1979; Pedersen et al. 1988). It should be pointed out that the transplacental gradient for cocaine or any other chemical agent is dependent upon several other factors: the pharmaco-kinetics of the drug, maternal and fetal plasma protein binding, the degree of drug ionization, placental and umbilical blood flows, the anatomic structure of the placenta, and fetal metabolism and tissue uptake of the drug. Data in figure 8 show the rapid placental transfer of cocaine from mother to fetus following a 2 mg/kg dose of cocaine. As early as 1 minute following IV infusion of cocaine to pregnant sheep, a measurable drug concentration appeared in the fetal arterial blood (Morishima et al. 1992). Furthermore, the steady-state levels of cocaine

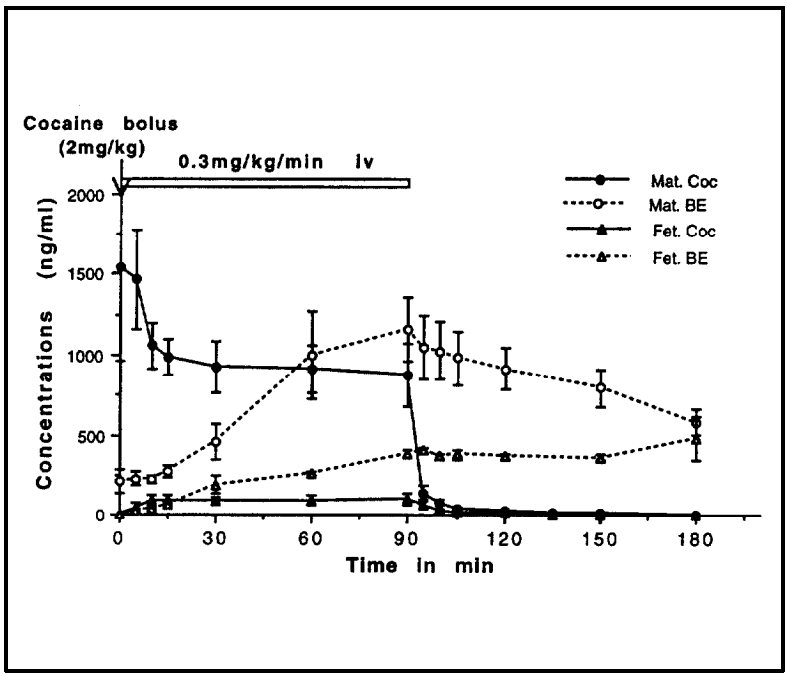

FIGURE 8. *Mean (±SE) values for plasma concentrations of cocaine (Coc) and benzoylecgonine (BE) in the mother (Mat.) and fetus (Fet.) following IV infusion of cocaine (0.3 mg/kg/min) to pregnant ewes (N = 7).*

were reached rapidly both in maternal and fetal blood. Since the concentrations of benzoylecgonine continued to increase both in the maternal and fetal blood while the cocaine concentration was declining, this indicated that cocaine was being metabolized continuously. Recent studies also demonstrate that the fetus is capable of metabolizing cocaine.

As shown in figure 9, when a bolus dose of cocaine (2 mg/kg) followed by a constant infusion of 0.3 mg/kg/min was administered directly into the fetal vein, both cocaine and its metabolite benzoylecgonine appeared in the fetal blood immediately after cocaine administration, and these concentrations remained 20 to 40 times higher than the maternal values during the infusion. In contrast, the maternal concentrations of cocaine and benzoylecgonine were not measurable at 10 and 30 minutes, respectively, following administration of cocaine to the fetus. These results indicate that placental transfer of the drug occurs from the fetus to the mother, and that the fetus is capable of metabolizing cocaine independently.

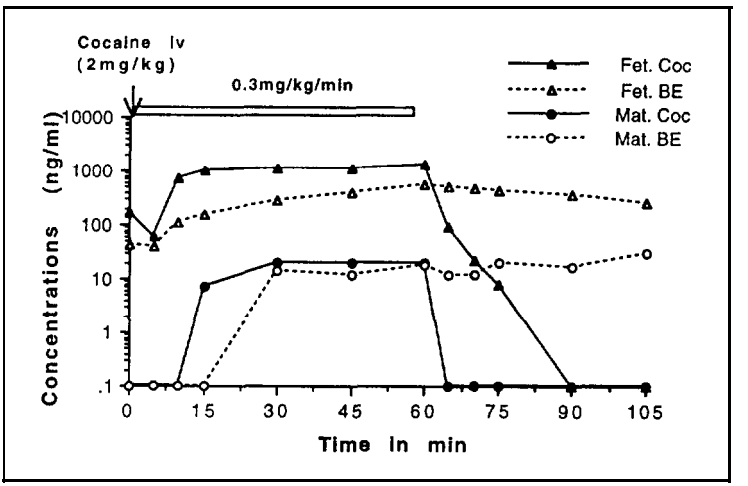

FIGURE 9. *Mean values for plasma concentrations of cocaine (Coc) and benzoylecgonine (BE) in the fetus (Fet.) and mother (Mat.) following IV administration of cocaine, 0.3 mg/kg/min (N = 4).*

To ascertain the distribution of cocaine in the maternal, placental, and fetal compartments, cocaine was infused in awake, term pregnant rats for 15 minutes at a rate of 0.3 mg/kg/min (Morishima et al., unpublished data). As shown in table 2, due to profound differences in maternal versus fetal plasma cocaine concentrations, the cocaine distribution ratio for the fetal/maternal compartment was 0.30. A considerably high concentration of the drug was also found in the amnion, and the levels exceeded those found on both sides of the placenta (placental barrier). Note that proper dissection of the maternal and fetal sides of the placenta was confirmed by pathological examination. These results suggest that cocaine is transferred into and from the amniotic cavity via diffusion across this membrane. Several other investigators have also reported the presence of cocaine in amniotic fluid in various species after maternal cocaine administration (Jain et al. 1993; Mahone et al. 1994; Moore et al. 1993; Sandberg and Olsen 1991*b*). Since the turnover of amniotic fluid is relatively slow, prolonged administration of cocaine may cause the accumulation of the parent compound as well as its metabolites in the amniotic fluid (Sandberg and Olsen 1991*b*). This would contradict a generally accepted concept that uptake and metabolism of drugs by the placenta, if they occur, would decrease the amount transferred to the fetus.

TABLE 2. *Mean (±SE) cocaine concentrations following a 15-minute IV infusion of the drug (0.3 mg/kg/min) to 8 term rats.*

Sample	Cocaine Concentration (µg/mL or µg/g/tissue)
Maternal plasma	2.14 ± 0.20
Fetal plasma	0.65 ± 0.04
Placenta	
Maternal side	6.65 ± 0.46
Fetal side	3.23 ± 0.37
Amnion	8.95 ± 1.05
Amniotic fluid	0.36 ± 0.04
Fetal whole body	1.96 ± 0.14

Studies with several anesthetics, including lidocaine, have shown limited uptake by the placenta (Finster et al. 1972; Geddes et al. 1972). However, the above findings in the rat study suggest that a considerable amount of cocaine is taken up by the placenta. A recent in vitro study by Simone and associates (1994) has shown that the placenta may serve as a large depot for cocaine due to considerable retention of the maternal cocaine dose in human placentae. These results also indicate a limited placental transfer of the drug to the fetus. Furthermore, the functions of cocaine target proteins such as the serotonin transporter, the noradrenaline transporter, and the sigma receptors in the placenta are significantly inhibited by the presence of cocaine. These findings suggest that the placenta may play an important role in the pathogenesis of cocaine-induced maternal and fetal complications (Ganapathy and Leibach 1994).

ROUTE AND MODE-RELATED INFLUENCE ON COCAINE DISPOSITION

Another factor that may have profound influence on cocaine disposition and pregnancy outcome is the route of administration. In animals, cocaine is most commonly administered by subcutaneous (SC) or intragastric (IG) route. However, neither of these routes mimics human IV or IN cocaine abuse. To assess the impact of various routes of administration on peak plasma cocaine concentrations, pregnant as well as nonpregnant rats were adminstrated cocaine, 20 mg/kg dose, SC or IG or 2.5 mg/kg dose IV. As seen in figure 10, peak cocaine concentrations were observed immediately after the IV route and declined rapidly. This was accompanied by a significant increase in both systolic and diastolic pressure that paralleled drug concentrations over time. In contrast, a gradual increase in plasma cocaine concentrations occurred following the SC or IG route along with a slower disappearance of the drug, resulting in a greater value of the area under the curve (AUC) when compared to the IV route.

Another study in rats designed to evaluate the influence of route as well as dose needed to produce lethal or nonlethal manifestations of cocaine showed no cocaine-induced adverse effects at 30 or 60 mg/kg when administered IG or SC (table 3) (Morishima et al. 1994). However, an IV infusion of 20 mg/kg of cocaine produced convulsions with toxic levels of cocaine in plasma and tissues. The IG or SC dose of cocaine at 120 and 240 mg/kg produced a high incidence of convulsions and circulatory collapse. These results suggest that cocaine in a dose range from 30 to

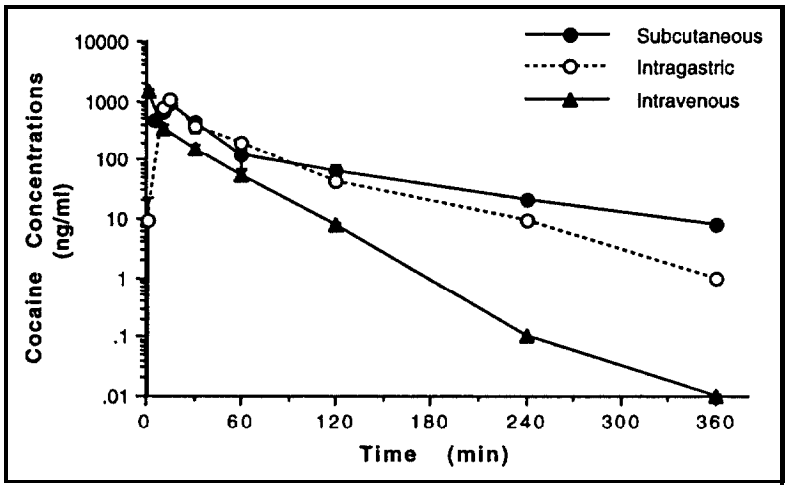

FIGURE 10. *The plasma concentration of cocaine following SC (20 mg/kg), oral (20 mg/kg), or IV (2.5 mg/kg) administration to nonpregnant adult female rats.*

TABLE 3. *Incidence (%) of cocaine-induced toxic manifestations depending on the route of administration to nonpregnant female rats.*

Route	IV	SC or IG			
Dose (mg/kg)	20	30	60	120	240
Agitation	71	100	86	56	57
Depression	43	0	0	57	71
Convulsions	57	0	0	78	71
Cir. collapse	0	0	0	56	86

KEY: IV = intravenous, SC = subcutaneous, IG = intragastric.

60 mg/kg when administered SC or IG does not produce plasma concentrations high enough to cause fetal toxic sequelae. On the other hand, if a higher dose of cocaine is administered in animals, it may cause maternal death or fetal hypoxemia due to a reduction in the uteroplacental

circulation. These pharmacokinetic and pharmacodynamic findings suggest that animal models which incorporate IV drug administration may provide fetal neurotoxicity data more relevant to human findings. Daily administration of cocaine via the IV route in small animals such as rats or guinea pigs can be accomplished by chronic implantation of a venous catheter.

The dose of cocaine required to produce various lethal or nonlethal manifestations in cocaine-exposed and naive adult female rats was also compared (Iso et al. 1994). These types of studies are of paramount importance because the effects of cocaine may differ due to alterations in half-life, disposition, and the development of tolerance (Ambre et al. 1988). Rats received a daily dose of 20 mg/kg cocaine by IG or SC route for 14 days. On day 15, cocaine was then infused intravenously at the rate of 2 mg/kg/min until the animal developed circulatory collapse. As shown in figure 11, smaller doses of cocaine were needed to produce lethal or nonlethal complications in chronically exposed as opposed to pharmacologically naive animals. Possible etiologies could include changes in cocaine's metabolic pathway as well as alterations in the rate of cocaine metabolism secondary to changes in liver function.

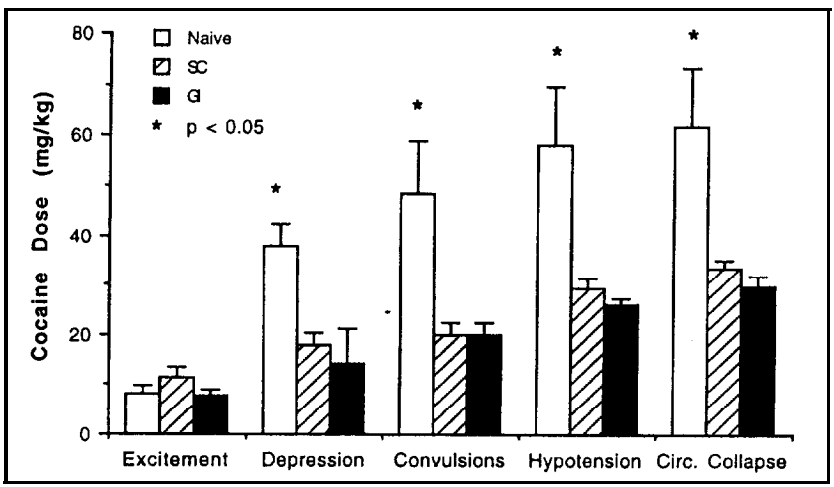

FIGURE 11. *Comparison of the mean (±SE) dose of cocaine necessary to produce major toxic manifestations in nonpregnant female rats chronically exposed to cocaine 20 mg/kg/day either SC or IG, and pharmacologically naive (naive) nonpregnantfemale rats.*

Alterations in hemodynamic responses both in rats and sheep were also observed following a cocaine exposure regimen that closely mimics human "binge" abusers (Morishima, unpublished data). The maximum increase in blood pressure in both species was greatest after the first exposure, and diminished with subsequent administrations. This attenuation of hemodynamic reponse to cocaine may be in part due to downregulation of catecholamine receptors or secondary to subsequent catecholamine depletion. These findings are important as they characterize the differential effects of a single cocaine bolus versus acute, repeated exposure (binge).

CONCLUSIONS

In summary, the pharmacokinetics, pharmacodynamics, and toxicity of cocaine may be greatly influenced by differences in species, gender, and pregnancy. The cocaine kinetics may also be affected by the route and mode of administration. Therefore, it is important that researchers investigating the effects of cocaine on various physiological systems take these biological factors into consideration when designing their experimental protocols.

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ACKNOWLEDGMENTS

This chapter was prepared with support, in part, from National Institute on Drug Abuse grant nos. DA-R01 06648 and 07588, and NIMH grant MHCRC 30906. Cocaine and its metabolites' assays were performed at Professor Thomas B. Cooper's analytical laboratories in New York State Psychiatric Institute, New York, NY.

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Effects of Morphine and Cocaine on Breathing Control in Neonatal Animals: A Minireview

George D. Olsen and Laine J. Murphey

INTRODUCTION

Breathing in mammals is under metabolic and neural control (Mines 1992), both of which factors may be altered in the adult, fetus, and neonate by acute or chronic exposure to drugs of abuse (Borison 1981; Derks et al. 1993; McGilliard et al. 1982; Murphey and Olsen 1994*a*; Olsen and Weil 1992; Olsen et al. 1981; Richardson et al. 1984; Szeto et al. 1988; Weese-Mayer and Barkov 1993; Weese-Mayer et al. 1992). Superimposed upon these basic control mechanisms are the alterations in breathing that occur as a consequence of normal development in the neonatal animal (Murphey and Olsen 1994*a*; Olsen and Weil 1992). Measurements of oxygen consumption and carbon dioxide production and their relation to ventilation while breathing room air indicate the metabolic demand for gas exchange and the appropriateness of the ventilatory response to this demand. Perturbations of breathing by hypercapnic or hypoxic challenge are techniques that permit a better comprehension of the neural control of breathing. The ventilatory response to carbon dioxide is mediated primarily through the central chemoreceptors in the medulla oblongata of the brain stem, which is the brain region important in regulation of breathing (Millhom and Eldridge 1986). The peripheral chemoreceptors in the carotid body are also responsive to hypercapnia. Under most conditions the response to carbon dioxide is the main driving force for changes in breathing (Millhom and Eldridge 1986; Mines 1992). However, hypoxia can also stimulate ventilation via the peripheral chemoreceptors in the carotid bodies and in some species, the aortic bodies as well (Honda 1992; Mines 1992). Drugs may affect the metabolic demands of the body to eliminate carbon dioxide and supply oxygen as well as the neural response to hypercapnia and hypoxia challenges. Two drugs of abuse will be considered in this minireview: morphine and cocaine.

Both morphine and cocaine are pharmacologically active with respect to breathing changes. In addition, these drugs are transformed in the body

to metabolites (Murphey and Olsen 1993, 1994*b*; Sandberg and Olsen 1991, 1992), some of which are active and may alter breathing (Murphey and Olsen 1994*a*; Pelligrino et al. 1989). Morphine and cocaine affect breathing differently. Morphine depresses breathing, whereas cocaine stimulates it. The breathing effects of postnatal administration of single doses of morphine, morphine-6-glucuronide (M-6-G), and morphine-3-glucuronide given to the neonatal guinea pig will be discussed, as well as the neonatal breathing effects of prenatal chronic cocaine exposure in both the guinea pig and rabbit neonatal models.

The outcome of animal model studies with drugs of abuse may advance knowledge of normal developmental physiology and promote the improvement of public health by providing a more thorough understanding of pathological conditions such as the apnea of prematurity and the sudden infant death syndrome (SIDS), which may be linked to drug abuse. With an appreciation of normal function and the sequence of drug-induced changes, prevention and treatment of the consequences of drug abuse become more likely.

VENTILATORY RESPONSE OF THE NEONATAL GUINEA PIG TO MORPHINE

The guinea pig has been used in the laboratory for developmental studies because its placenta, which is the hemomonochorial type, has permeability characteristics similar to the human placenta (Faber and Thornburg 1983; Olsen, in press-a; Olsen et al. 1989; Sandberg et al., in press) as well as a neurological maturity at birth similar to the human neonate (Dawes 1968). Furthermore, the guinea pig has a brain distribution of opioid receptors similar to primates (Mansour et al. 1988) and has a metabolite profile of morphine similar to the human; that is, both M-6-G and morphine-3-glucuronide are produced (Aasmundstad et al. 1993; Murphey and Olsen 1993, 1994*b*). In contrast, in sheep (Olsen et al. 1988) and rats (Aasmundstad et al. 1993; Rane et al. 1985) only morphine-3-glucuronide is produced.

To examine the effects of morphine and its metabolite upon ventilation, neonatal guinea pigs at various postnatal ages were injected subcutaneously with a dose of morphine or M-6-G while breathing room air (21 percent oxygen) or 5 percent carbon dioxide in 30 percent oxygen, balanced with nitrogen (Murphey and Olsen 1994*a*). The oxygen content in the latter gas mixture was increased to ensure that hypoxia did not play

a role in the observed ventilatory response to carbon dioxide. The design was a cross-sectional, randomized, placebo-controlled, dose-response study using a noninvasive computerized plethysmograph technique. Breathing effects of morphine and M-6-G were compared by dose-response analysis.

Morphine and M-6-G depressed inspiratory minute ventilation while breathing room air on day 7 and day 14, but not at 3 days of age. However, while breathing the elevated carbon dioxide mixture, both drugs lowered inspiratory minute ventilation at every postnatal age examined. The depression in ventilation was caused by a decrease in breathing frequency for the room air data and a decrease in both the frequency and tidal volume responses during the 5 percent carbon dioxide challenge. The mean inspiratory flow, a measure of neural output for respiratory effort, was depressed by both morphine and M-6-G. The most interesting aspect of the study, and an unexpected one, was that the potency of the two compounds were the same on day 3, whereas by day 7 M-6-G was eightfold more potent on a molar basis than was morphine as a respiratory depressant. The difference in potency was due to a shift in the M-6-G dose-response curve, while morphine-induced depression did not change with aging. Morphine-3-glucuronide, in a separate experiment, did not depress or stimulate respiration.

The developmental change in potency in the neonatal guinea pig appears not to be due to a change in systemic drug absorption or distribution to the brain (Murphey and Olsen 19946) nor due to a change in mu opioid receptor affinity in the pons and medulla (Murphey and Olsen, in press). These studies also demonstrated that M-6-G enters the brain, most likely by diffusion, and that the relative ability of the glucuronide to enter the brain in vivo compared to morphine is predicted in vitro by their respective high-performance liquid chromatography isocratic capacity factors (Carrupt et al. 1991).

VENTILATORY RESPONSE OF NEONATAL ANIMALS TO IN UTERO COCAINE EXPOSURE

It has been shown that like morphine, cocaine in the guinea pig has a metabolite profile similar to the human (Konkol et al. 1994; Murphey et al. 1993; Sandberg and Olsen 1991, 1992; Sandberg et al. 1993). The one published study on the ventilatory changes to hypercapnia in the neonatal guinea pig following cocaine exposure during the last half of

gestation (term is 70 days) showed that cocaine exposure during gestation increased neonatal tidal volume, inspiratory minute ventilation (35 percent), mean inspiratory flow, and the ventilatory response to carbon dioxide (25 percent) from day 3 until day 14 (Olsen and Weil 1992). The changes were reversible and not dose dependent. Neither breathing frequency nor heart rate was affected by cocaine. Arterial blood partial pressures of oxygen and carbon dioxide were not measured because of the noninvasive nature of the study method. Oxygen consumption and carbon dioxide production also were not analyzed. The increase in room air ventilation observed may be due to hyperpnea (a secondary physiological response to an increase in total body oxygen consumption and carbon dioxide production) or hyperventilation (induced by a primary increase in neural output from the central nervous system). The dose of cocaine used, 2 to 12 milligrams per kilogram (mg/kg), produced physiological effects such as stereotypic chewing movements and cardiovascular changes. Other reports have suggested that carbon dioxide production is indeed increased following in utero cocaine exposure in rabbit pups (Wasiewski and Willing 1990) and babies (McCann and Lewis 1991). The increased ventilation may be caused by a lingering effect of cocaine or active metabolites or by a cocaine withdrawal reaction.

Weese-Mayer and associates have examined the hypoxic ventilatory response in newborn rabbits following different cocaine treatment paradigms (Weese-Mayer and Barkov 1993; Weese-Mayer et al. 1992). The newborn rabbit is not as neurologically mature as the guinea pig at birth (Dawes 1968), or even at 6 days of postnatal age. In the first study, subcutaneous infusion of cocaine in pregnant rabbits, from day 10 of gestation to term (day 32), produced no effect on neonatal room air ventilation at various postnatal ages (Weese-Mayer et al. 1992). The normal ventilatory response to hypoxia, using inspired oxygen concentration reduced to 15, 10, and 8 percent, was eliminated by cocaine treatment.

The second study also examined the effects of in utero cocaine exposure plus hypoxia on neonatal respiratory function (Weese-Mayer and Barkov 1993). Pregnant rabbits received cocaine HCl at 30 mg/kg/day as a single daily subcutaneous injection beginning from gestation day 7 to 15 instead of day 32 (parturition). Room air ventilation and blood gases were not affected by cocaine nor were neonatal or maternal weight. Unlike the first study, cocaine-treated rabbit pups did respond normally with an increase in ventilation following an acute hypoxic challenge, but the

response to severe (8 percent oxygen) and prolonged (20 minutes) hypoxia was not sufficient to prevent a decline in blood oxygen saturation or heart rate, suggesting that there may be abnormalities such as a cocaine-induced increase in total body oxygen consumption and/or poor cardiovascular function for which the increase in ventilation cannot compensate. Collectively, these data suggest that maternal cocaine exposure in animals causes at least transient abnormal control of breathing in the newborn.

DISCUSSION

Epidemiological evidence has suggested that there may be an association between maternal substance abuse and SIDS. There are two recent reviews of the data concerning substance abuse and SIDS (Kandall and Gaines 1991; Kandall et al. 1993). In the review of SIDS cases from New York City between 1979 and 1989, Kandall and colleagues (1993) calculated that the risk ratio for SIDS in drug-exposed versus nondrug-exposed infants was elevated for heroin, methadone, and cocaine alone and for some combinations of those drugs. The report suggests that this increased risk in this group may eventually help to uncover the cause(s) of SIDS. Unfortunately, at this time no one knows the etiology of SIDS in any group, although many new hypotheses have been advanced (Emery et al. 1994; Gingras and Weese-Mayer 1990; Reid 1993). A leading hypothesis for some years has been that abnormal control of ventilation plays a role in the etiology of SIDS.

The association between SIDS and substance abuse is stronger for opioid drugs than for cocaine (Kandall and Gaines 1991; Kandall et al. 1993). In addition to the epidemiological data, there are other human data that implicate narcotic drugs in inducing prolonged abnormalities in ventilatory control. Martin and colleagues (1968) demonstrated that during morphine withdrawal, adults decreased their ventilatory response to carbon dioxide inhalation beginning about 7 weeks after the drug was stopped and that this abnormality persisted for as long as the study was continued, which was 30 weeks. Olsen and Lees (1980) reported that infants chronically exposed to methadone in utero had a reversible decrease in ventilatory response to carbon dioxide at birth, which persisted an average of 15 days and as long as 31 days after birth. McCann and Lewis (1991) suggested that the decreased response to carbon dioxide in babies at 8 weeks of age who had been exposed to both narcotics and cocaine in utero was due to a prolonged narcotic effect.

Newborn puppies exposed in utero to α-ℓ-acetylmethadol, a long-acting derivative of the narcotic analgesic methadone, have a shift to the right of their carbon dioxide ventilatory response curves 6 weeks after birth, which indicates decreased responsiveness to carbon dioxide (McGilliard et al. 1982). The opioid effects reported in neonates and newborn animals may be due to: (1) a prolonged effect of the parent drug and/or metabolites persisting in neonatal blood and brain after birth; (2) an alteration in neural control by a direct effect of drug on the development of breathing control since methadone, at least, decreases human fetal breathing movements in utero (Richardson et al. 1984); or (3) an indirect effect of drug upon maternal respiratory physiology with the retention of carbon dioxide due to respiratory depression such that the fetal brain develops in an atmosphere of high carbon dioxide (Olsen et al. 1977), which may alter the developing chemoreceptor.

Acute and probably chronic effects of morphine and M-6-G are due to interactions with opioid receptors. Three major classes of opioid receptors are currently recognized: mu, delta, and kappa. The mu receptor has been implicated in mediating the respiratory depressant effects of opioids including morphine (see Shook et al. 1990 and Yeadon and Kitchen 1989 for reviews). Mu receptors are present by 20 weeks of gestation in the human (Magnan and Tiberi 1989) and are likely responsible for decrease in fetal breathing movements in sheep during fetal infusions of morphine (Olsen and Dawes 1985; Szeto et al. 1988) and in humans after methadone exposure (Richardson et al. 1984). Endogenous opioid systems may be involved in normal fetal development of respiratory control, and perturbations of these systems by exogenous morphine or methadone exposure in utero may alter this control, leading to postnatal abnormalities. The relationships of the delta and kappa receptors to breathing control have not been as well studied as the mu receptors, although it is known that a delta agonist can affect fetal breathing movements in sheep (Cheng et al. 1992). It has been thought that kappa receptors do not influence breathing (Butelman et al. 1993; Howell et al. 1988); however, a recent study in the rhesus monkey indicates that at least one kappa agonist, U-69, 593, can decrease frequency and tidal volume (France et al. 1994).

The association of SIDS and prenatal exposure to cocaine, although not as strong as the association with narcotics (Kandall and Gaines 1991; Kandall et al. 1993), has been suggested by several studies (Chasnoff et al. 1989; Davidson Ward et al. 1990; Durand et al. 1990). There is at least one study (Bauchner et al. 1988), however, that found no increased

risk of SIDS among infants exposed in utero to cocaine. In addition to SIDS, breathing abnormalities in newborns of cocaine-abusing mothers have been reported (Chasnoff et al. 1989, Davidson Ward et al. 1986), including increased episodes of periodic breathing and apnea.

There are many potential mechanisms of action for cocaine neurotoxicity that might in turn affect the neural control of breathing (Olsen, in press-b). Cocaine and norcocaine block the voltage-gated sodium channel that regulates nerve conduction (Just and Hoyer 1977; Richie and Greene 1990). Although this effect requires doses higher than that for other effects of cocaine in the mature animal, the developing brain may be more sensitive to cocaine blockade and its consequences. Cocaine and norcocaine also are inhibitors of monoamine transporters for norepinephrine, dopamine, and serotonin, and cocaine exposure may affect these systems (Akbari et al. 1992; de Bartolomeis et al. 1994; Kilty et al. 1991; Minabe et al. 1992; Pacholczyk et al. 1991; Ritz et al. 1990; Shimada et al. 1991; Weese-Mayer et al. 1993). Release of epinephrine and norepinephrine from the adrenal medulla (Chiueh and Kopin 1978; Kuhn et al. 1990; Owiny et al. 1991; Stambler et al. 1993) is an effect of cocaine that has not received much attention in the cocaine literature. The released catecholamines, especially epinephrine, can increase oxygen consumption, glucose metabolism, and cardiovascular function. For example, in pregnant sheep, cocaine increases blood glucose significantly in both mother and fetus (Owiny et al. 1991). This is especially disturbing considering the data that suggest hyperglycemia may augment ischemia-reperfusion injury in the central nervous system (Hsu et al. 1994; Zhou et al. 1994). Cocaine stimulates fetal breathing movements in near-term fetal sheep (Derks et al. 1993), but the impact of this effect for neonatal physiology is not known.

The vascular effects of cocaine are well known. Cocaine is a vasoconstrictor (Tella e: al. 1992) which may affect placental transfer of nutrients and oxygen to the fetus by reducing maternal perfusion of the placenta (Plessinger and Woods 1993; Woods et al. 1987). Benzoylecgonine, a cocaine metabolite, is also a vasoconstrictor (Madden and Powers 1990). The vascular effects of cocaine can affect cerebral blood flow and metabolism directly (Dow-Edwards et al. 1988, 1990, 1993; Sharkey et al. 1991) and indirectly through norepinephrine and serotonin changes (Hertz 1992; Muir and Ellis 1993; Sharma et al. 1990). Seizures induced by cocaine (Tella et al. 1992) and cocaine metabolites (Konkol et al. 1992) may result in neurotoxicity mediated by calcium influx into central neurons (Pazdemik et al. 1992); however, calcium channel blockers do

not protect animals from seizures and respiratory arrest (Derlet and Albertson 1989; Derlet et al. 1994). Superoxide formation from cocaine-induced ischemia/reperfusion has been proposed for cocaine-induced peripheral toxicity (Fantel et al. 1992*a*, 1992*b*), but may result in neurotoxicity as well. Famel and colleagues (1992*a*, 1992*b*) indicate that during hypoxic periods there is accumulation of hypoxanthines and xanthines from the catabolism of adenosine triphosphate (ATP). It should be pointed out in this regard that Rognum and Saugstad (1991) found that hypoxanthine is elevated in SIDS infants. On the other hand, catabolism of ATP to adenosine has been proposed to be neuroprotective (Hsu et al. 1994; Zhou et al. 1994). Finally, it has been suggested that a metabolite(s) of cocaine inhibits cytochrome P-450 in pregnant guinea pigs (Sandberg et al. 1993), and it may be that the same metabolite inhibits other crucial enzymes during development.

Among the newer studies on mechanism of cocaine toxicity, there are indications that cocaine alters cortical architecture (Gressens et al. 1992), neurotrophic activity (Weese-Mayer et al. 1993), immediate early gene expression (Hope et al. 1992), and glycosphingolipid content of brain (Leskawa et al. 1994). There has been a great deal of interest in the relationship of cocaine to hypoxia, brain blood flow, and brain dopamine (Pastuszko 1994; Weese-Mayer et al. 1994; Yonetani et al. 1994) in several animal models. For example, Weese-Mayer and Barkov (1993) have suggested that alteration in central dopamine or serotonin systems and peripheral dopamine synthesis in the peripheral chemoreceptor may be related to cocaine-induced alteration in the ventilatory response to hypoxia, but there is no conclusive data to make the connection at this time. Cocaine, however, does not affect gene expression of dopaminergic pathways following prenatal exposure (de Bartolomeis et al. 1994).

The mechanisms leading to SIDS are unknown, although abnormal control of breathing leading to cessation of breathing is usually considered the cause of death. An interesting hypothesis has recently been advanced, suggesting that the connection between SIDS and impaired control of ventilation may be hormonal in nature (Emery et al. 1994). Using the sleeping infant primate model *(Macaca fasiculoris),* ventilatory patterns and the response to hypercapnia and hypoxia of castrated males were studied during quiet sleep before and after testosterone administration. The hypercapnic response was significantly depressed by testosterone. The hormonal treatment did not affect the incidence or length of apnea or the response to hypoxia. This intriguing study immediately raises at least two questions. The first question is,

What significance does this study have for the female newborn (for SIDS also occurs in females)? The second question is, What does morphine or cocaine exposure in utero do to sex hormone secretion in the newborn that could be linked to breathing control? There is evidence that morphine (Adams et al. 1993; Cicero et al. 1991, 1993; Johnston et al. 1992; Nock and Cicero 1991; Vathy and Katay 1992; Vathy et al. 1983, 1985) and cocaine (Raum et al. 1990; Vathy et al. 1993) can affect sexual development in several animal models including the rat and the guinea pig, but the relationship to breathing control has not been investigated. In fact, some of these studies in rats (Adams et al. 1993; Cicero et al. 1991) indicate that morphine reduces serum testosterone in the male rat, which is contrary to the hypothesis that testosterone secretion may be the link between morphine exposure in utero and depressed ventilatory response. Of course this link may be present in primates but not in rats. It should be noted that testosterone in amniotic fluid at birth for males born to cocaine users is decreased, but females were not affected (Ahluwalia et al. 1992).

SUMMARY

From observational studies in humans and experimental studies in animal models, there is a strong indication that prenatal exposure to morphine or cocaine causes at least transient abnormalities of breathing control in the neonate. If these abnormalities persist, sudden death may occur in the infant. Mechanisms responsible for alterations in breathing control by drugs of abuse and the relationship of these alterations to SIDS are not proven at this time. The complex nature of breathing control and neurodevelopment, as well as the confounding variables in human drug abuse, have so far prevented a definitive understanding of the link between drug abuse during pregnancy and SIDS. Further investigations are required to explore the biology of this important public health problem.

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ACKNOWLEDGMENTS

The guinea pig studies of morphine and cocaine discussed in this chapter were supported in part by the National Institute on Drug Abuse grants DA-04905 and DA-07912 to Dr. Olsen and grant DA-05516 to Dr. Murphey. Gary J. Sexton, Ph.D. and David L. Wilson, M.S., who assisted in the statistical design and analysis of the guinea pig respiratory studies, were supported in part by Public Health Service grant no. 5R01 HL45267.

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Cardiovascular Effects of Cocaine in Infant and Juvenile Piglets

Frank M. Scalzo and Lora J. Burge

INTRODUCTION

Use and abuse of licit and illicit substances such as ethanol, cocaine, heroin, and marijuana by pregnant women are major health concerns, as they can produce harmful effects on the developing fetus. Recent clinical reports describe increased incidences of cardiac anomalies and abnormal breathing patterns in cocaine-exposed infants in addition to impaired growth and development (Coles et al. 1992; Kandall et al. 1993; Lipshultz et al. 1991; Silvestri et al. 1991; Zuckerman et al. 1989). Preliminary findings report observing cardiac dysfunctions in cocaine-exposed infants that include arrhythmias, increased frequency of bradycardia, and cardiac malformations (Frassica et al. 1994; Lipshultz et al. 1991; Silvestri et al. 1991). At present, the long-term impact of these cardiac abnormalities is not known, nor are the underlying mechanisms understood.

Studies in adult animals show cocaine-induced alterations in heart rate (HR) and blood pressure (BP). The underlying mechanisms of cocaine's adverse cardiovascular effects appear to involve both central and peripheral sympathetic nervous systems (Branch and Knuepfer 1994; Dolkart et al. 1990; Gillis et al. 1991; Jones and Tackett 1990; Kiritsy-Roy et al. 1990; Raczkowski et al. 1991; Schindler et al. 1992; Tella et al. 1992, 1993; Wilkerson 1988).

Studies in neonates have shown that maternal care and delivery of nutrient are important in regulating the development of cardiovascular function in many species, including humans (Cohen et al. 1992; Myers and Scalzo 1988; Scalzo 1992; Shair et al. 1986). Thus, cocaine may influence the maturation of cardiovascular function through direct cardiotoxic effects and indirectly through perturbation in the nurturing process and in the nutritional needs during critical periods of development. Alterations in neurobehavioral development might also occur secondary to these effects.

To characterize the potential adverse effects of cocaine on the development of the cardiovascular system, the authors as well as other researchers have developed and used the piglet animal model due to its cardiovascular and gastrointestinal functional similarities with humans (Dodds 1982; Nowicki et al. 1986). Administration of a high dose of cocaine (17 milligrams per kilogram (mg/kg)) to newborn piglets induced mesenteric ischemia, suggesting that exposure to cocaine during early development may be important in the etiology of necrotizing enterocolitis (Hebra et al. 1993). These findings may also reflect the importance of cardiovascular responses to feeding in normal gastrointestinal development (Cohen et al. 1992; Scalzo 1992; Shair et al. 1986). Other recent studies in anesthetized newborns and awake neonatal piglets show cocaine-induced decreases in cerebral blood flow and oxidative metabolism in piglets (Anday et al. 1993; Kurth et al. 1993). Studies have shown that the pharmacokinetics of cocaine in swine are comparable to other species of similar size (Kambam et al. 1992; Scalzo et al. 1993); however, there appears to be an accumulation of norcocaine in porcine brain following repeated administration of cocaine (Anday et al. 1993).

To ascertain the effects of cocaine on cardiac function during development, the authors have conducted studies in piglets beginning at two different ages: infant (postnatal days 8 and 9) and juvenile (postnatal days 36 to 38). As discussed above, this animal model was selected because of its functional developmental similarities with the human infant. Postnatal day 8 was the youngest age studied to assess the effects of cocaine in an immature animal model. Earlier ages could have been selected, but not without greater health risks to the piglets and more elaborate postoperative care. Also, the authors' pilot studies in infant piglets have revealed significant BP and HR responses to cocaine. Postnatal day 36 was selected as this is the age at which the brain growth spurt ceases (Dobbing 1981). Also, by postnatal day 21, basal HR has begun to drop and BP has increased to near adult levels (Stanton 1986). Overall, the maturation of central regulation and peripheral functioning of the cardiovascular system occurs between postnatal days 30 and 60 (see Gootman 1986 for a review). Neuronal pathways to the heart and adrenal medulla are mature by postnatal day 30, and adult-like pressor and depressor responses are elicited through central vasomotor areas (Stanton 1986).

The selection of cocaine dose (4 mg/kg) was based on the authors' pilot findings, which showed a lack of dramatic cardiovascular and behavioral effects at this dose. The authors also examined whether tolerance or

sensitization to cocaine develops in piglets since the potential adverse effects of repeated cocaine administration on cardiovascular function in neonates are unclear at present.

EXPERIMENTAL MODEL

Infant piglets 8 to 9 postnatal days (males, N = 6) or juvenile pigs 36 to 38 postnatal days (males and females, N = 4) were implanted with catheters in a carotid artery and jugular vein using procedures described previously (Scalzo 1992). Briefly, piglets were anesthetized with a 2 to 3 percent isoflurane/oxygen mixture via spontaneous breathing, and under sterile conditions a microrenathane catheter was implanted into the jugular vein and carotid artery. The cannulas were flushed daily with heparinized saline, and 2 to 4 days after surgery each animal was placed in a nylon mesh sling for 15 minutes to acclimate the animal to the test situation. Cocaine was administered intravenously in a volume of 1 milliliter (mL) over 15 to 20 seconds and was followed by a 1 mL saline flush. BP was measured via the arterial catheter, which was connected to a pressure transducer. The pulse pressure signal was amplified and the signal digitized via an A/D converter. HR was determined from the BP signal.

Juvenile pigs (mean bodyweight 8.8±0.5 kg) and infant piglets (mean bodyweight 3.2±0.2 kg) were injected with a single dose of intravenous (IV) cocaine (4 mg/kg), and their blood pressure was monitored continuously for 45 minutes postcocaine dose. To examine whether tolerance or sensitization develops to repeated cocaine doses, infant pigs were injected with a daily dose of IV cocaine (4 mg/kg) for an additional 4 consecutive days, totaling five cocaine doses. The interval of 24 hours between doses was chosen based on the authors' earlier findings that the elimination half-life of cocaine is approximately 30 minutes in piglets (Scalzo et al. 1993). The animals were monitored continuously for 45 minutes following each dose of cocaine. A saline injection was not given to the infant pigs because no effect was observed after such an injection in the authors' pilot study. Juvenile pigs were dosed every 48 hours after the first dose. Data are presented as percentage of change from baseline. Baseline was determined by obtaining the median of mean BP (or HR) over 1 -minute periods for 5 minutes prior to saline or cocaine injection.

TIMECOURSE OF CARDIOVASCULAR ACTIONS OF COCAINE

Figure 1 shows an example of a BP tracing obtained from an infant piglet injected with cocaine as indicated at 5 minutes on postnatal day 10. Typically, BP increased immediately following injection and returned to baseline within 10 to 15 minutes postinjection. Baseline levels of both BP and HR were comparable between subjects and across days and no significant differences existed in baseline BP and HR for juvenile or infant pigs (table 1). However, there appears to be a trend for baseline HR to decrease and baseline BP to increase with age on the first treatment day.

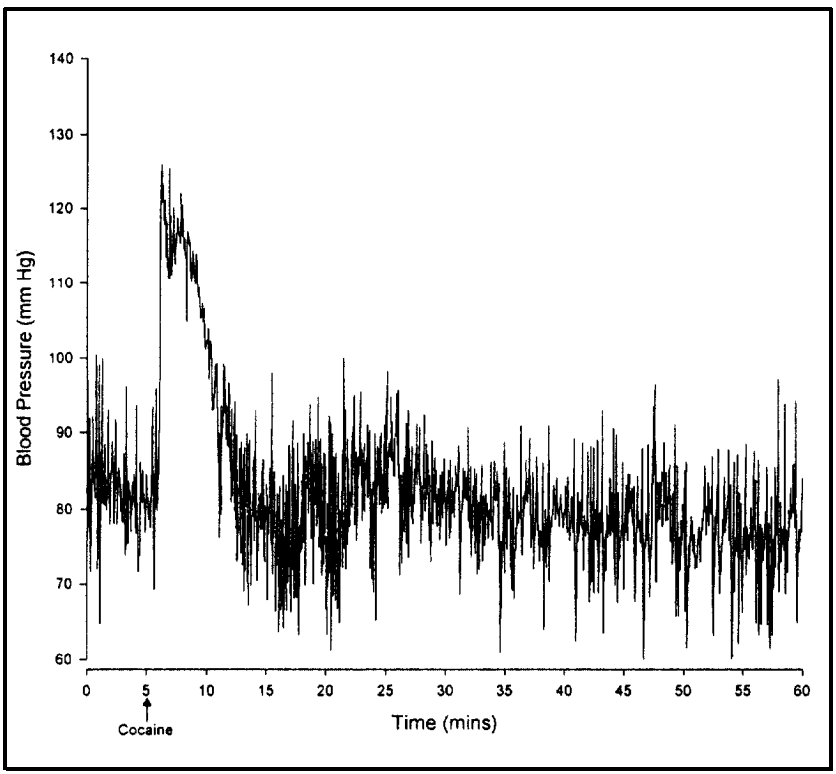

FIGURE 1. *Example of a blood pressure tracing from a postnatal day 10 piglet injected with 4 mg/kg cocaine at 5 min.*

TABLE 1. *Baseline blood pressure and heart rate in young and juvenile pigs (mean±SEM).*

	Infant		Juvenile	
	Treatment 1	Treatment 5	Treatment 1	Treatment 5
Heart rate (bpm)	203±23	189±13	173±21	148±10
Blood pressure (mm Hg)	92±7	96±5	104±3	82±13

Figure 2 depicts BP and HR following a saline injection in juvenile pigs. Saline injections had no effect on either BP or HR in juvenile piglets.

Figure 3 shows the effects of the first and fifth cocaine injections on BP in juvenile pigs. Following the first exposure to cocaine, the maximum increase in BP occurred 2 to 3 minutes after injection and returned to baseline levels within 12 to 15 minutes. Following the fifth cocaine treatment, a 13 mm Hg increase in BP comparable to the first cocaine exposure was observed; the BP returned to baseline by 15 minutes.

The HR data corresponding to the BP data (in figure 3) are shown in figure 4. The HR response following the first cocaine treatment is characterized by a 20-beat-per-minute (bpm) decrease that lasted for 4 to 5 minutes beginning immediately following injection. HR returned to baseline by 10 minutes postdosing and then increased above baseline, returning to near baseline levels at 40 minutes postdosing. The HR response following the fifth dose followed a different profile (i.e., the initial bradycardia was not as large and the increase in maximum HR was more pronounced, 70 versus 48 bpm). Also, HR did not return to baseline even 45 minutes following the fifth cocaine dose.

Figure 5 shows the effects of the first and fifth cocaine injections on BP in infant pigs. The BP response to the first cocaine injection is characterized by a maximum increase of 11 mm Hg above baseline that occurred 3 to 5 minutes following injection. BP returned to baseline 7 to 8 minutes following injection and remained 5 mm Hg below baseline for the remainder of the observation period. Following the fifth injection, the increase in BP was diminished and BP decreased 5 mm Hg below

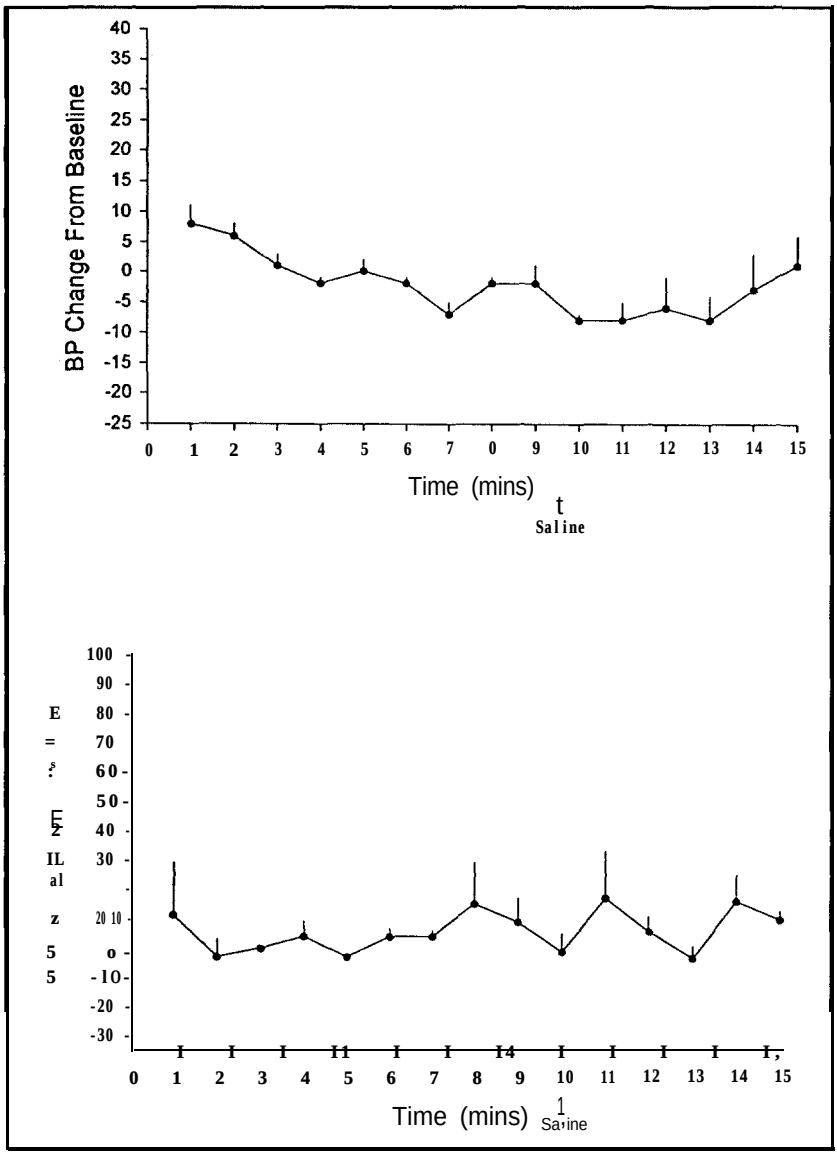

FIGURE 2. ***Effects of saline injection on baseline blood pressure (upper) and heart rate (lower) in juvenile piglets (mean + SEM).***

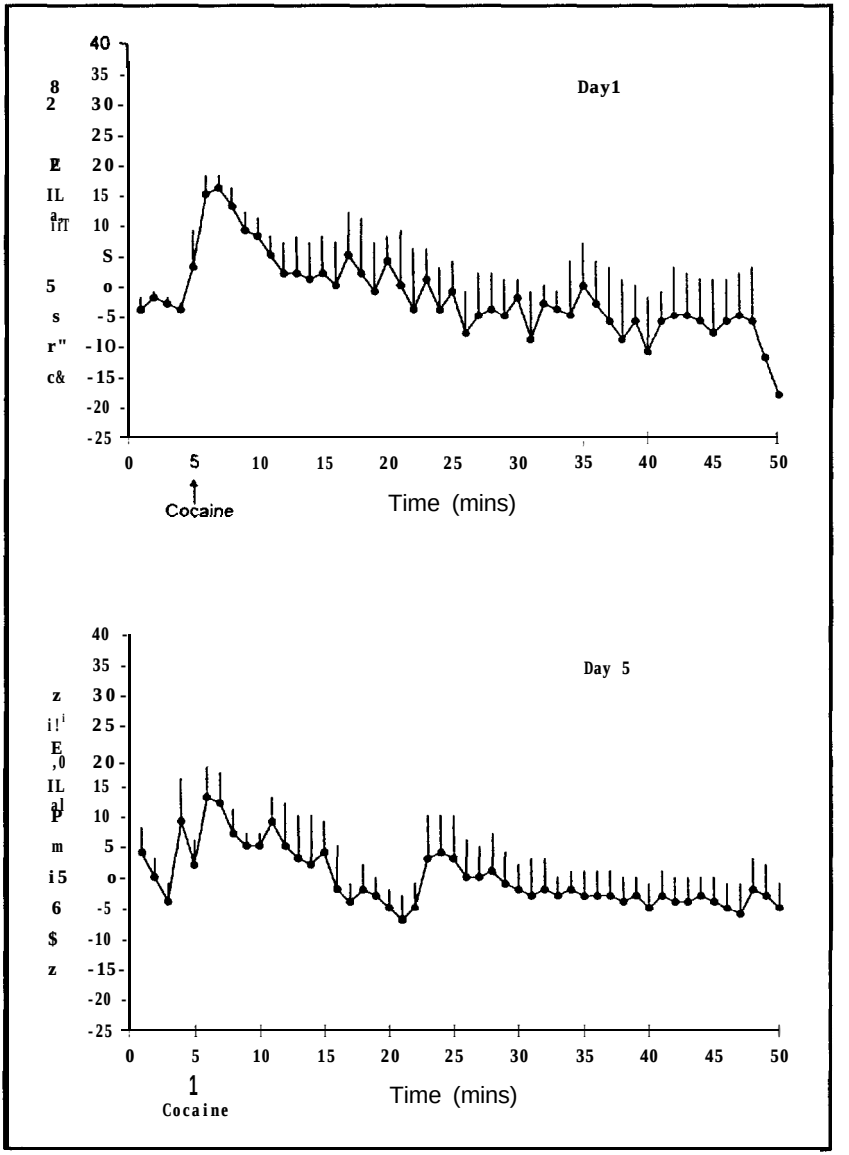

FIGURE 3. ***Mean change in blood pressure (mm Hg) from baseline following 4 mg/kg IV cocaine on treatment day 1 (upper) and treatment day 5 (lower) in juvenile piglets (mean + SEM).***

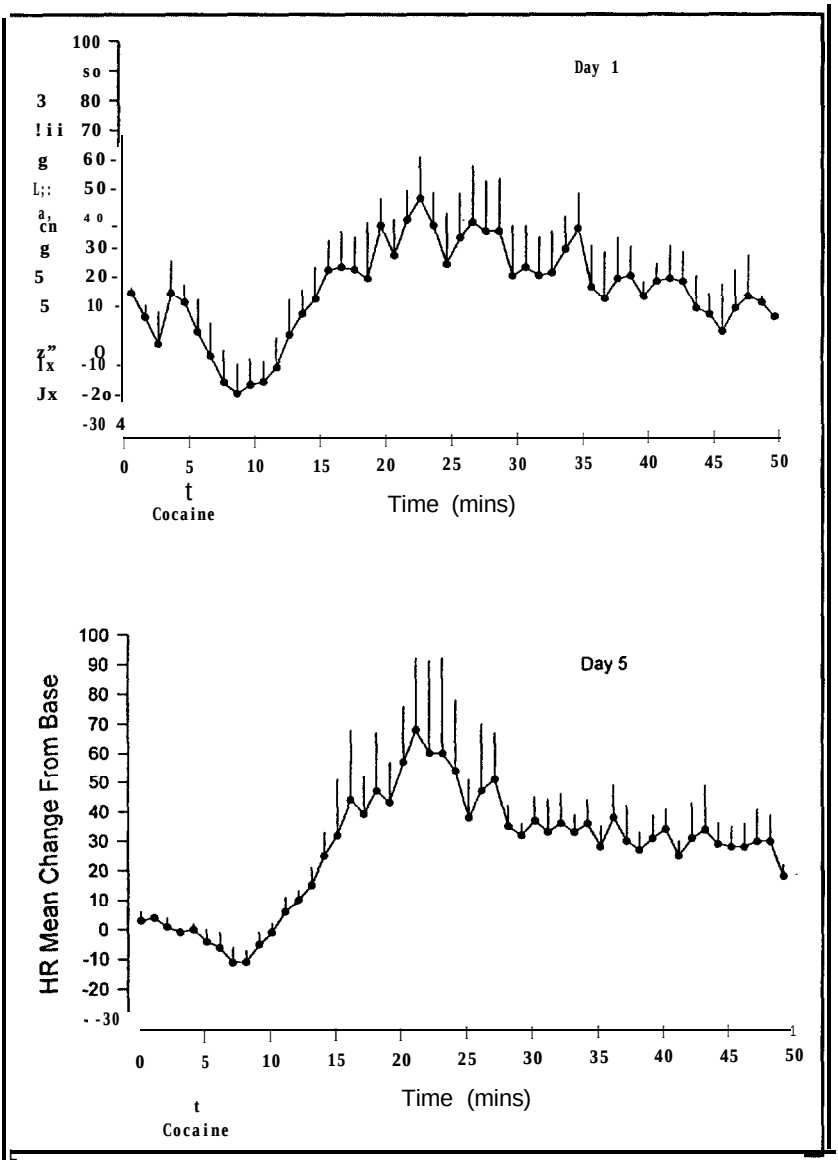

FIGURE 4. ***Mean change in heart rate (bpm) from basetine following 4 mg/kg IV cocaine on treatment day 1 (upper) and treatment day 5 (lower) in juvenile piglets (mean + SEM).***

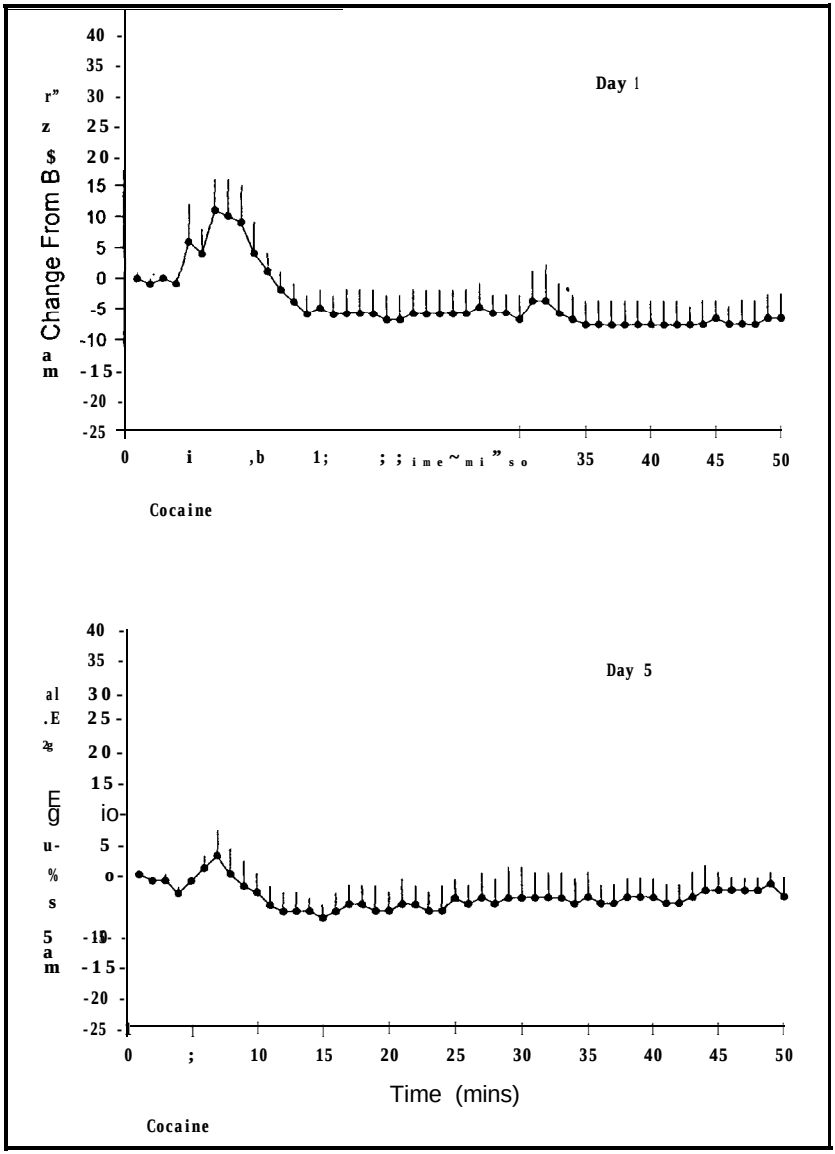

FIGURE 5. ***Mean change in blood pressure (mm Hg) from baseline following 4 mg/kg IV cocaine on treatment day 1 (upper) and treatment day 5 (lower) in infant piglets (mean + SEM).***

baseline 6 to 7 minutes postinjection. BP remained 4 to 5 mm Hg below baseline for the remainder of the observation period.

Figure 6 shows the HR data corresponding to the infant BP data presented in figure 5. HR initially decreased to 18 bpm below baseline 3 to 5 minutes postdosing and then gradually increased to a range of 30 to 40 bpm above baseline 10 to 20 minutes postdosing. HR declined thereafter reaching baseline values 33 minutes postdosing. The profile of HR change following the fifth injection of cocaine is shown in the lower panel of figure 6. Only a 5 bpm decrease in rate was observed upon dosing. Beginning 10 minutes postdosing, a sustained increase in HR to 55 to 75 bpm above baseline was observed. This tachycardia lasted 25 minutes, after which HR declined to 20 to 40 bpm above baseline.

DISCUSSION

These results demonstrate that acute and repeated doses of 4 mg/kg cocaine produced profound alterations in HR in infant and juvenile piglets. The data also show cocaine-induced pressor effects in piglets, although these were modest in magnitude and diminished with repeated exposure in infants. These findings suggest that administration of repeated high doses of cocaine during early development can influence the physiological maturation of the cardiovascular system.

The acute effects of cocaine on HR in both juvenile and infant pigs were similar and consisted of a biphasic response, that is, an abrupt decrease in rate, perhaps a reflex bradycardia, followed by a prolonged elevation in HR. On the other hand, the changes in BP were less pronounced in infant piglets compared to those seen in juveniles. The observed increases in BP at both ages are similar in magnitude to those seen in rats after 3 mg/kg cocaine (Tella et al. 1991), but less than those reported in rats after 5 mg/kg cocaine (Branch and Knuepfer 1994).

In juvenile pigs, the magnitude and duration of the cocaine-induced bradycardia were diminished and the magnitude of the subsequent tachycardia was increased after repeated dosing. In infants, no cocaine-induced bradycardia was observed following the fifth dose of cocaine; however, the magnitude of the cocaine-induced tachycardia was greater than that observed following a single dose of cocaine. Thus, the profile of the HR response to cocaine changed over the 5 days, although it is

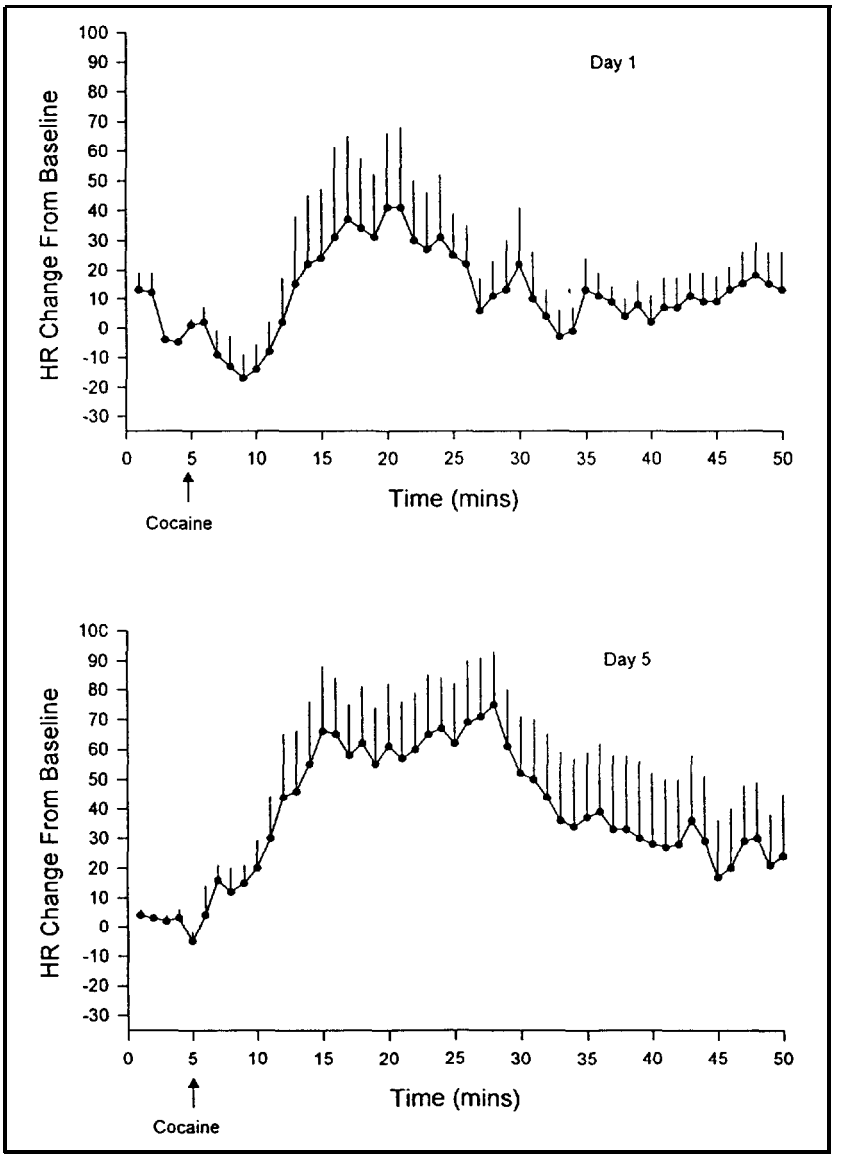

FIGURE 6. *Mean change in heart rate (bpm) from baseline following 4 mg/kg IV cocaine on treatment day 1 (upper) and treatment day 5 (lower) in infant piglets (mean + SEM).*

unclear whether this was due to a permanent change in cardiovascular responsivity or due to maturation. These data also suggest an enhanced responsivity to the tachycardia effects of cocaine after repeated exposure at both ages. Furthermore, in infant piglets this tachycardia is accompanied by a diminished response to the pressor effects of cocaine. The increase in the magnitude of tachycardia with repeated dosing observed in the present study is in contrast to a study in rats that did not show any change in magnitude of tachycardia with repeated dosing (Tella et al. 1991).

Repeated cocaine administration had little effect on the magnitude or profile of BP responses to subsequent doses in juvenile piglets. A comparison between ages in the BP response to the fifth cocaine injection reveals that the magnitude of the pressor response is less in the infants compared to juveniles, as only a modest increase in BP was seen in infant piglets. These data suggest that, in infant pigs, repeated exposure to cocaine produces a diminution in the pressor responses, which is in contrast to the sensitization in BP response observed in rats (Tella et al. 1991).

Tolerance to both the pressor and HR effects of cocaine has been reported in human studies (Ambre 1993). The observed diminution in BP response in infants after the fifth injection could be interpreted along these lines, although no evidence for tolerance to the HR response was found at either age. It is possible that the sustained increases in HR are related to increased locomotor activity typically observed within 5 to 10 minutes postdosing. This increased activity did not occur in all subjects and tended to be intermittent at best in most subjects.

Smith and colleagues (1993) reported a BP increase of 59.9 mm Hg following a 4 mg/kg cocaine dose in rats, and this pressor response was followed by a bradycardia and subsequent tachycardia that lasted 15 to 30 minutes. The present study revealed a similar pattern and range of tachycardia. However, compared to the present study, larger increases in fetal BP and HR were observed in response to a 2 mg/kg cocaine dose in fetal sheep (Dolkart et al. 1990). In sheep, a maximum increase of 37 mm Hg was observed 30 seconds after a 4 mg/kg cocaine dose. Similarly, the largest HR increases were observed 30 seconds postdosing (O'Brien et al. 1994). In conscious dogs, a 4 mg/kg cocaine dose produced approximately an 80 mm Hg increase in pressure and approximately a 50 bpm increase in HR (Wilkerson 1988). In the present study, the response profile for HR was similar at both ages to that observed in

squirrel monkeys at doses of 1 and 3 mg/kg cocaine (Schindler et al. 1992). The modest magnitude of the BP increases observed in the present study might reflect differences in basal sympathetic tone between species. It also underscores species differences in cardiovascular responses to a given cocaine dose and the importance of interspecies comparisons across a range of doses.

The piglet is useful as a model to study the cardiovascular effects of cocaine for several reasons, including the many similarities with human cardiovascular and gastrointestinal physiology and the timing of the brain growth spurt. A comparison of cocaine pharmacokinetic parameters between humans and pigs shows similar values for elimination half-life, volume of distribution, and clearance (table 2).

Taken together, these results show that acute or repeated exposure to cocaine can influence the maturation of the cardiovascular system in pigs. Furthermore, changes in BP and HR during critical periods of development may have implications for blood flow regulation to vascular beds (e.g., cerebrovascular and mesenteric) that is important for normal growth and development. Although the long-term consequences of these actions are not known, they may play a mechanistic role in the somatic and neurobehavioral deficits reported following developmental cocaine exposure in human and animal studies.

TABLE 2. *Species comparison of cocaine pharmacokinetics.*

Species (Reference)	Dose (mg/kg)	Route	AUC (mg/L x min)	C_{max} (mg/L)	Cls (L/min/kg)	Volume of Distribution (L/kg)[a]	t½[b] (min)
Piglet (Scalzo et al. 1993)	6.0	IV	148.9	27.9	0.041	1.543	29.4
Pig (Kambam et al. 1992)	3.0	IV	NR	1.0	0.078	2.939	24.2
Human (Javaid et al. 1983[b])	1.3	IV	24.6	NR	NR	7.49	88.8
Human (Bamett et al. 1991)	1.0-3.0	IV	NR	0.682-3.868	0.01-0.333	1.2-1.9	40.0-83.0
Pregnant sheep (DeVane et al. 1991)	3.0	IV	51.49	24.0	0.082	NR	4.0
Pregnant guinea pig (Sandberg and Olsen 1991)	6.0	IV	NR	2.41	0.059	3.4	48.0
Rhesus monkey (Duhart et al. 1993)	1.0	IM	16.0	0.043	NR	7.4	84.0
Pregnant macaque (Binienda et al. 1993)	1.0	IM	21.6	0.288	NR	4.7	72.0

KEY: NR = not reported; a = Specific type of volume of distribution reported differs between some labs; b = Based on data as calculated by Duhart et al. 1993; IM = intramuscular; C_{max} = maximum concentration; Cls = maximum clearance; t½ = half life.

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ACKNOWLEDGMENTS

This work was supported by National Institute on Drug Abuse grant no. DA-06319. The technical assistrance of Felicia Racey is gratefully acknowledged.

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Effects of Cocaine on Fetal Brain Metabolism and Behavioral State in the Sheep Model

David J. Burchfield

The use of cocaine by pregnant women remains a major public health concern. Although an earlier report (Mofenson and Caraccio 1987) of 10 to 15 percent use in pregnant women appears to overestimate the usage in the general population, recent studies show that certain socioeconomic subgroups approach the 10 to 12 percent user rate (Moser et al. 1993; Vega et al. 1993). Because of the high prevalence rate of cocaine use in pregnant women, a thorough understanding of its effects on the fetus, especially on the fetal brain, would seem to be of paramount importance.

For many years, the pregnant sheep has served as an excellent model for perinatal physiological studies. Unlike most other species, the pregnant ewe can undergo surgical hysterotomy and fetal instrumentation with a low incidence of subsequent postoperative abortion. Using this model, the physiological effects of drugs such as cocaine have been studied in the chronically instrumented fetal sheep during the postoperative period, at a time away from the stress of surgery and the possible confounding effects of anesthesia. In addition, the sheep fetus is of a size similar to humans and the blood volume is such that multiple blood withdrawals can take place without significant fetal compromise.

EFFECTS OF COCAINE ON FETAL BRAIN METABOLISM

Cocaine administered to pregnant sheep causes uterine artery vasoconstriction (Woods et al. 1987) and fetal hypoxemia in a dose-dependent fashion (Burchfield et al. 1991; Moore et al. 1986; Woods et al. 1987). In the author's study cocaine caused a 36 percent drop in fetal oxygen partial pressure (pO_2) following a 2 milligrams per kilogram (mgkg) dose of cocaine to the ewe (Burchfield et al. 1991). Simultaneously, it caused a 25 percent rise in fetal blood pressure.

Due to this combination of fetal hypoxemia and vasoconstriction, one might predict that oxygen delivery to the fetal brain is compromised during maternal cocaine exposure. To test this hypothesis, Gleason and

colleagues (Gleason et al. 1993) measured fetal cerebral blood flow following a 2 mg/kg bolus injection of cocaine to the near-term pregnant ewe using the radiolabeled microsphere technique. Although fetal hypoxemia ensued, cerebral blood flow increased 37 to 58 percent so that oxygen delivery to the fetal brain was unimpaired.

These results are in contrast to those subsequently published by Covert and colleagues (Covert et al. 1994). Using a smaller dose of cocaine to the ewe and estimating cerebral blood flow by measuring unilateral common carotid flow in the fetal lamb, this group reported an approximate 50 percent decrease in cerebral blood flow. The methodology of Covert and associates differed substantially from that used by Gleason and colleagues (Gleason et al. 1993) in that Covert and coworkers reported a "peak" change in carotid blood flow, which was a drop in carotid blood flow, within minutes of maternal administration of 1 mg/kg cocaine. This was followed by a rebound return of carotid blood flow to higher than baseline by 6 minutes. Gleason and coworkers reported no change in brain blood flow 2 minutes after maternal injection of 2 mg/kg cocaine, but a significant increase in flow to all cerebral regions by 5 minutes. Possibly, cocaine caused an acute transient decrease in cerebral blood flow due to the baroreflex from acute vasoconstriction, but within minutes this decrease was overcome through a catecholamine-driven increase in cardiac output.

The author examined cerebral blood flow and oxygen delivery in eight near-term (0.9 gestation) fetal sheep under two different paradigms: during continuous maternal infusion of cocaine and during continuous fetal infusion of cocaine (Burchfield et al., in press). This design was chosen to discern the direct versus indirect effects of cocaine on the fetus. Cocaine administered to the pregnant ewe produced a drop in fetal oxygenation; however, fetal cerebral oxygen delivery was unimpaired probably due to a small increase in brain blood flow. Cocaine administered directly to the fetus did not cause hypoxemia or changes in cerebral blood flow.

To determine the fetuses' ability to adapt to hypoxia through increasing cardiac output, additional studies in fetal sheep were conducted at an earlier gestation period, 0.7 term (Peña et al. 1994). At this earlier gestation, administration of maternal cocaine did not produce fetal hypoxemia as it did at 0.9 gestational period; cerebral blood flow and oxygen delivery were also unimpaired at this gestational age. From these studies as well as those of Gleason and associates, the author concludes

that fetal cerebral oxygen delivery is not impaired by maternal cocaine administration. Furthermore, the observed neurobehavioral effects of cocaine in cocaine-exposed newborn infants are probably not due to fetal hypoxia-ischemia.

Because cerebral glucose utilization is so closely linked to cerebral function (Abrams et al. 1987; Kennedy et al. 1979; Sokoloff 1981), several investigators have studied local cerebral glucose utilization (LCGU) in laboratory animals using the [^{14}C] 2-deoxyglucose method. Dow-Edwards and associates (1988) showed that prenatal cocaine exposure in rats produced increased LCGU when measured at age 60 days.

In an attempt to further understand the potential mechanisms of neonatal neurobehavioral abnormalities associated with prenatal cocaine exposure, this author studied LCGU in six fetal sheep during a direct intravenous (IV) infusion of cocaine and compared this with seven control fetuses (Burchfield and Abrams 1993). As seen in table 1, LCGU was globally depressed in the fetal sheep administered cocaine, with 33 of 34 structures showing lower LCGU than in control fetuses.

An explanation of the global depression in LCGU in cocaine-exposed fetuses may be due to cocaine's effect as a local anesthetic. Local anesthetics, including cocaine, prevent the generation and conduction of nerve impulses by decreasing the cell membrane permeability to Na^+ (Richie and Greene 1990). At high concentrations of cocaine, the function of both inhibitory and excitatory neurons is depressed. This depression may be the effect of acute cocaine administration to the fetus, that is, global reduction of the central nervous system activity through blockade of nonselective voltage-sensitive Na+ channels.

A paradox appears to exist in the knowledge concerning the effects of cocaine on fetal brain metabolism. On the one hand, oxygen delivery is unimpaired (Gleason et al. 1993; Peña et al. 1994) but LCGU is globally depressed (Burchfield and Abrams 1993). If both of these are true, then there must be an uncoupling of the normally tight relationship between tissue glucose and oxygen utilization.

TABLE 1. *Local cerebral glucose utilization (µmol/100 g/min) in control and cocaine-exposed feral sheep.*

	Control (N = 7)	Cocaine (N = 6)	P Value
Cerebral cortex			
Sensorimotor	33.4 ± 18.2	19.1 ± 7.0	0.09
Prefrontal	46.6 ± 29.0	19.6 ± 4.4	0.05
Auditory	38.3 ± 19.5	25.7 ± 10.0	0.17
Cingulate	47.2 ± 26.5	26.5 ± 6.8	0.09
Striate	41.1 ± 29.5	23.5 ± 3.4	0.17
Subcortical structure			
Amygdala	35.3 ± 19.0	20.4 ± 7.1	0.096
Hippocampus	46.0 ± 18.7	28.9 ± 7.1	0.06
Septal area	44.2 ± 26.4	18.6 ± 4.1	0.04
Caudate	42.3 ± 26.7	23.0 ± 4.0	0.10
Habenula	51.2 ± 28.0	26.5 ± 6.7	0.06
Subthalamus	82.6 ± 39.3	40.2 ± 14.2	0.03
Thalamus			
Main nuclei	55.3 ± 28.1	26.7 ± 8.9	0.04
Lateral genic. body	63.6 ± 30.4	39.4 ± 10.8	0.09
Medial genic. body	67.8 ± 33.8	39.3 ± 12.6	0.08
Hypothalamus			
Mammillary body	80.4 ± 36.3	40.8 ± 13.8	0.03
Medial area	33.4 ± 17.5	15.9 ± 3.7	0.05
Midbrain and brain stem			
Superior colliculus	58.8 ± 31.8	36.9 ± 11.9	0.13
Inferior colliculus	118.6 ± 60.5	71.0 ± 23.6	0.09
Lateral lemniscus	73.5 ± 40.0	41.1 ± 8.2	0.08
Periaqueductal gray	25.6 ± 17.7	14.4 ± 3.8	0.15
Superior olives	69.8 ± 31.2	46.1 ± 11.7	0.10
Inferior olives	80.5 ± 39.9	40.5 ± 17.2	0.04
Oculomotor n.	66.1 ± 43.4	33.9 ± 6.4	0.10
Vestibular n.	76.3 ± 44.5	44.3 ± 14.0	0.11
Cochlea n.	61.7 ± 28.6	34.8 ± 8.1	0.04
Reticular formation	42.8 ± 21.5	16.7 ± 3.0	0.05
Cerebellum			
Vermis. cell den. lay.	55.7 ± 24.4	35.1 ± 6.6	0.07
Nucler	63.7 ± 33.7	38.4 ± 13.6	0.10
Cortex	33.8 ± 17.2	16.7 ± 3.0	0.04
Cervical cord			
Ventral horns	40.8 ± 10.6	19.2 ± 4	0.02
White matter			
Uptic chiasm	18.8 ± 18.0	10.9 ± 3.5	0.10
Corona radiata	20.1 ± 10.2	16.3 ± 6.0	0.43
Periventricular white	7.5 ± 2.1	7.7 ± 4.6	0.92
Spinal cord, lat. col.	17.0 ± 6.8	9.2 ± 1.2	0.02

NOTE: Values are means±SD.

COCAIN'S EFFECTS ON FETA BEHAVIORAL STATE

Adult humans who chronically abuse cocaine spend less of their sleep time in rapid eye movement (REM) sleep (Richie and Green 1990)—a sleep state considered very important in fetal life (Roffwarg et al. 1966; Ruckebusch 1972; Szeto and Hinman 1985). REM sleep is theorized to be important in normal cerebral maturation and development (Roffwarg et al. 1966). Roffwarg and colleagues (1966) hypothesized that REM sleep provided intense stimulation to the central nervous system at a time when the developing brain received little external stimulation, that is, during prenatal and early postnatal life. Studies have shown that functional stimulation potentiates structural growth in the nervous system (Bennett et al. 1964; Weiskrantz 1958) and this stimulation may precede myelinization (Langworthy 1933).

The author investigated whether cocaine, a drug known to reduce REM sleep in adults, also interfered with REM sleep in the fetus (Burchfield et al. 1990). Using each fetus as its own control, either cocaine or saline was administered IV over 90 minutes to seven fetal sheep on alternate days to determine if cocaine altered the behavioral state pattern. By administering the cocaine directly to the fetus, the confounding effects of fetal hypoxemia were avoided. As seen in figure 1, saline infusion did not alter the percentage of time in REM or non-REM behavioral states. However, cocaine (dissolved in saline vehicle) reduced the percentage of time in REM from approximately 40 percent to 4 percent.

In an attempt to see if the fetus gains tolerance to cocaine's effect on behavioral state, additional studies were performed in which the cocaine infusion period was extended to 6 hours. In this study, a similar depression in REM sleep activity with no evidence for tolerance to this effect was seen. However, upon termination of the cocaine infusion at the end of 6 hours, fetuses showed evidence for "catch up" REM, spending approximately 55 percent of their sleep time in REM.

The mechanism for cocaine's curtailment of REM sleep may be through its known inhibitory properties on the locus ceruleus (Pitts and Marwah 1986), injuries to which lead to deceased REM sleep time (Jouvet and Delorme 1965). In adult volunteers, REM sleep deprivation leads to anxiety and irritability (Dement 1960; Hartman 1969); if newborn humans exposed in utero to cocaine truly demonstrate irritability and excessive crying (Bingol et al. 1987; Chasnoff et al. 1985; Madden et al.

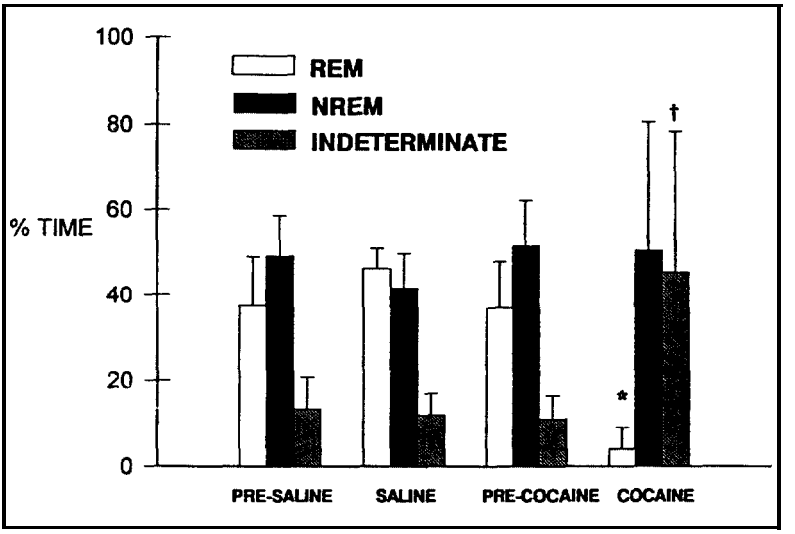

FIGURE 1. *Percent rime in the different behavioral states under the four experimental conditions. Values are means ± SD.*

KEY: * = $p < 0.0001$ compared to other REM periods;
† = $p < 0.002$ compared to other indeterminate periods.

1986), then a cause-and-effect relationship between cocaine's effect on REM sleep and subsequent neurobehavioral abnormalities can be extrapolated.

CONCLUSION

The ovine species serves as an excellent model for the study of cocaine on fetal physiological functions. Although data are conflicting, it appears that maternal cocaine administration does not cause, at least for any extended period, diminished cerebral oxygen delivery even though it may uncouple cerebral oxygen-glucose utilization in some manner. This deserves further study. Fetal REM sleep, thought to be important in normal fetal cerebral development, is diminished during cocaine exposure, but the fetus has the ability to catch up with extended periods of REM following cocaine removal.

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Fetal Cerebral Vascular Effects of Cocaine Exposure

Michael D. Schreiber

INTRODUCTION

In this country alone, over 25 million people have experimented with cocaine and 2 to 3 million are habitual abusers (Tarr and Macklin 1987). Prominent among these are pregnant women. Cocaine use during pregnancy has increased to epidemic proportions, and in certain populations over 20 percent of pregnant women abuse cocaine (Hadeed and Siegel 1989; Oro and Dixon 1987; Rosenak et al. 1990). Data from the author's patient population document an abuse rate greater than 35 percent in mothers delivering prematurely (Dusick et al. 1993). Accompanying this is an upsurge in the incidence of untoward neurologic sequelae of cocaine use in both the pregnant mother and her fetus. Numerous clinical reports in adult cocaine abusers have documented a significant incidence of cerebral vascular accidents, including cerebral infarctions, subarachnoid hemorrhages, and intraventricular hemorrhages. Recently, case reports have suggested similar consequences occur in fetuses born to cocaine-abusing mothers (Dixon and Bejar 1989; Hoyme et al. 1990; Spires et al. 1989; Tenorio et al. 1988).

The inherent complexity of any clinical study of perinatal cocaine abuse is magnified by the high-risk nature of the patients involved. That is, a woman who abuses cocaine places her fetus at risk through other mechanisms as well, including lack of prenatal care, poor nutrition, and polydrug abuse. Thus, animal models of prenatal cocaine exposure are necessary to accurately assess the role of cocaine in perinatal morbidity and mortality. Despite several animal studies examining the cerebral vascular actions of cocaine, a clear picture of cocaine's perinatal cerebral vascular effects has not yet emerged.

This chapter will briefly examine the available clinical data as well as the laboratory animal models used to investigate the problem of cerebral vascular complications of prenatal cocaine exposure. In recent years, several animal models have been developed to expose the fetus under controlled conditions to cocaine concentrations similar in quantity and timing to those experienced by the human fetus. The fetal laboratory

studies discussed will focus primarily on the work performed in the author's laboratory, though the important work of others is also included.

CEREBRAL VASCULAR COMPLICATIONS OF COCAINE IN HUMANS

The placenta offers little protection from cocaine exposure to the fetus as cocaine freely diffuses across the placenta with little significant metabolism (Schenker et al. 1993). Chasnoff and colleagues (1986) first reported a cerebral infarction in a full-term infant born to a mother who had abused cocaine prior to delivery. Subsequent reports have confirmed that the fetus exposed prenatally to cocaine is at risk for neurovascular sequelae including cerebral infarction (Dixon and Bejar 1989; Tenorio et al. 1988), intracranial hemorrhage (Hoyme et al. 1990; Spires et al. 1989), and cavitary lesions (Dixon and Bejar 1989; Tenorio et al. 1988). These studies in neonates parallel the reports in adults. Early case reports in adult cocaine abusers also described cerebral infarctions as well as subarachnoid hemorrhages (Golbe and Merkin 1986; Lichtenfeld et al. 1984; Schwartz and Cohen 1984), and recent larger studies have demonstrated the extent of these complications (Daras et al. 1991; Jacobs et al. 1989; Levine et al. 1990). Imaging studies of cocaine abusers have also documented cocaine-induced abnormalities in cerebral blood flow (Holman et al. 1991; Volkow et al. 1988).

CEREBRAL VASCULAR EFFECTS OF COCAINE IN FETAL AND NEWBORN ANIMALS

Studies in adult animals show cocaine-induced acute cerebral vascular effects which in some species appear to be artery specific (Haung et al. 1990; He et al. 1994; Madden and Powers 1990; Morishima et al. 1992; Stein and Fuller 1993). Cocaine-induced vasoactive changes have also been reported in immature cerebral vasculature following maternal exposure to cocaine; however, to date the results have been equivocal or conflicting due to use of different experimental paradigms. Cocaine has been shown to elicit fetal hypoxemia and systemic hypertension in chronically instrumented fetal sheep (Dolkart et al. 1990; Moore et al. 1986; Woods et al. 1987) and the mechanism of fetal hypoxia appears to be an a-adrenergic mediated decrease in uterine blood flow (Dolkart et al. 1990). However, the mechanism of cocaine-induced fetal hypertension is not understood at present.

CEREBRAL BLOOD FLOW OR HYPERTENSION?

To understand the pathogenesis of cocaine-induced fetal hypertension, the author and colleagues conducted several studies in intact chronically instrumented fetal sheep (Schreiber et al., unpublished data). Intravenous (IV) injection of 1 milligram per kilogram (mg/kg) cocaine into the awake pregnant ewe at 124 days gestation (term: 147 days) elicited a 41 percent increase in maternal arterial pressure with a concomitant decrease (31 percent) in uterine blood flow (figure 1), thus producing a 125 percent increase in calculated uterine vascular resistance. Fetal systemic arterial pressure increased 31 percent, while the cerebral blood flow was decreased by 51 percent (as estimated by a unilateral carotid artery ultrasonic flow transducer) following maternal cocaine injection (figure 2). Consequently, calculated cerebral vascular resistance, which is arterial pressure divided by blood flow, increased twelvefold. Within 10 minutes of cocaine injection, a rebound hyperemia was noted with an increase in cerebral blood flow 134 percent over peak reduction. The cocaine-elicited changes could be attenuated or totally eliminated when studies were performed under general anesthesia.

The present findings differ from those of Gleason and colleagues (1993), who observed an increase in fetal cerebral blood flow with concomitant fetal hypertension in chronically instrumented fetal sheep. Since the percentage increase in fetal systemic arterial pressure was greater than the rise in cerebral blood flow in their study, it would suggest an increase in cerebral vascular resistance. Also, the observed differences in cocaine-elicited fetal cerebral blood changes in the two studies (Gleason et al. 1993) may result from the techniques used to measure blood flow in the two studies. Radioactive microspheres assessment used by Gleason and colleagues (1993) allows for only discrete points of data analysis, whereas flow transducers used in the author's studies allow for continuous monitoring. The validity of flow transducers to accurately measure cerebral blood flow in sheep has been questioned in the past. Covert and colleagues (in press) as well as other researchers (van Be1 et al. 1994) have recently shown the validity of this technique in fetal sheep. Hence, maternal cocaine injection appears to increase fetal cerebral vascular resistance with sustained fetal hypertension and causes an initial decrease in cerebral blood flow followed by a rebound hyperemia.

To obviate the simultaneous changes in pressure and flow and potential cardiovascular reflexes, the in vitro effects of cocaine were examined on the fetal cerebral vasculature using cannulated, pressurized fetal sheep

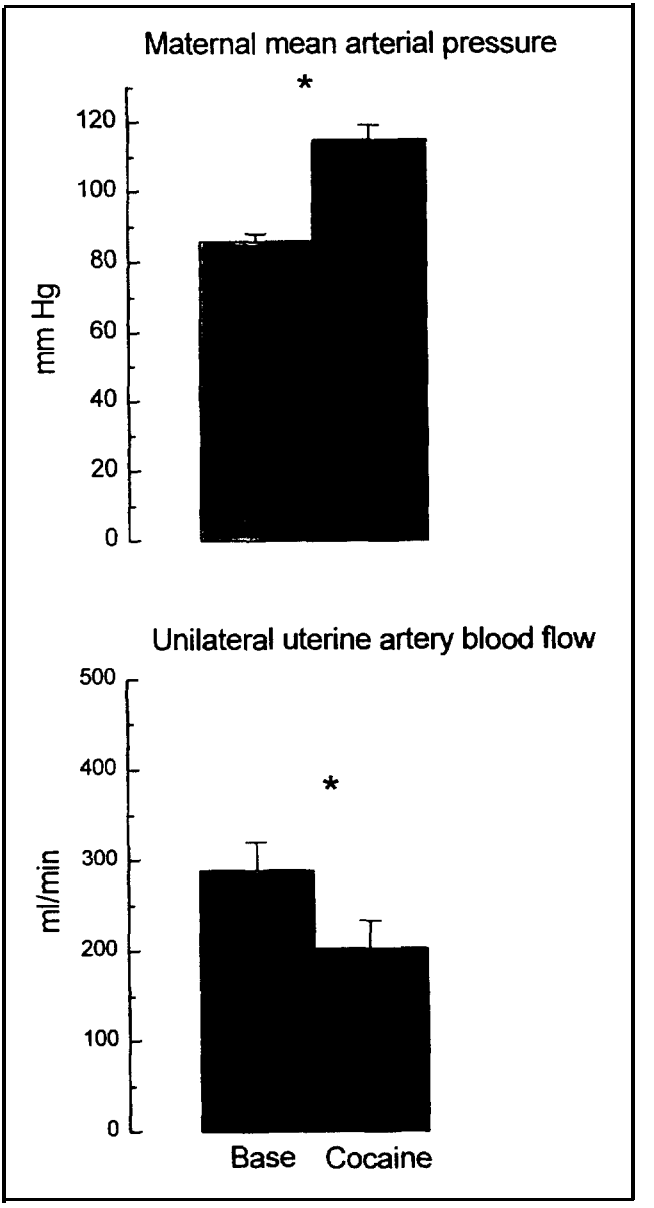

FIGURE 1. *Maternal cocaine injection (1 mg/kg IV) increases maternal mean arterial pressure and decreases uterine blood flow in pregnant ewes.*

KEY: * = $p < 0.05$.

SOURCE: Covert et al. 1994.

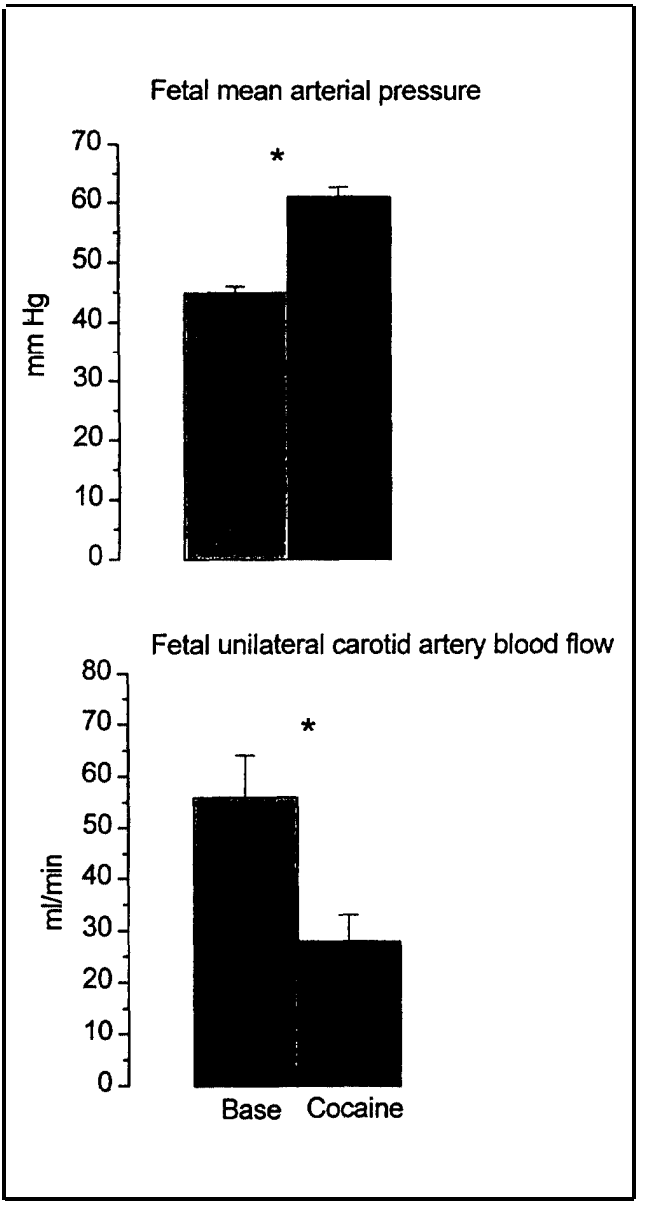

FIGURE 2. *Maternal cocaine injection (1 mg/kg IV) increases fetal mean arterial pressure and decreases carotid artery bloodflow in pregnant ewes.*

KEY: * = $p < 0.05$.

SOURCE: Covert et al. 1994.

cerebral arteries (Schreiber et al. 19946). This technique allows for the assessment of vascular smooth muscle isotonic constriction employing a videomicroscaler to measure changes in vessel diameter. Cocaine caused concentration-dependent constriction of fetal middle and anterior cerebral arteries (123 days gestation). Maximal constriction reached 57 percent of potassium chloride (KCl)-elicited constriction (figure 3). KCl causes direct, nonreceptor-mediated smooth muscle constriction. The KCl response was normalized to better control for slight variability in the amount of vascular smooth muscle between different vascular segments. As resistance (R) is inversely proportional to diameter (D) to the fourth power ($R \propto 1/D^4$), this change in diameter would account for an estimated sevenfold increase in cerebral vascular resistance.

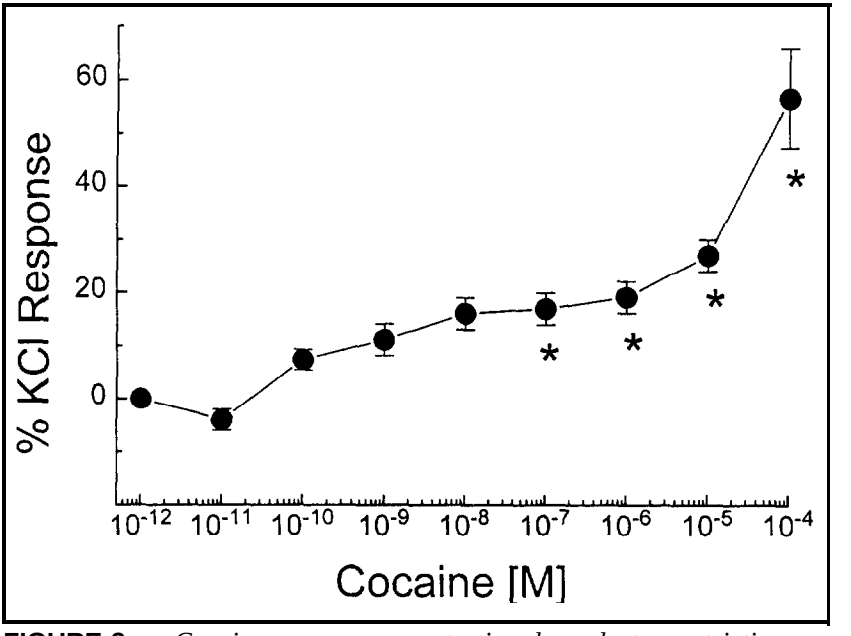

FIGURE 3. *Cocaine causes concentration-dependent constriction of cannulated, pressurized fetal sheep cerebral arteries.*

KEY: * = $p < 0.05$.

SOURCE: Schreiber et al. 19946.

In addition to eliciting vasoconstriction, cocaine was also shown to alter the response of fetal cerebral arteries to monoamine neurotransmitters (Schreiber et al. 1994*a*). The effect of cocaine was shown to be dependent on the cocaine concentration (figure 4). Cocaine at 10^{-5}M augmented norepinephrine-elicited vasoconstriction and increased sensitivity as demonstrated by a leftward shift of the norepinephrine concentration response curve. Drug sensitivity can be quantified by determining the log of the effective concentration which elicits 50 percent of maximal constriction (log EC,). Log EC, norepinephrine decreased from -6.63±0.09 to -7.11±0.03. At higher concentration, 10^{-4}M cocaine had the opposite effect with a 46 percent attenuation of maximal norepinephrine-elicited constriction, which suggests that at least two mechanisms

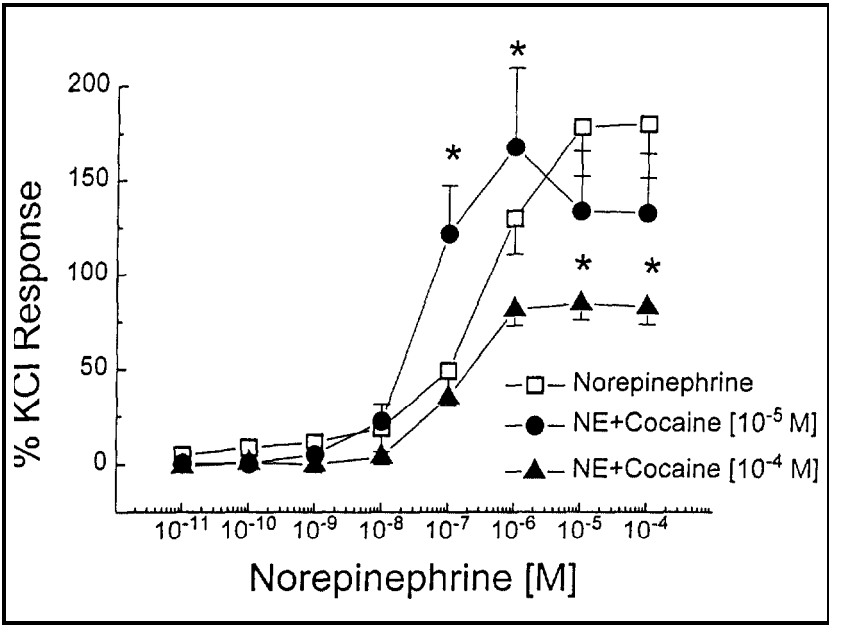

FIGURE 4. *Effect of cocaine on fetal cerebral artery response to norepinephrine. At 10^{-5}M, cocaine augments norepinephrine-elicited maximal response and sensitivity. At 10^{-4}M, cocaine attenuates norepinephrine-elicited maximal response.*

KEY: * = $p < 0.05$ versus same concentration of norepinephrine alone.

SOURCE: Schreiber et al. 1994*a*.

are operative. This author hypothesized that synaptic uptake inhibition at the lower concentration may be responsible for increased norepinephrine sensitivity and augmented constriction, while the local anesthetic properties of cocaine, through Na^+ channel inhibition, may be responsible for attenuated response at higher cocaine concentrations. On the other hand, Albuquerque and Kurth (1993) recently proposed that cocaine-elicited constriction may be mediated through Na^+ channel inhibition.

Cocaine-induced augmentation of norepinephrine-elicited constriction at 10^{-5}M was not monoamine agonist-specific as cocaine also increased serotonin sensitivity and augmented maximal serotonin-elicited constriction (figure 5). Log EC, serotonin decreased from -7.24±0.04 to -7.81±0.08. Cocaine-induced attenuation of serotonin-elicited constriction at higher cocaine concentrations (10^{-4}M) was also demonstrated, confirming that the mechanisms responsible for cocaine-induced response alterations are not specific to norepinephrine.

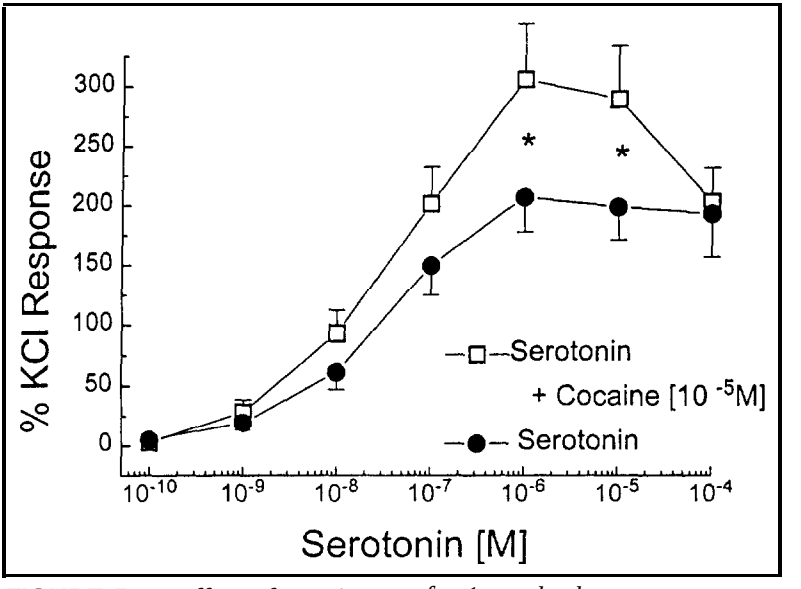

FIGURE 5. *Effect of cocaine on feta1 cerebral artery response to serotonin. At 10^{-5}M, cocaine augments serotonin-elicited maximal response and sensitivity.*

KEY: * = $p < 0.05$ versus same concentration of serotonin alone.

SOURCE: Schreiber et al. 1994*a*.

This effect was further demonstrated by measuring the response to single doses of norepinephrine (10^{-6}M) and serotonin (10^{-6}M) with and without cocaine (10^{-4}M) pretreatment. In these studies, cocaine decreased monoamine agonist response 26.5 percent and 40 percent, respectively (figure 6).

CEREBRAL VASCULAR EFFECTS OF COCAINE METABOLITES

Clinically, the cocaine-exposed fetus is subjected to the effects not only of cocaine but also of cocaine metabolites that accumulate in the fetal body and brain. In humans, acute cocaine administration produces serum cocaine concentrations up to 10 micromolars (µM) and has a serum half-life of 90 minutes (Gawin and Ellinwood 1988). Whether the cocaine kinetics in human pregnancy are altered is not yet known. In

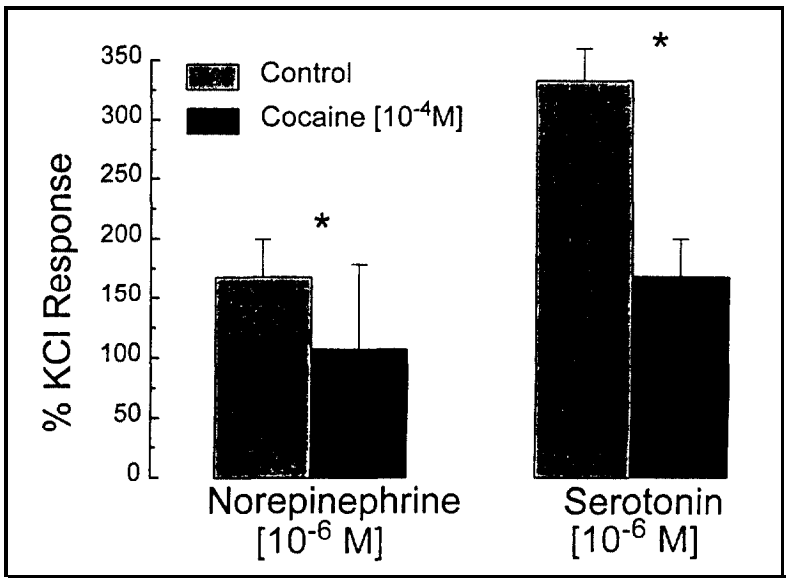

FIGURE 6. *Cocaine, 10^{-4}M, attenuates fetal cerebral artery response to norepinephn'ne and serotonin.*

KEY: * = $p < 0.05$.

SOURCE: Schreiber et al. 1994*a*.

animals, however, pregnancy-associated changes in cocaine disposition have been reported and are reviewed elsewhere (Morishima et al., this volume).

Cocaine can be metabolized enzymatically as well as nonenzymatically to produce benzoylecgonine (Stewart et al. 1979), which is the major cocaine metabolite routinely assayed in meconium of newborn babies exposed to cocaine in utero (Maynard et al. 1992; Ostrea et al. 1989, 1992; Schutzman et al. 1991). Studies in rats also show significant concentrations of benzoylecgonine in the fetal brain even when the fetal serum levels are lower compared to the maternal serum concentrations (DeVane et al. 1989; Spear et al. 1989; Spiehler and Reed 1985). Cocaine is also metabolized to ecgonine and ecgonine methyl ester (Stewart et al. 1979) and may undergo N-demethylation to produce norcocaine (Madden and Powers 1990; Stewart et al. 1979). Cocaine pharmacokinetics in the neonatal guinea pig are similar to human newborns (Sandberg and Olsen 1991, 1992).

To evaluate the pharmacological effects of benzoylecgonine, the major cocaine metabolite, the author examined its acute effects on cannulated, pressurized fetal sheep cerebral arteries using the technique described above for in vitro studies. Benzoylecgonine caused a similar degree of maximal constriction as cocaine (figure 7); however, the sensitivity of fetal cerebral arteries to benzoylecgonine was increased by almost a hundredfold (log EC, benzoylecgonine -8.79±0.36 versus log EC, cocaine -6.95±0.42). The observed flat characteristic of the concentration response curves suggests that more than one mechanism may be involved in the vasoconstriction effects of benzoylecgonine. Studies with adrenergic antagonist agents showed inhibition of benzoylecgonine-induced vasoconstriction by phentolamine, suggesting benzoylecgonine effects are mediated through stimulation of α_1-adrenergic receptors.

The responses of other cocaine metabolites on fetal cerebral vasoconstriction showed significant norcocaine-elicited constriction of cerebral artery segments, but only at 10^{-4}M. Ecgonine and ecgonine methyl ester caused no constriction at the concentrations tested (figure 8).

However, a small degree of dilation was observed with ecgonine methyl ester at 10^{-4}M. Comparison of cocaine and its metabolites' responses showed benzoylecgonine to be the most potent vasoconstrictor followed by cocaine >> norcocaine, ecgonine, ecgonine methyl ester.

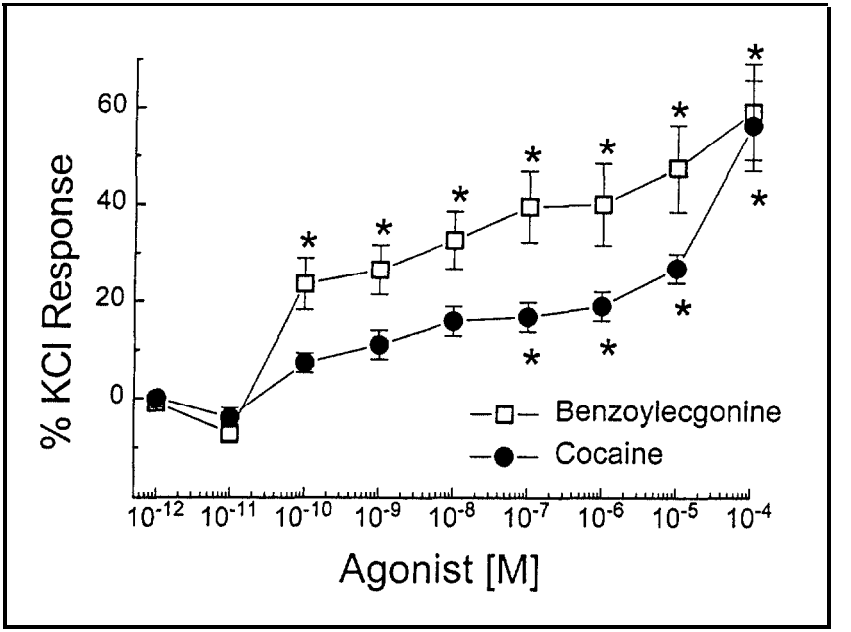

FIGURE 7. *Benzoylecgonine causes concentration-dependent constriction of cannulated, pressurized fetal sheep cerebral arteries. Response to cocaine is shown for comparison. Fetal cerebral arteries are more sensitive to benzoylecgonine than to cocaine.*

KEY: * = $p < 0.05$ versus 10^{-12}M.

SOURCE: Schreiber et al. 1994*b*.

The present results on the vasoactive effects of the cocaine metabolite benzoylecgonine are similar to those reported by others, though notable differences exist. These differences may be maturational, species, or methodological phenomena. For instance, benzoylecgonine, which does not inhibit synaptic monoamine uptake (Ritz et al. 1990), is the most potent cocaine metabolite in studies of adult cats (Madden and Powers 1990) and in fetal sheep (Schreiber et al. 1994*b*) but not in newborn piglets (Kurth et al. 1993). In immature animals, the difference may be due to the underdeveloped nature of sympathetic central and peripheral innervation (Shaul et al. 1990) or due to limited perivascular innervation of pial arterioles (Hardebo et al. 1986). Furthermore, the mechanism through which benzoylecgonine causes vasoconstriction is also controversial. In fetal sheep, α-adrenergic inhibition blocked

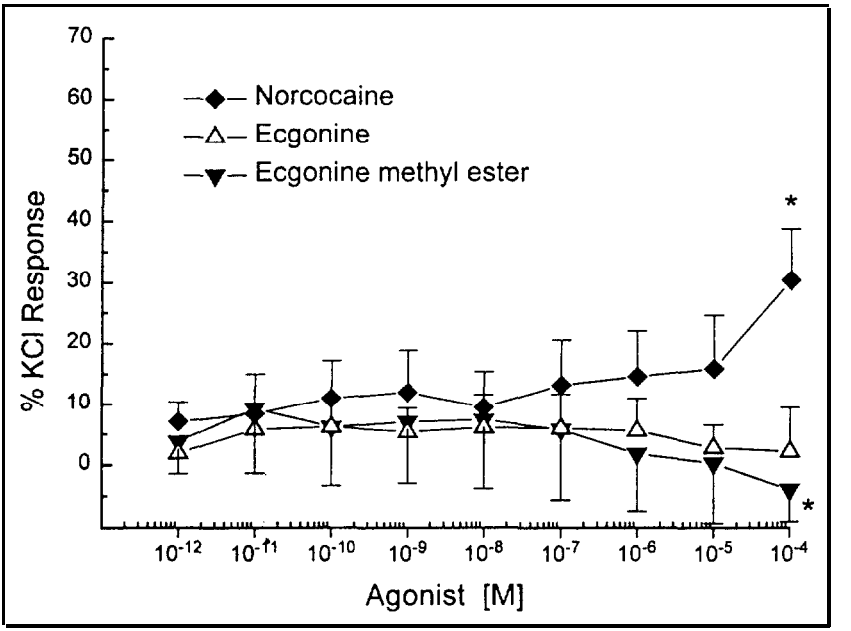

FIGURE 8. *Response of fetal sheep cerebral arteries to other cocaine metabolites.*

KEY: * = $p < 0.05$ versus 10^{-12}M.

SOURCE: Schreiber et al. 1994*b*.

benzoylecgonine-elicited constriction of cerebral arteries while it had no effect on benzoylecgonine-elicited constriction in newborn piglet pial arterioles (Kurth et al. 1993). In adult animals, benzoylecgonine-induced vasoconstriction appears to be dependent on influx of extracellular Ca^{2+} as reported by Madden and colleagues (1995).

The author and colleagues also evaluated the pharmacological effects of cocaethylene, a unique cocaine metabolite formed through hepatic transesterification in the presence of ethanol. Over 75 percent of cocaine abusers also use alcohol; thus, cocaethylene may be an important metabolite in the toxic effects of cocaine and ethanol. Also, the pharmacologic profile of cocaethylene is somewhat unique; that is, at high concentrations, it inhibits Na^{+} channels and is equipotent to cocaine as an inhibitor of the dopamine transporter, whereas it is less potent as an inhibitor of the norepinephrine and serotonin transporters.

Examination of cocaethylene in fetal and adult cerebral artery segments showed concentration- as well as age-dependent constriction (i.e., 70-day fetus > 120-day fetus > adults) (figure 9). In addition, even after correction for the cocaethylene-elicited constriction, cocaethylene significantly decreased maximal norepinephrine-elicited constriction by 35 percent and decreased sensitivity by nearly tenfold (figure 10).

Additional studies in awake, chronically instrumented fetal sheep showed significant levels of cocaethylene in fetal serum following maternal injection of cocaethylene and increased fetal systemic arterial pressure with concomitant decreased cerebral blood flow similar to cocaine (figure 11) (Covert et al. 1994). In contrast to cocaine-induced decrease in uterine artery blood flow and fetal oxygenation, cocaethylene increased uterine artery blood flow (figure 12) but had no effect on fetal

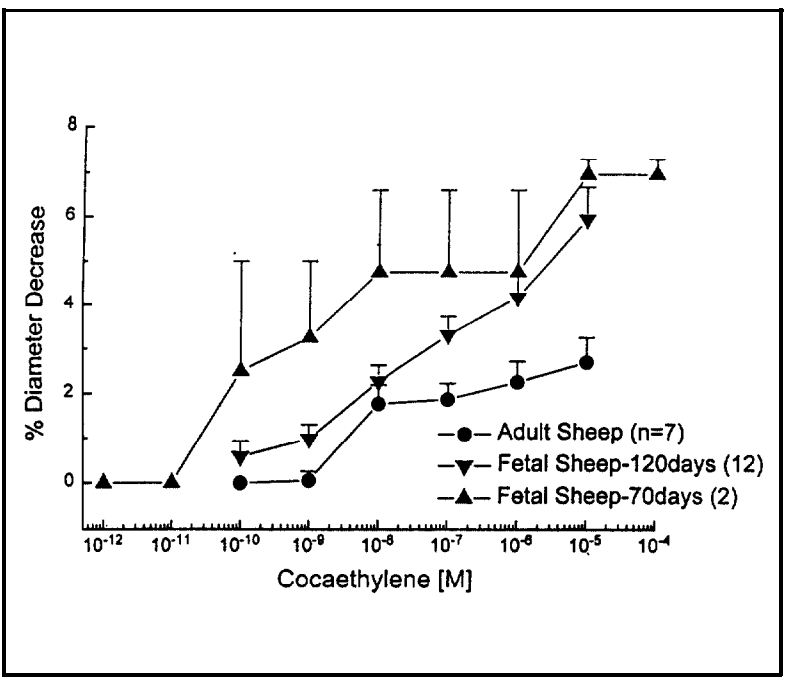

FIGURE 9. *Cocaethylene causes concentration-dependent constriction of fetal sheep cerebral arteries. Fetal sheep responses were greater than adult sheep ($p < 0.05$).*

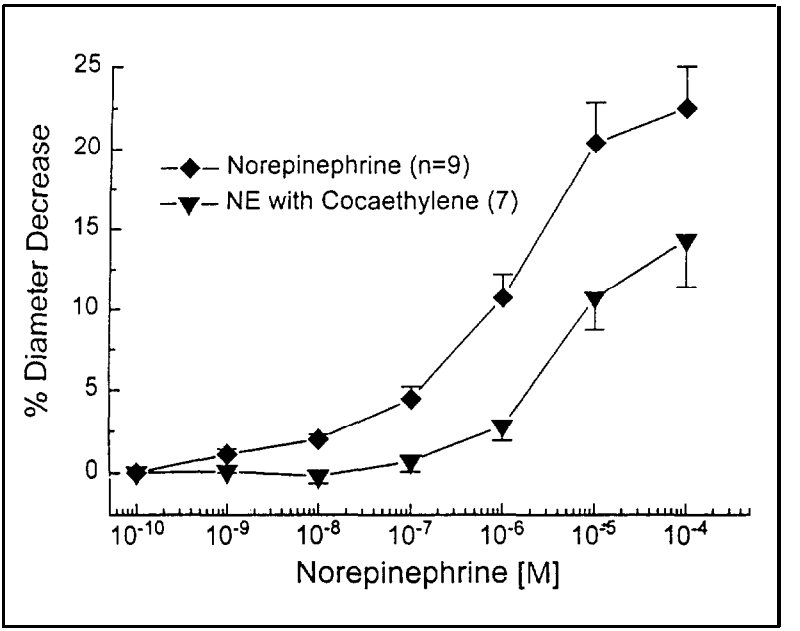

FIGURE 10. *Cocaethylene attenuates fetal cerebral artery maximal norepinephrine-elicited constriction and decreases norepinephrine sensitivity ($p < 0.05$).*

oxygenation. Although it has been suggested that cocaine and ethanol work independently on the cerebral circulation, the present results suggest that the combined metabolic product of these two drugs (i.e., cocaethylene) may play an important role in the pathophysiologic effects of cocaine.

CONCLUSIONS

In summary, the in vitro and in vivo studies, as well as findings from other laboratories, show significant cocaine-induced changes in the fetal cerebral vascular system. Although the patbogenesis of cocaine insult is not yet defined, it could involve a variety of mechanisms that could include the direct action of cocaine on synaptic monoamine uptake inhibition, Na^+ channel inhibition, direct vasoreactivity, and indirect effects through cocaine metabolites. Cocaine may also cause injury at several diverse sites within the fetal-maternal unit; for instance, the fetal insult through maternal or fetal hypertension, uterine vasoconstriction,

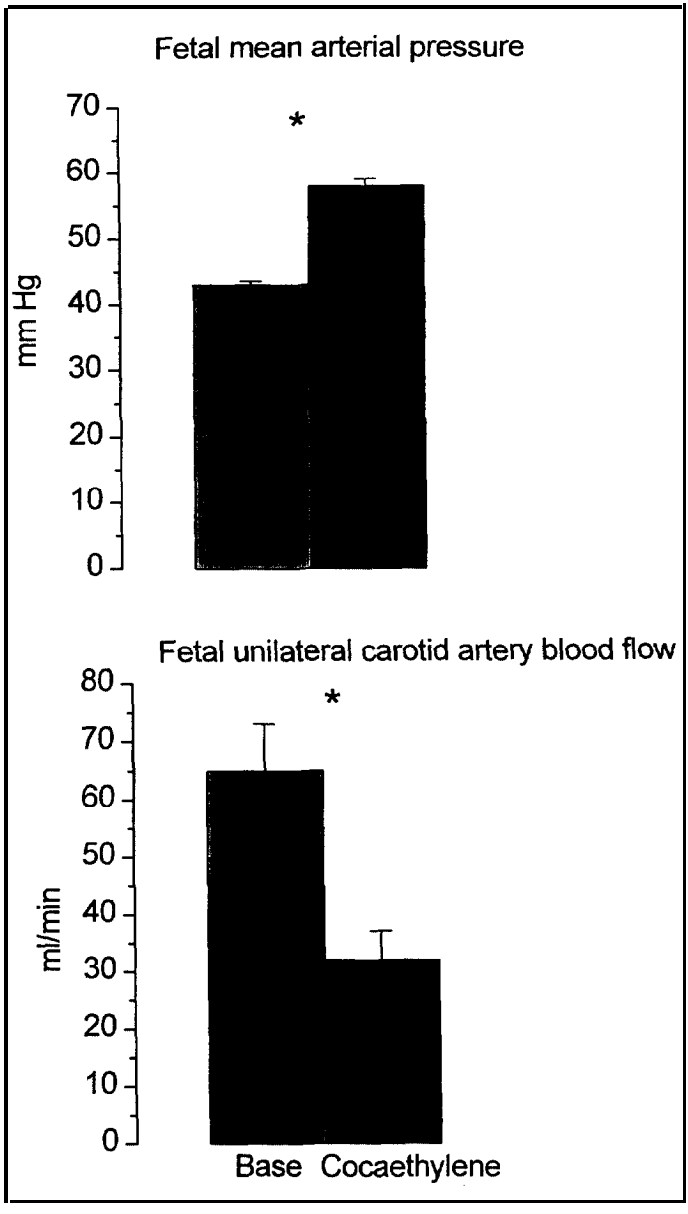

FIGURE 11. *Maternal cocaethylene injection (1 mg/kg IV) increases fetal mean arterial pressure and decreases carotid artery bloodflow in pregnant ewes.*

KEY * = $p < 0.05$.

SOURCE: Covert et al. 1994.

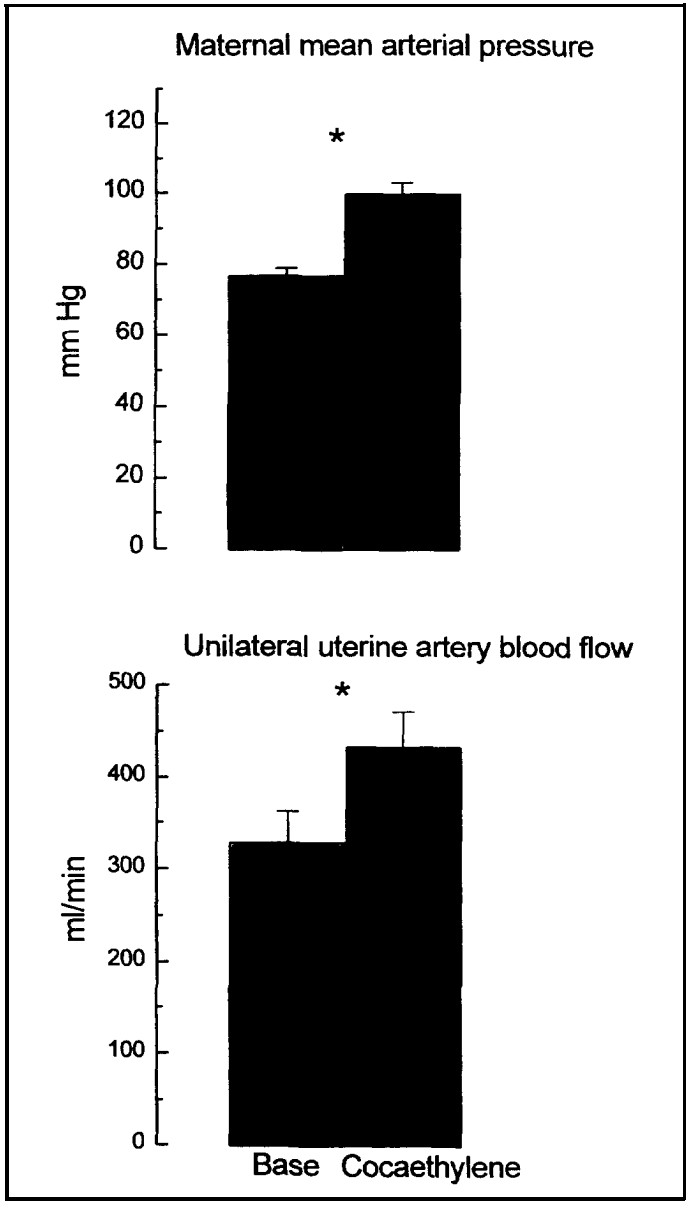

FIGURE 12. *Maternal cocaethylene injection (1 mg/kg IV) increases maternal mean arterial pressure and decreases uterine blood flow in pregnant ewes.*

KEY: * = $p < 0.05$.

SOURCE: Covert et al. 1994.

and fetal hypoxemia. Such alterations of the developing cerebral circulation, whether by a decrease or an increase in cerebral blood flow, are likely to contribute to, if not be fully responsible for, the reported perinatal neurovascular complications of prenatal cocaine exposure.

Although a typical cocaine abuser ingests cocaine throughout pregnancy, much of the current research is focused primarily on the acute effects of cocaine exposure during pregnancy. In addition to the catastrophic cerebral vascular complications reported in the newborn, it is not known whether more subtle effects occur in the fetus and in the newborn following in utero chronic cocaine exposure. Thus, additional clinical and animal studies are needed to address the confounding issues and to better delineate the effects of chronic cocaine exposure and its mechanisms.

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ACKNOWLEDGMENT

This work was supported by National Institute on Drug Abuse grant no. DA-07607.

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Effects of Prenatal Morphine and Cocaine on Postnatal Behaviors and Brain Neurotransmitters

Ilona Vathy

INTRODUCTION

The social and economic repercussions of drug use have received much attention in recent years. Of major concern are the problems of drug abuse among pregnant women and the potential adverse effects of psychoactive drugs on developing fetuses. Neuroactive compounds such as cocaine and opiates have been shown to readily cross the placenta and blood-brain barrier; thus, exposure to these drugs during pregnancy may modify critical developmental events in the brain of exposed offspring, resulting in permanent alterations in neural functions including behavior. Children born to women who abuse drugs during pregnancy are reported to have problems related to attention, self-discipline, responsibility, self-confidence, and peer relationships, as well as strong antisocial trends (Johnson and Rosen 1982; Wilson et al. 1973, 1979). The consequences of withdrawal of opiates are recognized (Perlmutter 1967, 1974), but the consequences of withdrawal of cocaine in the newborn are still being debated. Increased irritability, crying, vigorous suckling, and poor eating behavior, in addition to alterations in startle reflex, poor visual processing, and decreased organizational responses to environmental stimuli have been reported in newborn infants born to cocaine-addicted mothers (Chasnoff et al. 1987; Oro and Dixon 1987). These symptoms may represent a withdrawal syndrome that is characteristic of stimulants. They may also be a direct drug effect, because cocaine metabolites have been found in prenatally exposed neonates up to 7 days after birth (Oro and Dixon 1987). Unfortunately, the current clinical literature lacks longitudinal studies of children born consequent to maternal cocaine abuse.

Understanding the consequences of prenatal drug exposure for the development of the human central nervous system (CNS) and for behaviors is an extremely difficult task. Thus, animal studies are essential to correlate neurochemical and behavioral consequences of such prenatal drug exposure with clinical evidence. Indeed, in recent years

animal studies describing long-lasting effects of prenatal exposure to psychoactive drugs on postnatal development have appeared in increasing numbers (Hammer 1993; Zagon and Slotkin 1992).

It has been hypothesized that opiates and cocaine induce long-lasting alterations in the neurobehavioral development of exposed offspring by altering the same neural mechanisms affected when drugs are given to adults. When given acutely to adult animals, both morphine (the active component of heroin) and cocaine alter behaviors in exposed animals in part by interacting with a number of neurotransmitter systems, including the monoamines. For example, morphine and cocaine modify motor activities such as spontaneous motor behaviors including ambulation and motor coordination and open-field activities such as rearing, grid crossing, grooming, gnawing, sniffing, and nose poking. The striatal dopamine (DA) systems regulate many of these motor behaviors, and studies have correlated alterations in motor activities following morphine or cocaine administration to adult animals with alterations in striatal opioid and DA receptor subtypes (Hammer 1993; Lakoski et al. 1992). Therefore, one likely mechanism of action for both drugs is the ability to alter sttiatal DA neurotransmission.

In the adult brain, cocaine is also known to inhibit the presynaptic transporter mechanism (reuptake) which removes DA, norepinephrine (NE), and serotonin from the synapse, thereby potentiating the effects of these neurotransmitters (Taylor and Ho 1978). Opiates also affect central monoamines. Studies of CNS localization of endogenous opioid peptides and monoaminergic projections suggest that there is an anatomical basis for interaction between these peptides and the monoamine neurotransmitters. Many brain areas that contain endogenous opioid peptides are rich in tyrosine hydroxylase and dopamine beta hydroxylase immunoreactivity, the enzymes that synthesize DA and NE (Ge et al. 1993; Khachaturian and Watson 1982; Mitchell et al. 1988). Behaviors that are stimulated by catecholamines are often inhibited either by endogenous or exogenous opiates. These inhibitory actions have been correlated with inhibition of catecholamine neurotransmission (Szekely 1994). Thus, many investigators have assessed monoamine-regulated behaviors and neurochemistry in animals gestationally exposed to abused drugs.

Studies examining postnatal behavioral and biochemical alterations in prenatally morphine- or cocaine-exposed animals tend to focus on one of three developmental timeframes rather than investigating the entire lifespan. Thus, the studies reviewed here will be discussed in these

developmental timeframes: (1) from postnatal weeks 1 to 3 (from birth to weaning); (2) from postnatal weeks 4 to 8 (from weaning through adolescence, including puberty); and (3) from postnatal weeks 9 to 25 (from young adult to mature adult). Both neurobehavioral and neurochemical changes that occur during these developmental periods following morphine or cocaine exposure during gestation will be reviewed. It is important to note that variations in the dose, time, and route of administration of drugs, as well as the specific behavioral parameters measured by different laboratories, are likely contributors to the inconsistencies to be described below. Moreover, few of the studies examining brain neurotransmitters following prenatal opiate exposure include behavioral measures in their experiments.

EFFECTS OF PRENATAL OPIATE EXPOSURE

Neurobehavioral Effects

Postnatal Weeks 1 to 3. The discovery of endogenous opioid systems that regulate somatic and neural growth raises the possibility that exogenous opiates, when present at inappropriate times or in non-physiological concentration, can alter neural development. Endogenous opioids are thought to inhibit brain development, including neuronal growth (Hauser et al. 1989; Komblum et al. 1987; Zagon and McLaughlin 1986*a*, 1986*b*) and proliferation (Zagon and McLaughlin 1987, 1991), by opioid receptor-dependent mechanisms. Other studies demonstrated that prenatal morphine reduces neuronal packing density and the number of neurons in newborn rats without affecting cortical thickness (Ricalde and Hammer 1991; Seatriz and Hammer 1993). This suggests that prenatal morphine might act to restrict cortical cell proliferation and maturation. In prenatally opiate-exposed rat pups, brain deoxyribonucleic acid (DNA) synthesis, cell proliferation, and synaptogenesis have also been reported to be delayed (Hammer 1993).

In general, morphine-exposed animals exhibit delayed eye and ear opening (McGinty and Ford 1976). During the first 3 weeks of postnatal life, prenatal opiates also delay the development of righting, startle, and auditory reflexes as well as motor skills required for ambulation and coordination (Caza and Spear 1980; Friedler 1977; Zagon and McLaughlin 1978). These developmental and behavioral delays are often considered to result from drug withdrawal. However, it is also possible that repeated intrauterine exposure to opiates may have direct effects on

the development of the nervous system, because opiate drugs are readily distributed across the placenta to the fetus (Szeto et al. 1978, 1981, 1982).

neurochemical and neuroanatomical abnormalities as well as altered drug response, delayed behavioral development, altered motor activity levels,

1992, 1993; Slotkin et al. 1979; Zagon and McLaughlin 1978, 1982; Zagon et al. 1979*a*, 1979). The behavioral alterations appear to be transitory in nature since they disappear by the age of 4 weeks

the brain NE metabolizing enzyme monoamine oxidase (Tsang et al. 1986); deficits in the central uptake of serotonin, DA, and NE (Slotkin et

following perinatal exposure to methadone (Darmani et al. 1992).

Postnatal Weeks 4 to 8. The literature regarding the neurobehavioral effects of prenatal morphine during the time of weaning to adolescence is inconsistent at present. Some laboratories show increased activity in morphine-exposed animals (Davis and Lin 1972), others demonstrate reduced open-field ambulation and rearing (Friedler 1977), and still others observe no behavioral alterations (Castellano and Ammassari-Teule 1984; Sonderegger and Zimmerman 1978; Sonderegger et al. 1979). These discrepancies may be due to an age-related switch from hypoactivity in neonates to hyperactivity in adolescent, morphine-exposed offspring (Sobrian 1977). The normal developmental changes in motor activity have been correlated with the maturation of adrenergic hindbrain and cholinergic forebrain structures (Campbell and Mabry 1973; Campbell et al. 1969); a depletion in brain catecholamines has been shown to produce hyperactivity in 2- to 4-week-old rat pups (Shaywitz et al. 1976). Thus, it is possible that prenatal morphine depletes or temporarily modifies the normal development of brain catecholamines.

Another factor that may contribute to the inconsistent results is gender-related, postpubertal differences in circulating gonadal hormones, as puberty occurs in rats between 5 and 6 weeks of age. Thus, females tested after puberty may be at different stages of the estrous cycle, and it is known that open-field activity of female rats is influenced by the estrous cycle stage (Johnston and File 1991; Nomikos et al. 1987). Likewise, postpubertal males will have elevated levels of testicular androgens. Thus, it is critical that future studies include gender as a variable.

Postnatal Weeks 9 to 25. Studies examining adult behavioral and physiological consequences of fetal exposure to opiates are limited. This may result from the apparent transient nature of changes in activity and spontaneous motor behaviors observed during earlier postnatal

adult rodents exhibit increased treadmill running, ambulation, rearing, and locomotor activities, and sexually dimorphic changes in reproductive

Vathy and Katay 1992; Vathy et al. 1983, 1985; Ward et al. 1983; Zagon et al. 1979*a*). Ward and colleagues (1983) found that prenatal exposure

(lordosis) behavior in male rats. Johnston and colleagues (1992, 1994) demonstrated in hamsters that perinatal morphine exposure increases the

unchanged or enhanced. This author's work, as discussed below, demonstrates that prenatal morphine exposure inhibits female sexual

1992; Vathy et al. 1983, 1985). It is interesting that in both rats and hamsters, adult male and female sexual behaviors are differentially

Effects on Brain Neurotransmitters

There are too few biochemical studies to review each of the three developmental timeframes separately; therefore, they will be discussed together. A more exhaustive discussion of this literature may be found in Hammer (1993). Because chronic administration of opiates can modify brain opioid receptor binding in adults, several authors have examined the impact of neonatal opiate exposure on opioid receptor binding at different postnatal periods. For example, prenatal morphine administration is reported to have no effect on mu receptor binding in forebrain homogenates of 1-day-old rats (Bardo et al. 1982; Coyle and Pert 1976), whereas mu receptor binding is increased in the sttiatum and nucleus accumbens 6 months after morphine treatment (Handelmann and Quirion 1983). Prenatal morphine exposure is also reported to decrease mu receptor binding in the whole brain during the first postnatal week (Kirby and Aronstam 1983; Tempel et al. 1988) and to increase mu receptor binding at later ages (Iyengar and Rabii 1982; Tsang and Ng 1980). Studies administering morphine through gestation and lactation until postnatal day (PD) 5 demonstrate decreased mu receptor binding in the medial and lateral preoptic area (POA), but not in the sensory cortex (Hammer et al. 1991).

Tempel (1991) has shown that 4 days of postnatal morphine administration completely eliminates mu receptor binding sites on PD 5 in striatal patches and has a smaller effect on binding in the striatal matrix, nucleus accumbens, and amygdala. Thus, downregulation of mu receptor binding is regionally specific during the early postnatal period. In contrast, longer durations of morphine treatment (PDs 1 to 8) do not induce alterations in mu opiate receptor density on PD 9 in the neocortex, nucleus accumbens, basolateral amygdala, hippocampus, dorsomedial or ventromedial hypothalamus, substantia nigra, thalamus, periaquaductal gray, or locus coeruleus, areas that have moderate mu receptor binding levels in adult animals. These studies suggest that modifications of mu receptor binding by prenatal morphine are influenced by the duration of morphine exposure and the age of the animal when receptor binding is assayed.

During early development of the CNS, another monoamine neurotransmitter, serotonin, is thought to act as a signal for growth and synaptogenesis (Lauder 1990; Lauder and Krebs 1978; Whitaker-Azmitia et al. 1987). Serotonin may act as a regulatory substance for both the development of serotonin neurons and for the proliferation and maturation of other neural populations (Azmitia and Whitaker-Azmitia 1987; Chubacov et al. 1986; Jonakit et al. 1983; Lauder 1990; Lauder and Krebs 1978). Opiates have been reported to alter serotonin levels in adults (Gopalan et al. 1989). Thus, alterations in levels or distribution of serotonin may also result from prenatal opiate exposure.

EFFECTS OF PRENATAL COCAINE EXPOSURE

Neurobehavioral Effects

Postnatal Weeks 1 to 3. Prenatal exposure to cocaine has been shown to induce neurobehavioral abnormalities and structural alterations in the developing brain (Dow-Edwards et al. 1990; Gingras et al. 1992; Hutchings 1993; Hutchings et al. 1989; Spear et al. 1989*a*). The mechanisms underlying these pathophysiological changes may be direct or indirect effects of cocaine on the developing offspring. For example, cocaine produces placental and cerebral ischemia, depriving the fetus of oxygen and nutrients (Koegler et al. 1991; Mactutus and Fechter 1986; Woods et al. 1987). Cocaine can also impact directly on the survival of brain cells by inhibiting mitosis and retarding cell differentiation

(Albuquerque and Kurth 1993; Anderson-Brown et al. 1990; Zachor et al. 1994).

Prenatal cocaine exposure, like morphine, delays the acquisition of righting and startle reflexes (Henderson and McMillen 1990; Sobrian et *b*). Unlike morphine, it increases spontaneous locomotor activity during the first 3 weeks of postnatal life, during active (lights off) and inactive (lights on) periods (Henderson and McMillen 1990). However, at age 20 days, rats placed into a novelty cage may also display hypoactivity (Church and Overbeck 1990; Henderson and McMillen 1990). Additionally, prenatal cocaine exposure decreases learning of odor cues from milk. Cocaine-exposed offspring also exhibit reductions in foot-shock-precipitated wall climbing at PD 12, a behavior that has been related to levels of catecholamine activity at this age (Spear et al. 1989*a*, 1989*b*).

Postnatal Weeks 4 to 8. Behavioral studies demonstrate inconsistencies in spontaneous motor and open-field behaviors such as rearing, grid crossing, and grooming. Riley and Foss (1991*a*) found that animals exposed prenatally to cocaine do not differ in any locomotor activity from controls. Pet-is and colleagues (1992) showed that prenatally cocaine-exposed females, but not males, display increased spontaneous locomotor behaviors. Vathy and colleagues have shown that cocaine-exposed females rear about 50 percent less than controls, while cocaine-exposed males exhibit the same or somewhat more rearing than saline controls (Vathy et al. 1993). The behavioral inconsistencies in these three studies may have resulted from the use of different doses of cocaine and different gestational regimens for cocaine exposure.

Postnatal Weeks 9 to 25. Investigations of cocaine-exposed animals during adulthood show reductions in rearing, grid crossing, and nose pokes in the open field (Peris et al. 1992). In the same study, a cocaine challenge injection in prenatally cocaine-exposed females or control males failed to alter behavioral activity, whereas it increased activity in saline-exposed females. By contrast, Giordano and colleagues (1990) observed no effects on locomotion and stereotypy in prenatally cocaine-exposed rats. Riley and Foss (1991*a*, 1991*b*) demonstrated no changes in locomotor activities or in acquisition of passive avoidance, active avoidance, and spatial navigation tasks by animals exposed prenatally to cocaine.

Prenatal cocaine exposure in the same animals at PDs 60, 90, and 180 appears to produce different behavioral changes. Cocaine-exposed offspring with no obvious behavioral alterations at PD 60 show unusual sensitivity to environmental stimuli at later ages (McMillen et al. 1990; Miller and Seidler 1994). They exhibit abnormal startle reactions, irritability, even fearfulness such that they refuse to enter an open field and take longer to find a submerged platform than controls. Miller and Seidler (1994) also found that female offspring of saline-exposed dams display greater motor activation to a cocaine injection than male offspring at age 60 days. However, these sex differences were completely eliminated by prenatal exposure to cocaine. That is, female rats receiving cocaine during the prenatal period show the same motor activation to an acute cocaine challenge as do males exposed to saline in utero. In other studies, Raum and colleagues (1990) showed alterations in adult male scent-marking behavior in prenatally cocaine-exposed male rats. Vathy and colleagues have shown that prenatal cocaine exposure differentially affects adult male and female sexual behavior (Vathy et al. 1993). These behavioral studies, therefore, strongly suggest that it is essential to conduct lifespan experiments.

Effects on Brain Neurotransmitters

Studies in this section are reviewed together. Striatal DA receptors have long been implicated in the regulation of locomotor behavior, including open-field activities (Fishman et al. 1983; Furchtgott et al. 1961; Hutchings et al. 1989). Some laboratories have demonstrated changes in

1

(1989*a*

Prenatal cocaine exposure has also been reported to increase glucose utilization in the hypothalamus and limbic region in 60-day-old female but not male rats (Dow-Edwards et al. 1988; Freed et al. 1988)

tions in DA release, measured in the striatum by in vivo microdialysis, have been shown at PD 12, but not 1 or 2 months after birth (Keller et al. 1991). Vathy and colleagues (1993) have shown that prenatal cocaine increases NE and DA content in the POA of young adults males, but has no effects in females.

Prenatal cocaine exposure may also alter the density and distribution of central catecholamine fibers. On postnatal day 28 it increases tyrosine hydroxylase immunoreactivity in the rat hippocampus, where a rich NE innervation is normally seen, and in the cortex (Akbari and Azmitia 1992). The same authors also examined the effects of prenatal cocaine exposure on the development of serotonin systems. Prenatal cocaine inhibits the normal growth of serotonin fibers in the cerebral cortex and hippocampus but not in the spinal cord (Akbari et al. 1992). The density of serotonin fibers in the cortex and hippocampus is decreased at PDs 1 and 7. By postnatal week 4, no significant effect was observed, indicating that the effects of prenatal cocaine on serotonin fiber density are transient. The effect of prenatal cocaine on the development of striatum has also been examined. Snyder-Keller and Keller (1993) saw no obvious changes in the development of the nigrostriatal DA system, as assessed by the distribution of DA neurons in the substantia nigra and DA terminals in the striatum. Despite the lack of effects on DA in growth of the striatum (between PDs 18 to 24, 30 to 40, or after 45 days of age), prenatal cocaine treatment does alter serotonergic ingrowth. A hyperinnervation by serotonin-immunoreactive fibers is observed in the striatum of adult, prenatally cocaine-treated rats. The 50 to 200 percent increase in fiber density was comparable in both males and females, and in both Long-Evans and Sprague-Dawley strains (Snyder-Keller and Keller 1993). Thus, cocaine-induced changes in serotonin fiber density could potentially account for long-term behavioral changes.

PRENATAL EXPOSURE TO DRUGS AFFECTS ADULT REPRODUCTIVE BEHAVIORS AND BRAIN NEUROTRANSMITTERS

Due to the relative dearth of information on drug-induced changes in male and female reproductive behaviors and their regulation by central

catecholamines, the author has pursued earlier findings to further examine the gender-specific effects of prenatal exposure to morphine or cocaine on these behaviors and their neurochemical correlates.

Effects of Prenatal Morphine Exposure on Adult Sexual Behavior and Hypothalamic NE

In most of the experiments pregnant rats were injected with 10 milligrams

18 (Vathy et al. 1983, 1985). This prenatal morphine exposure does not

offspring. Early work by Vathy and colleagues demonstrated that adult,

reproductive behavior in response to the ovarian steroids estradiol and

observed even when pups were raised by control dams, and were not attributable to decreased access of steroid to the brain or to decreases in steroid receptor binding in the POA or hypothalamus. In contrast, male rats exposed to morphine in utero exhibit facilitated reproductive behavior (Vathy et al. 1985).

In a more recent study, Vathy and Katay replicated earlier findings that female offspring of rats given morphine during mid to late gestation (days 11 to 18) are impaired in their ability to exhibit estrous behavior in response to ovarian steroids administered in adulthood (Vathy and Katay 1992). Moreover, this study also examined adult male sexual behavior in detail after the same in utero morphine exposure, and tested the hypothesis that prenatal morphine alters catecholamine content of adult male and female rat hypothalamus (Vathy and Katay 1992). Male and female rats were tested for adult sexual behavior, and the behavioral effects were correlated with NE and DA levels and turnover in the hypothalamus and other brain regions.

Exposure to morphine on gestational days 11 to 18 differentially altered adult sexual behavior and brain catecholamines in male and female rats (Vathy and Katay 1992). Female rats exposed prenatally to morphine exhibit a dramatic inhibition in sexual behavior relative to controls (table 1). This behavioral modification is unlikely to reflect global deficiencies in the hypothalamic-pituitary-gonadal axis, because prenatal morphine does not alter estrous cyclicity or the timing of puberty (Vathy et al. 1985). In contrast, males exposed to morphine in utero show facilitated reproductive behavior. They mount and intromit significantly more

TABLE 1. *Effects of prenatal morphine on adult female reproductive behavior.*

Week of Test	Prenatal Treatment	N	Lordosis Quotient
1	Saline	15	70±2
	Morphine	14	10±1*
2	Saline	15	90±1
	Morphine	14	13±1*

NOTE: Lordosis quotient is the percentage of lordosis responses exhibited in a 10-mount test (number of lordosis/number of mounts x 100). Values are means±SEM.

KEY: * = $p < 0.001$ versus saline.

SOURCE: Data are modified from Vathy and Katay 1992.

frequently, but need more time to achieve an ejacu-lation than saline-treated males. Morphine-exposed male offspring also have shorter postejaculatory intromission latencies than saline-treated males (table 2). Higher mounting and intromitting frequencies may reflect increased sexual motivation; however, it could also result from decreased penile sensitivity or erectile capacity. This might suggest an effect of prenatal morphine on the spinal cord. Stimulation of opioid systems in adult male rats can disrupt copulation, and the site of this action is the spinal cord (Komisaruk 1982; Pfaus and Gorzalka 1987; Wiesenfeld-Hallin and Sodersten 1984). If prenatal morphine alters sensory signals coming from an intromission, this may explain why morphine-exposed offspring take longer to ejaculate relative to controls. This author, in collaboration with McKenna, Matson, and Eaton, has already started to examine this possibility in prenatally drug-exposed animals (McKenna et al. 1994).

The concentration and turnover rate of brain catecholamines in the hypothalamus, frontal cortex, striatum, and cerebellum of adult male and female rats exposed to morphine prenatally were then measured to test whether prenatal morphine modifies catecholamine content and/or turnover in a specific brain area (e.g., hypothalamus) or has widespread

TABLE 2. *Effects of prenatal morphine on adult male reproductive behavior.*

Week of Test	Prenatal Treatment	N	Behavioral Measures PEI (sec)	EL (sec)
1	Saline	9	382±1	1246±52
	Morphine	13	198±6**	1654±88
2	Saline	9	398±6	760±20
	Morphine	13	147±6**	1173±53*
3	Saline	9	420±7	736±36
	Morphine	13	142±5**	957±31*

NOTE: Values are means±SEM.

KEY: PEI = postejaculatory intromission interval; EL = ejaculation latency; * = $p < 0.05$ versus saline; ** = $p < 0.01$ versus saline.

SOURCE: Data are modified from Vathy and Katay 1992.

effects on the catecholamine systems. Morphine exposure in utero permanently alters adult hypothalamic NE levels in both male and female rats, and these effects are sexually dimorphic. In the hypothalamus of male offspring, NE content is increased 95 percent, whereas in the hypothalamus of female offspring, it is decreased 57 percent relative to controls (Vathy and Katay 1992).

NE and DA turnover rates were then estimated in the same brain areas following alpha-methylparatyrosine (AMPT) administration. NE turnover in the hypothalamus of morphine-exposed rats increases 50 percent in males and decreases 50 percent in females (figure 1). Prenatal morphine has no effects on NE turnover in the male POA, but in female rats NE turnover in the POA decreases approximately 60 percent (Vathy et al. 1994). Prenatal morphine has no effect on striatal, frontal cortex, or cerebellar NE or on basal levels or turnover of DA in any brain region. These results demonstrate that prenatal morphine alters not only NE content but also NE turnover rate in a sexually dimorphic manner in specific brain regions of male and female rats. This sexually dimorphic

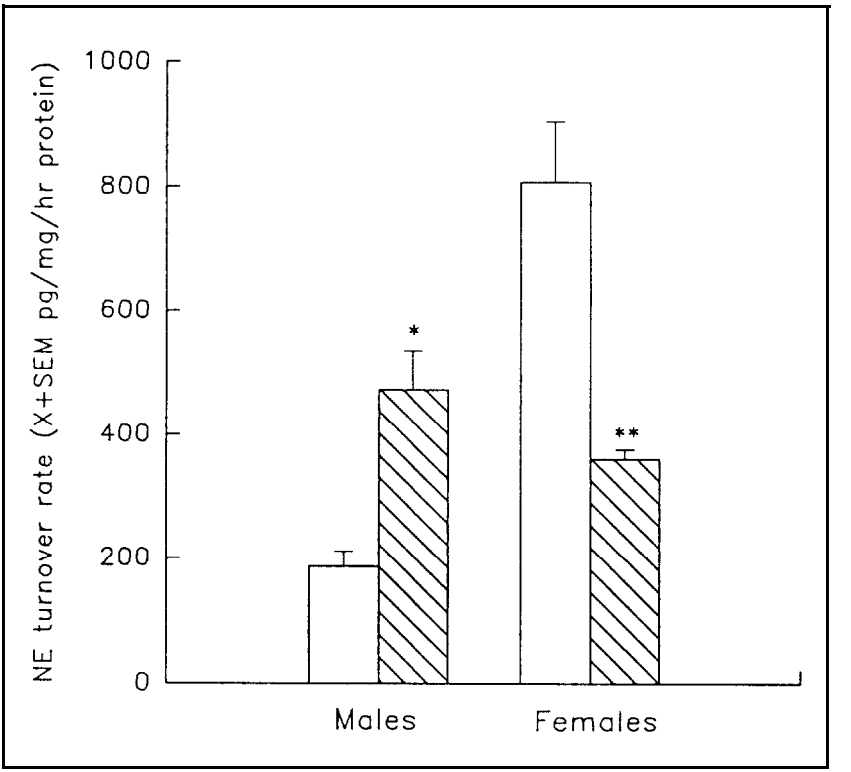

FIGURE 1. *Effects of prenatal morphine on NE turnover rates in the hypothalamus of male and female rats. The open bars represent saline- and striped bars morphine-exposed animals. Values are means±SEM of N = 5-6.*

KEY: * = $p < 0.05$ versus saline-exposed males; ** = $p < 0.01$ versus saline-exposed females.

SOURCE: Modified from Vathy et al. 1994.

change in NE turnover rate does not simply reflect altered basal hypothalamic NE levels, because the rate constant in drug-exposed animals also differs from saline-exposed animals. Likewise, when NE utilization over the 8 hours following AMPT administration is expressed relative to basal NE, morphine-exposed males utilize more NE than saline-exposed males, and morphine-exposed females utilize less NE than saline-treated females. These sexually dimorphic alterations in hypothalamic NE induced by prenatal morphine may be related to changes

observed in adult male and female sexual behavior (Vathy and Katay 1992).

Effects of Prenatal Morphine Exposure on the Developing Catecholamine Systems

Further examination of hypothalamic and extrahypothalamic NE and DA at different PDs (prepubertal = PD 16 and 23; pubertal = PD 33; postpubertal = PD 45) showed that prenatal morphine alters the development of both NE and DA in the hypothalamus, POA, striatum, and cerebellum in a sexually dimorphic manner, with some changes appearing prior to puberty. Prenatal morphine increases hypothalamic NE concentration in males prepubertally (PD 23), and in females during the pubertal period (PD 33). In the POA, prenatal morphine enhances peak levels of NE in males prepubertally, but has no effect on NE content in females before puberty (table 3). DA content is also affected in a sexually dimorphic manner. At PD 16, prenatal morphine increases hypothalamic DA only in males, while it reduces the content of DA in female but not in male POA (table 4). At PD 45, prenatal morphine increases DA in the hypothalamus of females and decreases it in males. Thus, prenatal morphine, unlike diazepam, affects the content of NE and DA prior to puberty (Kellogg and Retell 1986). These alterations in NE and DA content are sexually dimorphic, occur at distinct ages in developing rats, and are imposed on a sexually dimorphic ontogeny of NE and DA content in the hypothalamus and POA. Moreover, DA neurons are affected by prenatal morphine earlier than NE neurons (Vathy et al. 1995).

Effects of Prenatal Morphine Exposure on Opiate Receptors of Adult Rats

The above studies indicate that opiate exposure during mid to late gestation selectively interferes with NE transmission in the hypothalamus and perhaps the POA. CNS localization of endogenous opioid peptides suggests an anatomical basis for interactions between these peptides and the monoamine neurotransmitters. This is especially true in the case of beta-endorphin projections, which coincide with NE projections in many brain areas including the hypothalamus and POA (Khachaturian and Watson 1982; Mitchell et al. 1988). It has also been suggested that several CNS monoaminergic systems are inhibited by tonically active opioid input. Thus, it is possible that prenatal morphine exposure alters the development of endogenous opioid systems in the hypothalamus,

TABLE 3. *Effects of prenatal morphine on NE content.*

Brain Area	Sex	Age			
		PD16	PD23	PD33	PD45
HYP			Peak		
	Male		M >> S		
				Peak	
	Female			M >> S	
POA			Peak		
	Male		M >> S		
				Peak	
	Female			M >> S	
CER					Peak
	Male				M >> S
	Female				
CX					
	Male				
	Female				
STR					
	Male	M > S			
	Female				

KEY: M = prenatal morphine; S = prenatal saline; HYP = hypothalamus; POA = preoptic area; CER = cerebellum; CX = cortex; STR = striatum. N = 5-7.

For example, the expression of mu opioid receptors, which inhibit NE release, may have been affected. To evaluate this possibility, the binding capacity (B_{max}) and the affinity (K_d) of mu opioid receptors in several brain regions including the hypothalamus, POA, ventral tegmental area, striatum, cortex, and cerebellum were measured using the highly specific ligand [^{3}H]-D-Ala-Gly-N-Methyl-Phe-Gly-ol (Rimanoczy and Vathy 1994). Females were ovariectomized at least a week prior to sacrifice, and some were injected with 3 micrograms (µg) estradiol benzoate (EB) 48 hours before sacrifice, a dose used routinely to prime female reproductive behavior.

TABLE 4. *Effects of prenatal morphine on DA content.*

Brain Area	Sex	Age			
		PD16	PD23	PD33	PD45
HYP	Male	M > S			
	Female				M > S
POA	Male				
	Female	M < S			
CER	Male	M > S			Peak M > S
	Female				
CX	Male			M > S	
	Female				
STR	Male				
	Female				

KEY: M = prenatal morphine; S = prenatal saline; HYP = hypothalamus; POA = preoptic area; CER = cerebellum; CX = cortex; STR = striatum. N = 5-7.

In saline controls, there was a gender-related difference in mu receptor binding in the hypothalamus. Males had significantly higher B_{max} than females regardless of estrogen treatment. Prenatal morphine in males did not alter the B_{max} or the K_d of mu opioid receptors in any brain region (Rimanoczy and Vathy 1994). In ovariectomized females, prenatal exposure to morphine did not alter the K_d, but it reduced the B_{max} of mu opioid receptors about 25 percent in the hypothalamus. A 3 µg estradiol benzoate injection increased the B_{max} of mu receptors in the hypothalamus of morphine-exposed but not saline-exposed females. This estrogen-induced increase in mu opioid receptors in morphine-exposed females resulted in a level of mu opioid receptors that was higher than that in morphine-exposed, ovariectomized females. The binding capacity of mu opioid receptors was not altered in other brain areas. The increased mu

opioid binding in the hypothalamus of morphine-exposed females following estrogen administration may be related to the inhibition of estrous behavior and to reduced NE content and turnover rates in the hypothalamus of these animals.

Effects of Prenatal Cocaine Exposure on Adult Sexual Behavior and Brain Catecholamines

Because cocaine, another highly abused drug, induces many of its behavioral alterations by blocking the reuptake of catecholamines, Vathy and colleagues also tested the effects of prenatal cocaine on adult sexual behavior and brain catecholamine levels (Vathy et al. 1993). The dose and the timing of the prenatal exposure to cocaine were the same as those in the author's morphine studies. Like morphine, prenatal cocaine modifies adult sexual behavior and brain catecholamines in a sexually dimorphic fashion. Females exposed to cocaine were significantly inhibited in their sexual behavior. In male rats, prenatal cocaine exposure, like prenatal morphine, increases mounting and intromitting behaviors and decreases postejaculatory latencies. However, prenatally cocaine-exposed male rats do not show longer ejaculation latencies or reduced copulatory latencies as do morphine-exposed males. Cocaine-exposed male rats had significantly higher NE and DA levels in the POA, and females had unchanged NE and DA content in all brain regions examined, when compared to same-sex controls. No drug-related differences in catecholamine content were found in hypothalamus, striatum, cortex, or cerebellum. These results suggest that exposure of developing animals to modest doses of cocaine during mid to late gestation results in long-lasting, sexually dimorphic alterations of adult sexual behavior and brain catecholamines in rats. Nevertheless, the effects of prenatal cocaine and morphine on adult sexual behavior and brain catecholamines are not identical.

SUMMARY AND CONCLUSION

There are several possible mechanisms that may explain how psychoactive drugs affect brain and behavioral development. During early development of the CNS, neurotransmitters are thought to act as signals for growth and synaptogenesis (Lauder 1990; Lauder and Krebs 1978; Whitaker-Azmitia et al. 1987). Fetal or early neonatal exposure to opiates or cocaine may cause an overall inhibition of brain growth and development due to inappropriate neural response to hormones and

neurotrophic signals during this critical period of CNS development. Because exposure to opiates and cocaine prenatally can alter opioid and catecholamine receptor density and distribution, this in turn could affect the development of neural connections by delaying or accelerating neural outgrowth during fetal and/or postnatal periods (Bardo et al. 1982; Hammer et al. 1989; Spear et al. 1989*a,* 1989*b;* Tempel et al. 1988; Tsang and Ng 1980). Another possibility may be alterations in monoamine neurotransmitter levels during gestation. Alterations in levels or distribution of these neurotransmitters may interfere with the mechanisms involved in the establishment of neural connections and behavior patterns.

From this and other studies examining the impact of prenatal drug exposure on adult behaviors and brain catecholamine systems, three themes emerge: (1) Exposure of the developing fetus to psychoactive drugs produces long-term alterations in adult behaviors and brain catecholamines, (2) these neurobehavioral and neurochemical alterations are sexually dimorphic, and (3) the hypothalamic catecholamine systems seem particularly vulnerable to prenatal drug exposure. Thus, it is clear that to gain understanding of the impact of prenatal drug exposure on adult brain and behaviors, lifespan studies are essential. These lifespan studies should consider gender as an important variable, because the occurrence of long-term neurobehavioral changes in this author's and other studies strongly suggests that prenatal drug exposure modifies adult male and female brain and behavioral development differently. Additionally, researchers should be aware of the particular sensitivity of the hypothalamus to perturbation by developmental drug exposure. Obviously, understanding the consequences of prenatal drug exposure for the development of the human CNS and behavior is an extremely complicated and difficult task. However, animal models have already proved useful and will be essential to correlate neurochemical and behavioral consequences of prenatal drug exposure with clinical evidence.

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ACKNOWLEDGMENT

This chapter was prepared with support from the National Institute on Drug Abuse grant DA-05833 and from the Department of Psychiatry, Albert Einstein College of Medicine.

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Prenatal Cocaine Produces Biochemical and Functional Changes in Brain Serotonin Systems in Rat Progeny

George Battaglia, Theresa M. Cabrera, and Louis D. Van de Kar

EFFECTS OF PRENATAL COCAINE

Overview of Clinical Studies

Cocaine abuse among pregnant women has received a great deal of scientific and media attention in recent years. Studies have estimated the prevalence of cocaine use among pregnant women to be 3 to 50 percent (Chasnoff et al. 1990; Osterloh and Lee 1989; Zuckerman et al. 1989), with the large variability attributable to methodological considerations such as patient population and drug reporting methods. Numerous clinical investigations that have attempted to characterize the teratogenic potential of cocaine in humans have been frequently complicated by polydrug abuse, socioeconomic factors, inaccurate self-reporting of drug use, and differences in the quality of prenatal care. Despite these limitations, increasing evidence indicates that prenatal exposure to cocaine can produce adverse effects in offspring. Clinical data indicating complications arising from cocaine use during pregnancy include intrauterine growth retardation, decreased head circumference, and preterm delivery with fetal distress (Church et al. 1991*b;* Griffith et al. 1994). While no long-term followup studies have been performed in offspring exposed to stimulant drugs, some evidence suggests that visual-motor coordination is selectively impaired during the first year and fine motor development is compromised as the children mature (Dixon 1989). Furthermore, infants exposed to cocaine during gestation exhibit increased irritability, abnormal sleep patterns, retained primitive reflexes, tremors, and hypertonia (Dixon 1989; Oro and Dixon 1987; Schneider and Chasnoff 1992). These data suggest that in utero exposure to cocaine may be associated with a number of neuropathologic consequences, some of which may be temporary while others may only become apparent as the child matures.

Overview of Animal Studies

In animal studies, prenatal cocaine exposure has been shown to produce adverse postnatal consequences in progeny (Dow-Edwards et al. 1990; Henderson and McMillen 1990; Heyser et al. 1992; Sobrian et al. 1990). In general, various studies have revealed that prenatal exposure to moderate doses of cocaine (10 to 30 milligrams per kilogram (mg/kg/day)) does not alter progeny maturation and growth parameters such as birth weight, crown-rump length, pinna detachment, fur emergence, and time to eye and vaginal opening (Cabrera et al. 1993*b;* Church et al. 199 1 a; Fung et al. 1989; Henderson and McMillen 1990). Despite the general lack of physical terata at moderate doses of cocaine, behavioral abnormalities such as learning deficits (Heyser et al. 1992; Spear et al. 1989*a*), alterations in locomotor activity (Church et al. 199 1 a; Spear et al. 1989*a*), righting reflex (Henderson and McMillen 1990; Sobrian et al. 1990), avoidance acquisition (Church and Overbeck 1990), and startle reflex (Sobrian et al. 1990) have been reported. These studies suggest the presence of neurochemical alterations in the absence of visually apparent physical terata. However, there is currently limited information available regarding the magnitude and extent of long-term central nervous system (CNS) alterations following prenatal exposure to cocaine or other widely abused psychostimulants.

Some neurochemical alterations in brain following gestational exposure to slightly higher doses of cocaine (i.e., $\geq$ 30 mg/kg/day) include increased density of alpha adrenergic receptors (Seidler and Slotkin 1992), noradrenergic hyperactivity (Seidler and Slotkin 1992), decreased glucose metabolism in specific brain regions (Dow-Edwards et al. 1990), increased dopamine (DA) type 1 (D_1) receptor density (Dow-Edwards et al. 1990) and sensitivity (Segal et al. 1989), and decreased dopamine type 2 (D_2) receptor density (Henderson et al. 1991). With respect to changes in serotonergic systems, Henderson and colleagues (1991) reported a lack of alterations in cortical 5-HT_{2A} serotonin receptors in adult male progeny, while Akbari and associates (1992) reported decreased cortical and hippocampal ^{3}H-paroxetine binding to serotonin (5-HT) uptake sites in neonatal rat pups prenatally exposed to cocaine.

COCAINE EFFECTS ON 5-HT SYSTEMS IN ADULT RATS

The pharmacology and actions of cocaine have been the subject of extensive reviews in recent years (Dackis and Gold 1987; Johanson and

Fischman 1990). While cocaine is well known for producing changes in catecholamine systems, an extensive literature has been accumulating concerning the contribution of 5-HT systems to the pharmacological and neuroendocrine effects of cocaine (Levy et al. 1994*a*). Unlike other stimulants, such as methamphetamine or 3,4-methylenedioxy-methamphetamine (MDMA), cocaine does not cause neurotoxicity in 5-HT systems (Seiden and Kleven 1988; Yeh and DeSouza 1991). Cocaine, nevertheless, produces profound effects on 5-HT metabolism and neuronal communication. In adult rats, cocaine can inhibit 5-HT synthesis, inhibit 5-HT uptake, and suppress the firing of 5-HT neurons (Cunningham and Lakoski 1988; Dackis and Gold 1987; Galloway 1990;

evidence indicates that fluoxetine, a 5-HT uptake blocker, reduces intravenous (IV) cocaine self-administration in rats (Carroll et al. 1990).

systems in the neurochemical and possibly the addictive properties of cocaine. Since cocaine can cross the fetal-placental barrier (Spear et al. 1989*b*
produce changes in fetal 5-HT metabolism comparable to those observed in adult animals. Consistent with this hypothesis, the presence of central 5-HT recognition sites (e.g., 5-HT uptake sites, 5-HT_{1A} $_{2A}$, and 5-$_{2C}$ receptors) (Bruinink et al. 1983; Ivgy-May et al. 1994; Roth et al. *a*) in fetal rat

5-HT systems in adult animals have potential sites of action in the fetus.

THE ROLE OF 5-HT IN FETAL DEVELOPMENT

Prior to assuming its role as a neurotransmitter in the CNS, 5-HT exerts trophic influences on the growth and maturation of 5-HT pathways in

presence of active 5-HT uptake and release processes in fetal brain, indicate that serotonin concentrations may be critically regulated during

5-HT during fetal brain development may affect the growth and maturation of 5-HT perikarya and target tissues that receive serotonergic

that cultured raphe neurons of the rat exposed to 5-HT exhibited an inhibition of neurite outgrowth. In contrast, Liu and Lauder (1991)

rat embryonic neurons increased the area of the neuronal soma, the

number of neurites, and neurite branching in 5-HT immunoreactive cells. In addition to influencing the growth of 5-HT neurons, Lauder and Krebs (1978) demonstrated that 5-HT exerts a maturational influence on 5-HT target tissues. Depletion of 5-HT by p-chlorophenylalanine (PCPA) delayed the differentiation of postsynaptic 5-HT target tissues (Lauder and Krebs 1978) and the early postnatal decline of 5-HT_1 receptors in forebrain (Whitaker-Azmitia et al. 1987). Some data also suggest that these effects may be mediated by stimulation of 5-HT_1 receptors on astroglial cells that produce neuron trophic factors (Whitaker-Azmitia and Azmitia 1989). In cultures of fetal cortical neurons in the absence of glial cells, stimulation of 5-HT_{1A} receptors produces reductions in neurite branches with little effect on somal area or cell viability (Sikich et al. 1990). The D_1 DA receptor agonist SKF38393 has also been reported to influence the in utero development of 5-HT systems (Whitaker-Azmitia et al. 1990*b*). Therefore, cocaine may alter the development of 5-HT systems via stimulation of 5-HT and catecholamine receptors due to blockade of 5-HT and DA uptake mechanisms. Since DA and 5-HT may play multiple roles in 5-HT neuronal development, the magnitude, direction, and extent of postnatal changes in 5-HT pathways following cocaine-induced perturbations to fetal 5-HT systems would be difficult to predict. Nevertheless, likely candidates for prenatal cocaine-induced biochemical and functional changes in 5-HT systems in progeny would include terminal 5-HT uptake sites and postsynaptic serotonin receptors, including 5-HT_2 and subtypes of 5-HT_1 receptors. Therefore, the studies described in this chapter investigated the effects of prenatal cocaine exposure on the biochemical and functional status of pre- and postsynaptic components of 5-HT pathways in male and female rat progeny. Since dysfunction of 5-HT pathways has been implicated in various clinical disorders including depression, anxiety, obsessive-compulsive behaviors, and in drug-seeking behaviors (Siever et al. 1991), data provided by the present studies may have significant clinical implications.

METHODS

Prenatal Exposure Paradigm

For all of the studies described, gravid Sprague-Dawley rats arrived in the laboratory on either gestational day (GD) 5 (experimental animals) or 7 (foster dams). All experimental animals were placed on a nutritionally balanced liquid diet as described previously (Cabrera et al. 1993*b*)

beginning on embryonic day (E) 8 and continuing throughout the drug exposure period. The foster rats had ad libitum access to rat chow and water. Foster dams were weighed and received animal care identical to the experimental dams; however, foster dams were not subjected to any experimental procedures. Beginning on E13 and ending on E20, experimental dams received injections of either 0.9 percent saline (1 mL/kg) or (-)cocaine hydrochloride (15 mg/kg) subcutaneously (SC) twice daily. The injection sites were varied to keep skin irritation to a minimum (Church et al. 1988; Spear et al. 1989*a*). Preliminary data from this laboratory and a report by Clow and colleagues (1991) indicate that a dose of cocaine identical to that used in the present studies did not result in a decrease in maternal weight gain during pregnancy, suggesting that a pair-feeding protocol was not compulsory. However, some investigators have reported a small decrease in maternal weight gain during the administration of cocaine at higher doses or for longer time periods. Therefore, as a precautionary measure, dams in the present studies were placed on a liquid diet to facilitate monitoring their daily food intake, which could then be correlated with maternal weight gain during pregnancy.

Rationale for the Prenatal Cocaine Exposure Paradigm

chronic SC doses of 10 to 40 mg/kg cocaine in dams produces plasma cocaine concentrations found within the range of, or exceeding, those reported in human cocaine users (Spear et al. 1989*b*).

One specific concern that has been raised regarding the SC administration of cocaine is that any tissue pathology that it may produce can act as a long-term stressor in the rats outlasting the stress response produced by cocaine itself (e.g., elevated adrenocorticotropic hormone (ACTH) and corticosterone levels). Preliminary studies from this laboratory have shown that while the cocaine exposure paradigm utilized in the present study produced some degree of tissue pathology in a small number of dams (i.e., ~ 12 percent), it did not alter basal levels of the stress hormones ACTH or corticosterone, nor did it alter the ability of 5-HT releasers to increase ACTH and corticosterone when dams were tested at postpartum day 3 or 4 (Battaglia et al. 1994). Taken together, these observations indicate that it would be unlikely that any effects of cocaine on biochemical or functional parameters in the offspring would be the result of such long-term stressor effects due to tissue pathology that may have been produced by the route of drug administration.

Rationale for the Biochemical Measurements and Neuroendocrine Challenge Tests

Since alterations in fetal 5-HT can influence the development and maturation of 5-HT neurites as well as target tissues receiving 5-HT innervation, changes in the density of 5-HT recognition sites such as 5-HT uptake sites and 5-HT receptor subtypes can provide an index of alterations in the biochemical status of pre- and postsynaptic 5-HT systems. In the present studies, pre- and postsynaptic 5-HT recognition sites were measured in conjunction with functional indices of 5-HT systems provided by neuroendocrine challenge tests.

Serotonergic neurons that innervate a number of forebrain regions also send collaterals to the hypothalamus where they mediate changes in plasma ACTH, corticosterone, renin, and prolactin secretion via specific 5-HT receptor subtypes. (For review, see Van de Kar 1991.) Serotonin-releasing drugs such as p-chloroamphetamine (PCA) enter serotonergic nerve terminals primarily via the 5-HT transporter and facilitate the release of endogenous stores of 5-HT (Kuhn et al. 1985). The released 5-HT can then stimulate one or more distinct postsynaptic 5-HT receptor subtypes (e.g., 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A}/5-HT_{2C} receptors) to elevate plasma concentrations of ACTH, corticosterone, prolactin, and renin

(Fuller and Snoddy 1980; Van de Kar 1991). Thus, the magnitude of the

functional status of hypothalamic serotonergic systems. As previously mentioned, since 5-HT neurons that innervate the hypothalamus also send collaterals to various forebrain regions, dysfunction of serotonergic fibers innervating the hypothalamus may likely be representative of alterations in the serotonergic terminals in extrahypothalamic brain regions as well. Thus, neuroendocrine challenge tests can provide a peripheral marker of

Furthermore, the profile of alterations in various hormone responses to the 5-HT releaser PCA can provide some measure of pre- and/or

of postsynaptic 5-HT receptor subtypes can be examined directly by investigating the ability of selective 5-HT receptor agonists to elicit

(PCA) and a 5-HT_{1A}, receptor agonist 8-hydroxy-2-(di-n-propylamino) (8-OH-DPAT) were utilized in neuroendocrine challenge tests to assess the functional status of pre- and postsynaptic components, respectively, of brain 5-HT pathways in offspring exposed prenatally to cocaine.

Offspring. In order to eliminate the possible influence of drug-induced differences in nurturing, at birth (i.e., postnatal day (PD) 0), all offspring from each of the experimental groups were fostered to the untreated, lactating dams. Al the time of fostering, the litters were culled to no more than 9 pups per litter (5 males, 4 females) and all pups were weaned on PD 21. At the time of weaning, males and females were housed separately in groups of two or three rats per cage and had free access to food and water. For each of the postnatal treatment groups, pups from different litters were used to achieve the required number of animals whenever possible. Male and female progeny were subjected to pharmacological challenges as described below at either PD 28 to 30 (males and females) or at PD 70 (males). These timepoints represent pre- and postpubescent ages, respectively.

Postnatal Experimental Measures: Study IA. In the initial study, the long-term functional consequences of prenatal exposure to cocaine on 5-HT systems were investigated in adult male progeny by measuring the ability of a 5-HT releaser to elevate plasma hormones. Male rats were sacrificed by decapitation at PD 70, 1 hour after a single IP injection of either saline or the 5-HT releaser PCA (8 mg/kg). Trunk blood was collected into centrifuge tubes containing 0.5 mL of 0.3 M EDTA (pH 7.4) for subsequent analysis of plasma ACTH, corticosterone,

prolactin, and renin levels. All plasma hormones were measured by radioimmunoassay according to previously published protocols (ACTH and prolactin, Li et al. 1993; corticosterone and renin, Richardson Morton

dissected for subsequent radioligand binding analyses. Serotonin

Cabrera 1994; Cabrera and Battaglia 1994; Cabrera et al. 1993*b*).

Study IB. For comparative purposes and to preclude complications due to hormonal cycling, female progeny were investigated only at a prepubescent timepoint (PD 30) using the same protocol as described earlier for the adult male progeny. As data from these initial studies suggested potential gender differences and the possibility of deficits due to alterations in either pre- andfor postsynaptic 5-HT-mediated neuroendocrine function following prenatal exposure to cocaine, subsequent studies were carried out at the same postnatal developmental time (study II).

Study II. This study was designed to more directly assess prenatal

postsynaptic 5-HT receptor systems in prepubescent male and female

5-HT receptor systems in progeny were determined by measuring the

saline or the selective 5-HT_{1A} receptor agonist 8-OH-DPAT tetralin; 0.5 mg/kg in male and female progeny at PD 28. Progeny were sacrificed 30 minutes postinjection by decapitation, and trunk blood was again collected for subsequent analyses of plasma hormones. The dose of 8-OH-DPAT was chosen based on previous reports that 0.5 mg/kg 8-OH-DPAT elicits maximal increases in ACTH and corticosterone (Li et al. 1993). Biochemical changes in the density of 5-HT_{1A} receptors were measured in the hypothalamus, frontal cortex, and midbrain by radioligand binding assays to assess whether any alterations in function could be attributed to alterations in receptor number. Neuroendocrine data were analyzed for significant differences using two-way (studies IA and IB) or three-way (study II) analysis of variance (ANOVA) and a Newman Keuls test.

RESULTS

Effects of Cocaine Administration in Pregnant Dams

As shown in figure 1A, SC administration of 15 mg/kg (-) cocaine hydrochloride twice a day to gravid dams from E 13 to 20 did not significantly alter liquid diet intake compared with saline-treated dams. Likewise, as shown in figure 1B, there were no significant differences in maternal weight gain during gestation among saline-, cocaine-, or noninjected chow-fed foster dams. Since maternal growth parameters were not altered, it is unlikely that any biochemical or functional alterations observed in the cocaine-exposed offspring would be the result

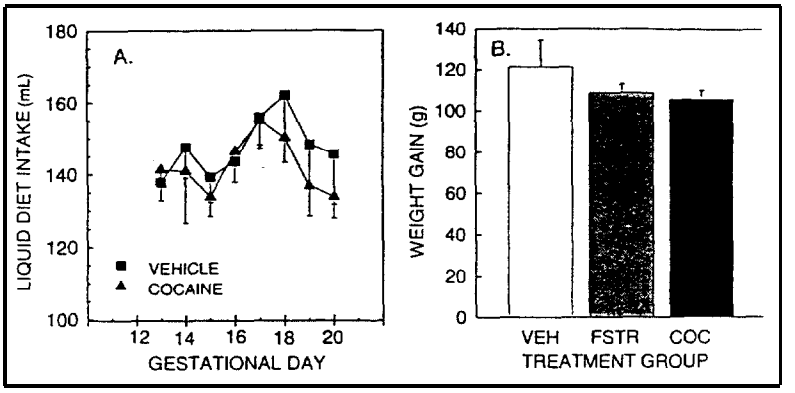

FIGURE 1. *(-) Cocaine administration (1.5 mg/kg SC, b.i.d.) from E 13-20 did not significantly decrease (A.) the amount of liquid diet consumed or (B.) the total amount of weight gained during the in utero cocaine exposure paradigm utilized in this study. Likewise, there was no difference in the amount of weight gained during pregnancy between foster chow-fed and experimental dams. Data represent the means±SEM from 5 to 10 dams and were evaluated by a repeated measures analysis (liquid diet intake) or one-way ANOVA weight gain.*

KEY: VEH = vehicle, FSTR = foster chowfed, COC = cocaine

SOURCE: Cabrera et al. 1993*b*.

of altered nutritional status in the mother. Likewise, alterations in 5-HT systems in the cocaine-exposed progeny would unlikely be attributed to drug-induced differences in nurturing as all the offspring were fostered to untreated lactating dams at birth.

Prenatal Cocaine Effects of Progeny Birth Parameters

No significant differences were observed between saline- and cocaine-exposed progeny in the number of male (6±1 and 5±1) or female (7±1 and 7±1) offspring per litter or in the total progeny number per litter (12±1 versus 14±1). At birth, no visually apparent physical terata were observed. As shown in table 1, all progeny had similar crown-rump lengths, and birth weights indicated that late gestational exposure to cocaine did not alter the physical development of either male or female pups. Also, no differences were observed in anogenital distances between treatment groups for each of the genders (table 1). Together, these data demonstrate a lack of visually apparent physical terata as a

TABLE 1. *Lack of effect of in utero cocaine on progeny growth parameters at birth.*

Treatment Group	Crown-Rump Length (mm)		Anogenital Distance (mm)		Birth Weight (g)	
	Males	Females	Males	Females	Males	Females
Control	49.2±1.6	47.5±1.6	2.8±0.2	1.3±0.1*	6.9±0.2	6.48±0.28
Cocaine	48.3±0.5	47.8±0.7	2.6±0.1	1.2±0.1*	6.7±0.2	6.33±0.13

NOTE: Data represent the mean±SEM from 5 to 10 litters per group. Litters were composed of 3 to 13 rats per gender. There was a significant main effect of gender on anogenital distance ($p < 0.05$). However, there was no significant main effect of in utero exposure group on crown-rump length, anogenital distance, or birth weight. Data were analyzed by a two- way ANOVA followed by a Newman Keuls test.

KEY: * = Significantly different ($p < 0.05$) from corresponding male progeny.

SOURCE: Cabrera et al. 1993*b*.

result of the in utero cocaine exposure paradigm utilized in this study. In general, these results are consistent with previous reports (Dow-Edwards 1989; Henderson and McMillen 1990; Spear et al. 1989*b)*. However, despite the lack of changes in physical parameters at birth, prenatal cocaine exposure did produce marked alterations in the biochemical and functional status of brain 5-HT systems at both pre- and postpubescent ages in progeny.

Selective Attenuation of Neuroendocrine Responses to a 5-HT Releaser in Adult Male Progeny: Evidence for Long-Term Deficits in Brain Serotonergic Systems

ACTH and Corticosterone. Plasma ACTH and corticosterone levels following IP injection of 8 mg/kg PCA in adult male rats at PD 70 are shown in figure 2. While basal ACTH levels did not differ between groups (figure 2A), cocaine-exposed male progeny exhibited a significant decrease (-43 percent; $p < 0.05$) in the magnitude of the ACTH response to PCA in comparison with the saline-exposed/PCA-challenged group. As this dose of PCA produces submaximal ACTH responses, these data indicate a change in either the median effective dose (ED,) or maximum effective dose (Emax) for the 5-HT-mediated ACTH response. Similar to ACTH, basal levels of corticosterone were not altered by in utero exposure to cocaine (figure 2B). However, PCA elevated plasma corticosterone above basal values to a comparable extent in both control (i.e., vehicle) and cocaine-exposed progeny. This differential effect of prenatal cocaine exposure on ACTH versus corticosterone could be explained by the fact that while the ACTH response to PCA was lower in cocaine versus control progeny, the actual levels of ACTH attained in cocaine progeny (> 200 picograms per milliliter (pg/mL)) were, nevertheless, sufficient to saturate the adrenal ACTH receptors and elicit a maximal corticosterone response (Bagdy et al. 1989; Engeland et al. 1981; Kaneko et al. 1980, 1981). However, these data do not preclude the possibility that prenatal cocaine exposure altered the ED_{50} for corticosterone stimulation in response to challenge with the 5-HT releaser.

Renin. As observed for basal levels of ACTH and corticosterone, basal levels of plasma renin did not differ between treatment groups in adult male progeny (figure 3). However, while PCA significantly elevated plasma renin above basal values in both progeny groups, in utero exposure to cocaine produced a 50 percent attenuation in the magnitude of the plasma renin response to 8 mg/kg PCA. The attenuated renin

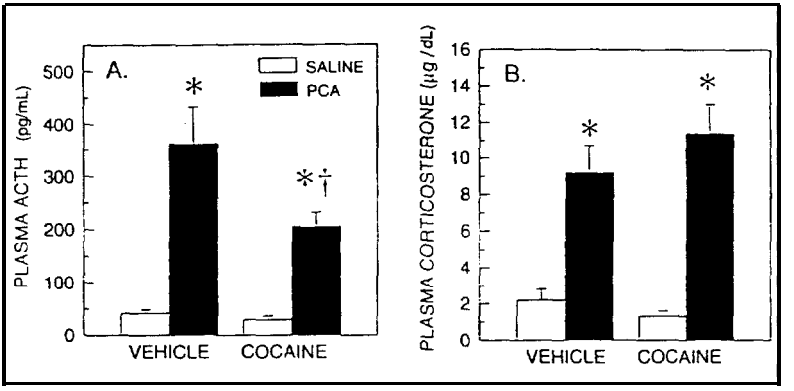

FIGURE 2. *In utero exposure to cocaine attenuates the increase in plasma adrenocorticotropic hormone (A.) without altering the corticosterone (B.) response to PCA (8 mg/kg, IP) in male PD 70 progeny. Data represent group means±SEM from 8 to 10 rats per group, The ACTH response to PCA (363.2± 70.1 pg/mL for vehicle) was significantly attenuated (-43 percent) in male progeny prenatally exposed to cocaine.*

KEY: * = a significant difference from the saline-injected group ($p < 0.01$); † = a significant difference from the corresponding vehicle/PCA-injected group ($p < 0.05$).

SOURCE: Cabrera et al. 1993*b*.

response may reflect a change in the ED_{50} and/or the Emax of the 5-HT mediated elevation of plasma renin in cocaine-exposed progeny, since the dose of PCA used is known to produce a submaximal renin response (Levy et al. 1994*a*).

Prolactin. Consistent with the lack of effect of prenatal cocaine exposure on basal levels of the other hormones, basal levels of plasma prolactin in PD 70 male progeny were not altered by prenatal cocaine exposure. However, unlike ACTH and renin, the magnitude of the prolactin response to PCA was similar in saline- and cocaine-exposed male progeny groups (Cabrera et al. 19936; data not shown).

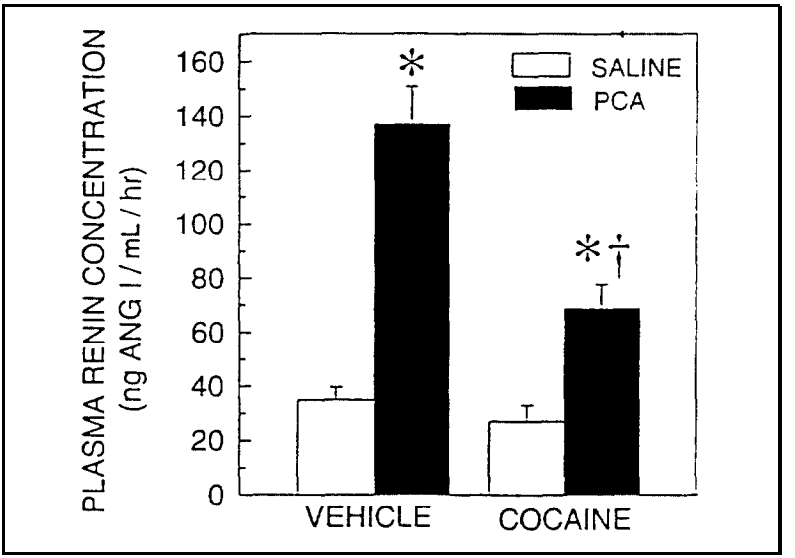

FIGURE 3. *In utero exposure to cocaine attenuates the ability of 8 mg/kg PCA to increase plasma renin concentration in male progeny at PD 70. Data represent group means±SEM from 7 to 10 rats per group. in utero exposure to cocaine resulted in a significant attenuation of the PCA-induced increase in plasma renin (-50 percent: $p < 0.05$) compared to the vehicie/PCA-injected group (137.8±13.8 nanograms (ng) ANG I/mL/hr).*

KEY: * = a significant difference from the saline-injected group ($p < 0.01$); † = a significant difference from the corresponding vehicle/PCA-injected group ($p < 0.05$).

SOURCE: Cabrera et al. 1993*b*.

Implications of the Attenuated 5-HT-Mediated Neuroendocrine Responses in Adult Male Progeny

Overall, the altered neuroendocrine profile of responses to the 5-HT releaser PCA demonstrates that prenatal cocaine exposure produces deficits in central serotonergic function in adult male progeny. Since brain serotonin systems play a role in mediating the neuroendocrine response to various stressors (Van de Kar et al. 1991), these data suggest that cocaine-exposed male offspring may exhibit an altered

neuroendocrine profile in response to physiologic and/or environmental stressors. Alternatively, since other neurotransmitter systems are also involved in mediating the neuroendocrine response to stress, the net response to nonpharmacological stressors may not be adversely affected, despite specific alterations in 5-HT function. These data indicate the importance of subsequent experiments to assess whether the observed alterations in brain serotonin systems following pharmacological challenge render the offspring hyper- or hyposensitive to environmental stressors. To date, behavioral studies in rats have provided some evidence of altered responsiveness to stressful situations following prenatal exposure to cocaine (Bilitzke and Church 1992; Johns et al. 1992*a,* 1992*b;* Spear et al. 1989*a*).

Since renin is the rate-limiting enzyme in the production of angiotensin II (ANG II), it plays a significant role in the regulation of blood pressure. Renin is produced in the juxtaglomerular cells located in the afferent arterioles of the kidney and catalyzes the conversion of angiotensinogen to ANG I, which is then rapidly cleaved by ANG converting enzyme to produce ANG II. Renin is released into the blood in response to various physiological stimuli including decreases in renal perfusion pressure, blood volume, or sodium concentration (e.g., hypotension, hemorrhage, and/or hypovolemia). In addition, increased sympathetic outflow to the kidneys also stimulates renin secretion. Therefore, renin and ANG II play an important and integral part in the maintenance of blood pressure and plasma volume. Consequently, alterations in the serotonergic regulation of renin secretion may contribute to, or be indicative of, compromised cardiovascular function in cocaine-exposed offspring. This possibility remains to be investigated.

Alterations in 5-HT Uptake Sites and 5-HT Receptors

Since decreases in the number of presynaptic 5-HT terminals or 5-HT uptake sites could be responsible for the attenuated neuroendocrine responses to the 5-HT releaser, the density of 5-HT uptake sites was measured in hypothalamic and cortical homogenates. Prenatal exposure to cocaine did not significantly change the density of 5-HT uptake sites either in the hypothalamus or in the cortex in adult male progeny (Cabrera et al. 1993*b*). The lack of reduction in the density of 5-HT uptake sites in either the hypothalamus or the cortex suggests a lack of gross regional deficits in 5-HT innervation to these areas. However, these data do not preclude the possibility that more discrete deficits in hypothalamic or extrahypothalamic 5-HT terminals could exist. For

example, in the hypothalamus, the majority of 5-HT uptake sites are not localized to 5-HT terminals but, rather, are predominantly localized in regions containing 5-HT axons of passage (Battaglia et al. 1990). Thus, marked changes in 5-HT uptake sites on terminals projecting to specific hypothalamic nuclei mediating the neuroendocrine responses (e.g., paraventricular nucleus) would not be detected in homogenate assays. Alternatively, the attenuation of ACTH and renin responses to PCA in cocaine-exposed male progeny may be the result of functional deficits in 5-HT uptake and release processes or decreased numbers of postsynaptic 5-HT receptors responsible for elevating plasma hormones. Therefore, densities of 5-HT receptor subtypes were measured in various brain regions as an index of hypothalamic receptor changes, since the limited amount of tissue precluded measuring all 5-HT receptor subtypes in hypothalamic homogenates. Reductions in 5-HT receptor subtypes in hypothalamus or extrahypothalamic brain regions would provide evidence supporting a role for postsynaptic alterations in the 5-HT-mediated neuroendocrine deficits revealed by challenge with the 5-HT releaser PCA.

In an initial study, no differences were observed in 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, or 5-HT_{2C} receptors between saline- and cocaine-exposed progeny in homogenates of whole cerebral cortex (Cabrera et al. 1993*b)*. A subsequent study revealed no change in hypothalamic 5-HT receptors but increases in the density of 5-HT_{1A} receptors (+9.6 percent; $p < 0.05$) and 5-HT_{2A}/5-HT_{2C} receptors (+17 percent) in homogenates of frontal cortex of adult male progeny exposed in utero to cocaine. Likewise, an increase in midbrain 5-HT_{1A} receptors in male cocaine-exposed offspring was observed at PD 70 (table 2). While hypothalamic and cortical 5-HT receptors may be differentially regulated, the lack of reduction in any of the 5-HT receptor subtypes indicates that the attenuated ACTH and renin responses to a 5-HT releaser do not appear to be due to any reductions in postsynaptic 5-HT receptors mediating the secretion of these hormones. These data suggest that the reduced PCA-mediated neuroendocrine responses in PD 70 male progeny are the result of presynaptic deficits, despite the lack of observable changes in 5-HT uptake sites in homogenates. These data also suggest that there are some receptor compensatory changes in adult progeny. However, as previously mentioned, the present data do not preclude the possibilities of changes in receptor number in discrete hypothalamic or other extrahypothalamic loci or of postreceptor alterations resulting in the attenuated hormonal responses. Since changes in the density of presynaptic 5-HT uptake sites and/or postsynaptic receptors in discrete neuroanatomic loci are beyond

TABLE 2. *Delayed onset of 5-HT receptor alterations in male progeny following in utero exposure to cocaine.*

Progeny Group	Hypothalamus	Frontal Cortex	Midbrain
5-HT_{1A}			
PD 28 males	0	0	0
PD 70 males	ND	+9.6 %	+17 %
$5\text{-HT}_{2A}/5\text{-HT}_{2C}$			
PD 28 males	0	0	0
PD 70 males	0	+14 %	ND

KEY: 0 = no change; ND = not determined. 5-HT_{1A} receptors were labeled using ^{3}H-8-OH-DPAT while $5\text{-HT}_{2A}/5\text{-HT}_{2C}$ receptors were labeled using ^{125}I-DOI.

the detection limit of homogenate assays, autoradiographic studies are currently being carried out to address the possibility of changes in 5-HT innervation and/or 5-HT receptor densities in discrete hypothalamic and extrahypothalamic brain regions. Alternatively, functional deficits in 5-HT terminals may be present in the absence of any alterations in the density of 5-HT uptake sites as previously observed during recovery from MDMA or fenfluramine-induced destruction of 5-HT pathways (Battaglia 1990; Battaglia et al. 1990, 1991; Zaczek et al. 1990). Therefore, it is possible that prenatal cocaine may have produced functional deficits in presynaptic 5-HT terminals in hypothalamus in the absence of changes in the number of hypothalamic 5-HT uptake sites. Consistent with the data reported here, Dow-Edwards and associates (1990) reported marked decreases in 2-deoxyglucose utilization in the hypothalamus of rats prenatally exposed to cocaine, indicating dysfunctional metabolic processes in the hypothalamus.

Differential Profile of Prenatal Cocaine-Induced Neuroendocrine Alterations in Prepubescent Female Rats

ACTH and Corticosterone. For comparative purposes, in study 1B, female progeny were also examined for functional alterations in brain serotonin systems. However, females were tested only at a prepubescent timepoint to preclude estrus cycle changes from introducing additional variables which could confound interpretation of the data. As observed

in adult male progeny, in female progeny basal ACTH levels were not changed by prenatal cocaine exposure (figure 4A). A single IP injection of the 5-HT releaser PCA (8 mg/kg) significantly elevated plasma ACTH above basal values in both vehicle and cocaine-exposed progeny (figure 4A). However, the magnitude of the ACTH response to PCA was significantly attenuated (-28 percent) in cocaine-exposed female progeny in comparison with the saline-treated/PCA-challenged group.

Likewise, single IP injection of PCA significantly elevated corticosterone levels above basal values in both progeny groups (figure 4B). In contrast to adult male progeny, but consistent with the attenuated ACTH response to PCA in female cocaine-exposed progeny, the corticosterone response to PCA was also significantly attenuated (-43 percent; $p < 0.05$) in female cocaine-exposed progeny compared to the corresponding control/PCA-injected group. These data suggest that in female progeny, prenatal exposure to cocaine may have altered the adrenal gland's responsiveness to ACTH-induced corticosterone stimulation since the absolute ACTH levels in cocaine-exposed progeny (> 200 pg/mL) should have been sufficient to produce a maximal elevation of corticosterone in these animals. Since the hypothalamic-pituitary-adrenal-cortical axis plays a key role in the endocrine response to stress, these data indicate that, like male offspring, female cocaine-exposed offspring may exhibit a compromised ability to respond to both physical and environmental stressors (e.g., trauma, hemorrhage, infection, hypoglycemia, novelty). This compromise may be comparable to or greater than that predicted in male progeny, since ACTH and corticosterone levels were both attenuated in female progeny prenatally exposed to cocaine.

Renin. Basal levels of plasma renin in female progeny were not altered by prenatal cocaine exposure (data not shown). However, in contrast to the attenuated renin response to PCA observed in adult male progeny, there were no between-group differences in the ability of PCA to elevate plasma renin in female cocaine-exposed progeny (Cabrera et al. 1994*b*).

Since the dose of PCA used in these experiments produced a submaximal renin response, the lack of effect on the magnitude of this response between prenatal treatment groups suggests that prenatal cocaine produced no change in either the ED_{50} or the Emax for renin in pre-pubescent female progeny. Differences in the renin responses in cocaine-exposed male and female progeny could be due to differences in postnatal age or may represent a true gender difference in response to prenatal exposure to cocaine.

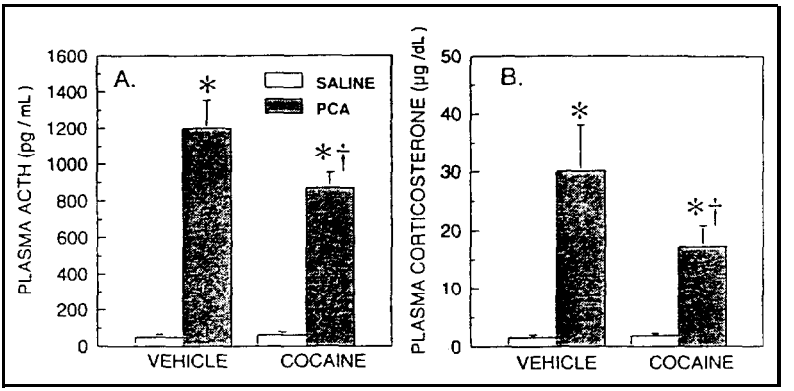

FIGURE 4. *In utero exposure to cocaine attenuates the ability of 8 mg/kg PCA IP to increase both (A.) plasma ACTH and (B.) plasma corticosterone secretion in female progeny at PD 30. Data represent group means±SEM from 7 to 10 rats per group. Basal levels of progeny ACTH (48±17 pg/mL for vehicle) and corticosterone (1.5 ± 0.5 micrograms per deciliter (µg/dL) for vehicle) were not altered by in utero exposure to cocaine. Both the ACTH (1201±152 pg/mL for vehicle) and corticosterone (30.1±8.0) responses to PCA were significantly attenuated (-28 percent and -43 percent, respectively) in female progeny prenatally exposed to cocaine.*

KEY: * = a significant difference from the saline-injected group ($p <: 0.01$); † := a significant difference from the corresponding vehicle/RCA-injected group ($p < 0.05$).

SOURCE: Reprinted from Cabrera, T.M.; Levy, A.D.; Li, Q.; Van de Kar, L.D.; and Battaglia, G. Cocaine-induced deficits in ACTH and corticosterone responses in female rat progeny. *Brain Res Bull* 34:93-97, 1994*b*, with kind permission from Elsevier Science Ltd., The Boulevard, Langford Lane, Kidlington 0X5 1GB, UK.

Implications of the Differential Neuroendocrine Responses in Male and Female Progeny

The present data indicate that prenatal cocaine exposure produces a differential profile of 5-HT-mediated neuroendocrine deficits in adult male (i.e., attenuated ACTH and renin responses) and prepubescent female progeny in response to challenge with a 5-HT releaser (i.e., attenuated ACTH and corticosterone but not renin responses) (table 3). Definitive conclusions regarding gender-specific alterations in response to cocaine exposure cannot be drawn from the data obtained in these studies, as male and female offspring were examined at different postnatal ages. However, these data indicated the necessity for subsequent studies investigating biochemical and/or functional measures in each gender at the same postnatal time. Therefore, study II investigated gender differences at the same developmental age using a selective 5-HT_{1A} receptor agonist to determine whether the differential hormone responses thus far represented true gender differences in 5-HT-mediated neuroendocrine responses.

TABLE 3. *Differential neuroendocrine profile of deficits following prenatal exposure to cocaine or methamphetamine in offspring following challenge with a 5-HT releaser.*

Progeny Group/ Prenatal Treatment	Attenuated ACTH Response	Attenuated Corticosterone Response	Attenuated Renin Response
Male (PD 70)			
Cocaine[a]	Yes	No	Yes
Methamphetamine[b]	No	No	Yes
Female (PD 30)			
Cocaine[c]	Yes	Yes	No
Methamphetamine[b]	No	No	Yes

NOTE: Basal levels of ACTH, corticosterone, and renin were not significantly altered in male or female progeny by either prenatal cocaine or methamphetamine exposure.

KEY: [a] = Male cocaine data summarized from Cabrera et al. 1994*a*.
[b] = Methamphetamine data summarized from Cabrera et al. 1993*a*.
[c] = Female cocaine data summarized from Cabrera et al. 1994*b*.

Interestingly, the attenuated ACTH response in male progeny and the attenuated ACTH and corticosterone responses observed in female cocaine-exposed progeny also contrast with previous findings following in utero exposure to the stimulant methamphetamine (table 3; Cabrera et al. 1993*a*). Prenatal exposure to methamphetamine (5 mg/kg SC, b.i.d., from E 13 to 20) did not significantly alter the ACTH or corticosterone responses to the 5-HT releaser PCA (8 mgkg, IP) in prepubescent female progeny (i.e., PD 30) or adult male progeny (i.e., PD 70). However, prenatal exposure to methamphetamine produced a significant attenuation of the renin response to PCA in both adult male and prepubescent female progeny (Cabrera et al. 1993*a*). Basal levels of ACTH, corticosterone, and renin were not affected by either prenatal administration of cocaine or methamphetamine. In adult animals, administration of methamphetamine or cocaine has been reported to inhibit 5-HT synthesis (Bakhit et al. 1981; Hotchkiss and Gibb 1980) and inhibit 5-HT uptake into nerve terminals (Ricaurte et al. 1980). However, despite some similarities in the neurochemical properties of these drugs in adult rats, prenatal exposure to each of these drugs produces a distinct profile of central serotonergic alterations in offspring. Taken together, these studies indicate that prenatal administration of cocaine or other psychostimulants that disrupt serotonergic systems during development can produce long-term functional changes in central serotonin systems in progeny. However, the specific changes produced by each of the psychostimulants may differ with respect to the gender and/or the postnatal developmental time investigated. These changes may be of significant clinical importance as dysfunction of 5-HT systems has been implicated in various psychiatric disorders including depression, anxiety, aggression, and drug-seeking behavior.

5-HT_{1A} Receptor-Mediated Neuroendocrine Responses in Prepubescent Male and Female Progeny: Evidence for Gender Differences

ACTH and Corticosterone. As shown in figure 5, prenatal exposure to cocaine produced a differential effect on 5-HT_{1A} receptor-mediated ACTH, but not corticostererone, responses in male and female prepubescent progeny (PD 28). Basal levels of ACTH in male and female progeny were not altered by prenatal exposure to cocaine. A single injection of the 5-HT_{1A} agonist 8-OH-DPAT significantly elevated plasma ACTH levels above basal values in both saline- and cocaine-exposed progeny. The magnitude of the ACTH response to a maximally effective dose of 8-OH-DPAT was significantly potentiated (+28 percent;

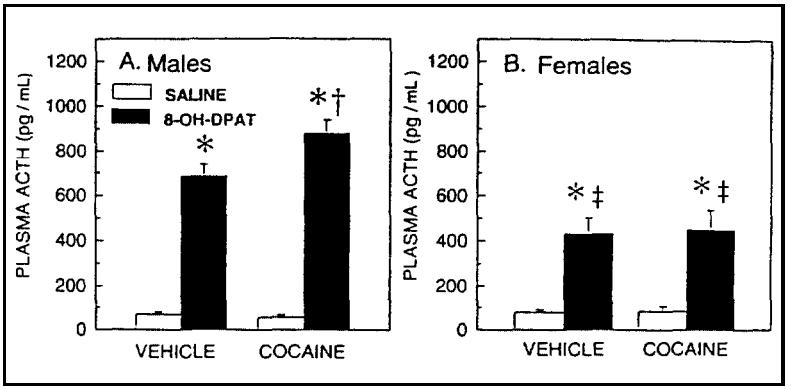

FIGURE 5. *The effect of prenatal exposure to cocaine on basal and stimulated plasma ACTH levels following a single injection of either saline or 8-OH-DPAT (0.5 mg/kg, SC) at PD 28 in (A.) male and (B.) female progeny prenatally exposed to either saline or cocaine (15 mg/kg, SC, to dams from E 13 to 20). Data represent group means ±SEMfrom 6 to 8 rats per group. The ACTH response to 8-OH-DPAT was significantly potentiated (+28 percent) in male but not female cocaine-exposed progeny compared with saline-exposed progeny. Data were analyzed by a S-way ANOVA followed by a Newman Keuls test.*

KEY: * = significantly different than the corresponding saline-injected group ($p < 0.05$);. † = significantly different than male vehicle-exposed/8-OH-DPAT-injected group ($p < 0.05$); ‡ = significantly different than corresponding male challenge groups ($p < 0.05$).

SOURCE: Battaglia and Cabrera 1994.

$p < 0.05$) in male progeny of cocaine-treated dams compared to 8-OH-DPAT-injected control progeny (figure 5A). In contrast, the magnitude of the ACTH response to 8-OH-DPAT was comparable in control and cocaine-exposed female progeny (figure 5B). Corticosterone was significantly elevated by 8-OH-DPAT, but the magnitude of the plasma corticosterone response to 8-OH-DPAT challenge was similar in control and cocaine-exposed male offspring (Battaglia and Cabrera 1994). Likewise, in females, the magnitude of the corticosterone

response to 8-OH-DPAT was not altered by prenatal cocaine exposure. Basal levels of corticosterone were similar across all progeny groups (Battaglia and Cabrera 1994).

Renin. The basal and 5-HT_{1A} receptor-stimulated levels of plasma renin in prepubescent male and female progeny prenatally exposed to saline or cocaine are shown in figure 6. As observed for all other hormones

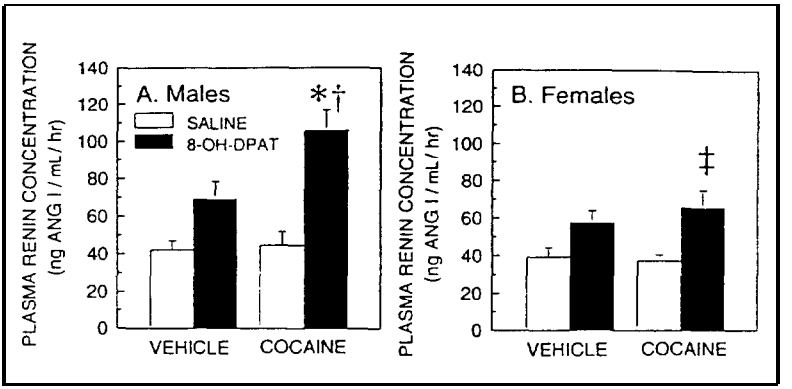

FIGURE 6. *Basal and stimulated plasma renin levels following a single injection of either saline or 8-OH-DPAT (0.5 mg/kg, SC) at PD 28 in (A.) male and (B.) female progeny prenatally exposed to either saline or cocaine (15 mg/kg, SC, to dams from E 13 to 20). Data represent group means±SEM from 6 to 8 rats per group. 8-OH-DPAT significantly elevated plasma renin above basal values only in cocaine-exposed male progeny and the magnitude of the renin response was significantly potentiated in comparison to vehicle-exposed/8-OH-DPAT-injected progeny. Data were analyzed by a S-way ANOVA followed by a Newman Keuls test.*

KEY: * = significantly different than the corresponding saline-injected group ($p < 0.05$); † = significantly different than male vehicle-exposed/8-OH-DPAT-injected group; ‡ = significantly different than corresponding male challenge group ($p<0.05$).

SOURCE: Battaglia and Cabrera 1994.

measured, basal levels of plasma renin were unaffected by prenatal cocaine exposure regardless of gender.

The 5-HT_{1A} agonist 8-OH-DPAT did not significantly elevate plasma renin levels above basal values in either male (figure 6A) or female (figure 6B) control progeny groups. These data are consistent with results previously reported in adult rats in which activation of 5-HT_{1A} receptors did not significantly elevate renin above basal levels (Van de Kar 1991). However, 8-OH-DPAT significantly elevated plasma renin above basal levels in cocaine-exposed male offspring. The magnitude of the renin response to 8-OH-DPAT was therefore significantly potentiated (+58 percent) in male cocaine-exposed rats in comparison to the response obtained in saline controls (figure 6A). However, the plasma renin response to 8-OH-DPAT was not significantly elevated in female cocaine-exposed progeny (figure 6B).

These data indicate gender differences in the effects of prenatal cocaine on 5-HT_{1A} receptor-mediated function in progeny. Furthermore, these data suggest that despite previous studies indicating a lack of 5-HT_{1A} involvement in the stimulation of renin in adult male rats, renin in male rats may indeed be elevated by 5-HT_{1A} agonists, since 8-OH-DPAT significantly elevated plasma renin in male rats exposed to prenatal cocaine. These data, which indicate that prenatal cocaine may "unmask" the ability of 5-HT_{1A} agonists to elevate plasma renin, contrast with the inability of chronic administration of cocaine in adult rats to produce a 5-HT_{1A}-mediated elevation of plasma renin (Levy et al. 1994*b*). On the other hand, the current data on the 5-HT_{1A}-mediated increases in renin are consistent with fluoxetine-induced changes in 5-HT_{1A}-mediated plasma renin responses. In adult male rats exposed chronically to fluoxetine, the 5-HT_{1A} agonists 8-OH-DPAT and ipsapirone produced a marked stimulation of renin significantly above basal values (Li et al. 1993, 1994), whereas no significant 5-HT_{1A}-mediated renin response was detected in nontreated animals. It is of interest that, in the present study, renin was not significantly elevated by 8-OH-DPAT in female progeny of either saline- or cocaine-exposed dams, suggesting again a differential effect of prenatal cocaine exposure on male and female 5-HT receptor systems.

Since the potentiation of neuroendocrine responses to 8-OH-DPAT may have been mediated by alterations in the number of either postsynaptic or presynaptic (somatodendritic) 5-HT_{1A} receptors, the effects of prenatal cocaine exposure on 5-HT_{1A} receptors in homogenates of midbrain,

hypothalamus, and frontal cortex were investigated in male progeny (table 2). In contrast to the elevated 5-HT_{1A} receptor density observed in frontal cortex and midbrain of adult (i.e., PD 70) male offspring, there was no significant increase in the density of 5-HT_{1A} receptors at PD 28 in male offspring prenatally exposed to cocaine (Battaglia and Cabrera 1994; table 2). These data suggest that the potentiated neuroendocrine responses to 8-OH-DPAT at PD 28 cannot be attributed to increases in 5-HT_{1A} receptors but are more likely due to postreceptor alterations. Alternatively, changes in 5-HT_{1A} receptor density in specific hypothalamic nuclei may be present but not detected, since changes in such discrete areas would be beyond the detection limit of homogenate assays used in the present studies. In addition, these data suggest that the 5-HT_{1A} receptor alterations observed at PD 70 do not persist from birth but rather occur only upon maturation of the animal. A similar profile of delayed onset of alterations in 5-HT receptors has been previously observed in offspring exposed in utero to the antidepressant and 5-HT uptake inhibitor fluoxetine (Cabrera and Battaglia 1994).

Implications of the Potentiated 5-HT Receptor-Mediated Neuroendocrine Responses

Data from study II suggest that male progeny may be more susceptible than female progeny to cocaine-induced changes in 5-HT receptor function since 5-HT_{1A}-mediated neuroendocrine responses are selectively potentiated in male offspring. However, it has recently been observed that prenatal cocaine can also potentiate 5-HT_{2A}/5-HT_{2C},-mediated neuroendocrine responses in prepubescent progeny (Cabrera et al. 1994*a*). The potentiation of 5-HT_{2A}/5-HT_{2C} receptor-mediated neuroendocrine responses in both male and female progeny exposed prenatally to cocaine contrasts with the gender-specific potentiation in 5-HT_{1A}-mediated neuroendocrine responses. Taken together, the potentiated 5-HT_{1A}-mediated neuroendocrine responses, but attenuated neuroendocrine responses following challenge with a 5-HT releaser are consistent with prenatal cocaine-induced alterations in both the pre- and postsynaptic components of brain 5-HT systems. Furthermore, preliminary data that indicate a reduction in 5-HT content in some brain regions following prenatal cocaine exposure (data not shown) support the presence of a deficit in 5-HT perikarya and/or terminal regions. Therefore, the increase in 5-HT_{1A} responsiveness in male progeny observed in the present study may be compensatory to discrete presynaptic deficits. It is noteworthy that a putative compensatory adaptation in 5-HT_{1A}-mediated effects was not observed in female

progeny, suggesting potential gender differences in 5-HT receptor compensatory mechanisms. Gender-specific neurochemical and neurobehavioral consequences of prenatal cocaine exposure have been observed by other laboratories, though neither males nor females have been reported to be more susceptible to the teratogenic potential of cocaine in all cases (Maecker 1993; Peris et al. 1992; Smith et al. 1989). Prenatal cocaine exposure can produce long-term biochemical and functional alterations in brain 5-HT pathways in male and female progeny. The major findings from the studies presented are that prenatal exposure to cocaine produces: (1) attenuated neuroendocrine response to a 5-HT releaser in postpubescent male and prepubescent female progeny; (2) a potentiated neuroendocrine response to the 5-HT_{1A} agonist 8-OH-DPAT in male but not female progeny at a prepubescent age; and (3) increases in 5-HT receptors in select brain regions of adult, but not prepubescent, male progeny.

SUMMARY AND CONCLUSIONS

These studies demonstrate that prenatal cocaine produces differential changes in neuroendocrine responses following challenge with a 5-HT releaser versus a 5-HT_{1A} agonist and suggest differential functional alterations in both pre- and postsynaptic components of 5-HT pathways. The attenuated neuroendocrine responses in adult male progeny following challenge with a 5-HT releaser, in the absence of reductions in 5-HT receptors, provide additional evidence in support of a presynaptic 5-HT deficit in adult male cocaine-exposed progeny. Furthermore, since prenatal cocaine produced a differential profile of alterations in 5-HT-mediated neuroendocrine responses in adult male (i.e., ↓ ACTH and renin) versus prepubescent female (i.e., ↓ ACTH and corticosterone) progeny following challenge with a 5-HT releaser, these data indicate that the differences could be due to gender and/or postnatal developmental ages. Gender differences in prenatal cocaine effects on postsynaptic receptor function were more clearly shown in study II, which demonstrated that at the same postnatal age, 5-HT_{1A}-mediated neuroendocrine responses were significantly potentiated in male but not female cocaine-exposed progeny. In summary, the data presented in this chapter indicate that the biochemical and functional changes in 5-HT systems observed following prenatal exposure to cocaine are unique with respect to pre- versus postsynaptic alterations, pre- versus postpubescent developmental times, and differences between genders.

A number of general conclusions can be drawn from the data presented. The presence of marked neurochemical deficits at both pre- and postpubescent timepoints, in the absence of any visually apparent physical terata, emphasizes the importance of investigating the neurochemical teratogenic potential of cocaine and other psychostimulants. Furthermore, data from these studies demonstrate the importance of investigating male and female progeny separately, as prenatal cocaine exposure may produce gender-specific alterations in some, but not all, aspects of brain neurotransmitter systems. Another important point that can be discerned from the present data is the necessity of subjecting cocaine-treated animals to challenge tests in order to reveal alterations that might not be readily apparent from measuring basal values for specific biochemical or functional parameters (e.g., basal hormone levels). In addition, the differential biochemical and functional changes in 5-HT systems, manifested at pre- versus postpubescent times, suggests that prenatal cocaine may adversely affect the normal maturational changes occurring in 5-HT systems. This may be of consequence in evaluating developmental stages in human offspring exposed to cocaine in utero. Furthermore, the ability of prenatal cocaine to alter 5-HT-mediated ACTH and renin responses in progeny suggest that offspring may exhibit alterations in their response to physiologic stimuli such as stress. Since neuroendocrine challenge tests can be performed in humans, the present data indicate the potential clinical utility of this approach to provide peripheral markers that can be used to identify changes in brain 5-HT pathways in human offspring exposed in utero to cocaine. Prenatal cocaine-induced alterations in brain 5-HT systems may be of significant clinical importance as dysfunction of 5-HT systems has been implicated in various psychiatric disorders including depression, anxiety, aggression, and drug-seeking behavior.

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ACKNOWLEDGMENTS

This work was supported in part by US Public Health Service grant no. DA 07741 from the National Institute on Drug Abuse. Theresa M. Cabrera is a recipient of a National Science Foundation Minority Graduate Fellowship, NSF GER 925375. The authors thank Francisca Garcia, Kayoko Kunimoto, Qian Li, Wilfred Pinto, and Joseph M. Yracheta for their assistance with the neuroendocrine challenge experiments.

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Ontogeny of Methamphetamine-Induced Neurotoxicity in the Rat Model

Charles V. Vorhees and Cunfeng Pu

INTRODUCTION

The purpose of this chapter is to review the effects of exposure to d-methamphetamine (MA) when administered during development and to contrast these effects to those seen in adult animals. The emphasis will be on effects in rats and the focus will be on three dependent variables: behavior, biochemical measurement of catecholamines, and immunohistochemistry.

METHAMPHETAMINE NEUROTOXICITY

It is well established that in adult rats and mice, high doses of MA induce reductions in striatal dopamine (DA) and forebrain serotonin (5-HT) concentrations (Bakhit et al. 1981; Ricaurte et al. 1980; Seiden et al. 1976, 1988; Wagner et al. 1979), and decrease tyrosine and tryptophan hydroxylase activities (Bakhit et al. 1981; Buening and Gibb 1974; Hotchkiss and Gibb 1980; Hotchkiss et al. 1979; Kogan et al. 1976). Other studies report MA-induced reductions in DA (Wagner et al. 1980) and 5-HT (Brunswick et al. 1992) high-affinity uptake sites and degeneration of striatal dopaminergic nerve terminals without affecting cell bodies (Ricaurte et al. 1982). DA terminal degeneration has also been found based on tyrosine hydroxylase (TH) immunoreactivity (IR) (Hess et al. 1989; Pu and Vorhees 1993). By contrast, reductions in forebrain 5-HT-IR are transient in most animals, showing recovery within 1 to 2 weeks (Axt and Molliver 1991). Investigations in mice (Hess et al. 1989) and rats (Pu and Vorhees 1993) have demonstrated reactive astrogliosis using glial fibrillary acid protein (GFAP)-IR in striata of adult MA-treated animals. However, no comparable astrogliosis has been reported in forebrain areas where MA induces reductions in 5-HT-IR, nor is there silver-degeneration staining in these regions (Ricaurte et al. 1982). This suggests that 5-HT changes do not represent the same kind of degenerative changes as are seen for TH-labeled nerve terminals.

Furthermore, it is known that the DA reductions in the striatum persist for extended periods of time. Although partial recovery occurs, significant reductions have been found for 2 months (Wagner et al. 1980) to 6 months (Ricaurte et al. 1980) after cessation of treatment.

METHAMPHETAMINE DEVELOPMENTAL EFFECTS

Despite these clear histological and biochemical effects of MA exposure in adult animals, few behavioral effects have been reported in such exposed animals (Seiden and Kleven 1989). Moreover, the few effects that have been seen are subtle, only being obtained under conditions of drug or behavioral challenge (Ando et al. 1984; Bittner et al. 1981; Fischman and Schuster 1977).

Paradoxically, neurotoxic doses of MA administered during development produce smaller changes in DA and 5-HT than those seen in adults after similar doses (Lucot et al. 1982; Wagner et al. 1981) when immunohistochemically examined at 20, 40, 60, and 80 days (Pu and Vorhees 1993). No changes are seen in TH-IR on days 20 or 40 or in striatal GFAP-IR on day 20 (Pu and Vorhees 1993). Similarly, no apparent changes were found in 5-HT-IR at 20 or 40 days (Pu and Vorhees, unpublished observations). By contrast, both prenatal and early postnatal exposure to neurotoxic doses of MA induce a number of behavioral effects in the offspring. Prenatal exposure to MA induces increased acoustic startle reactivity in the offspring as adults, as well as eye abnormalities and locomotor activity changes (Acuff-Smith et al. 1992). Early postnatal exposure to MA produces even more striking effects, inducing delays in the ontogeny of olfactory orientation responses to home cage bedding, increased acoustic startle reflex reactivity, altered locomotor activity patterns, and in those exposed on postnatal days 11 to 20, impaired spatial navigation acquisition in a Morris hidden platform maze (Vorhees et al. 1994*a*, 1994*b*). Thus, the paradox is that adult MA exposure induces substantial neurotransmitter changes and neuropathological changes, particularly in the neostriatum, with no clear behavioral correlates, while developmental exposure to similar doses induces clear behavioral abnormalities with no clear neurotransmitter or neuropathological correlates. This paradox is at the core of the authors' research into MA's effects. The goal is to try to elucidate what the behavioral effects are of adult MA exposure as well as the drug's mechanism of action, and to identify the cellular basis of the behavioral effects that have been characterized in rats developmentally exposed to MA. This chapter will

focus on one of these objectives (i.e., the mechanism of MA-induced TH-IR reduction and GFAP-IR increase in the neostriatum of adult rats). The authors began here as a way of trying to understand the shift in MA's neurotoxic effects as a function of age. The authors initially thought that standard markers of MA-induced neurotoxicity (TH, GFAP, and arguably 5-HT) would show changes in young animals exposed to neurotoxic doses of MA. However, as the evidence has unfolded, it is important to understand the changes that confer resistance upon younger animals, and ultimately to provide some insight into which systems are vulnerable to MA after early exposure.

TIMECOURSE OF METHAMPHETAMINE-INDUCED DEVELOPMENTAL EFFECTS

Initial experiments into the immunohistochemical effects of MA exposure were conducted on developing rats (Pu and Vorhees, unpublished observations). Rats exposed to MA (30 milligrams per kilograms (mg/kg) x twice a day) administered on days 1 to 10 or 11 to 20 postnatally showed no changes in TH, 5-HT. or GFAP-IR in any brain region. In order to determine whether the methods being used were sensitive enough to detect changes in adult rats as previously shown, adult rats were administered MA using two dosing regimens. One was that of Sonsalla and associates (Sonsalla et al. 1989), in which 10 mg/kg is given 4 times at 2-hour intervals on a single day, and the animals examined 72 hours later. In the second method (Marek et al. 1990*a*), a large dose (100 mg/kg) is given once and the animals examined 3 days later. As previously reported, the repeated, spaced dosing regimen was more effective than the large-dose approach at depleting neostriatal TH-IR based on a small experiment conducted by the authors (Pu and Vorhees, unpublished observations).

The authors then examined MA-induced changes in TH and GFAP-IR at 20, 40, 60, and 80 days of age in rats 72 hours after administration of 10 or 20 mg/kg x 4 MA (Pu and Vorhees 1993). Results at 60 and 80 days at both doses were the same and are represented in figure 1A, B. As can be seen, neostriatal TH-IR was markedly reduced and some of the fibers were swollen and twisted (figure 1A). Also noteworthy is that the TH-IR reduction is not uniform, with the largest depletion seen in the ventral portions of the neostriatum (not visible in this section). Furthermore, the ventral-lateral zone was the most specifically affected subregion. As can

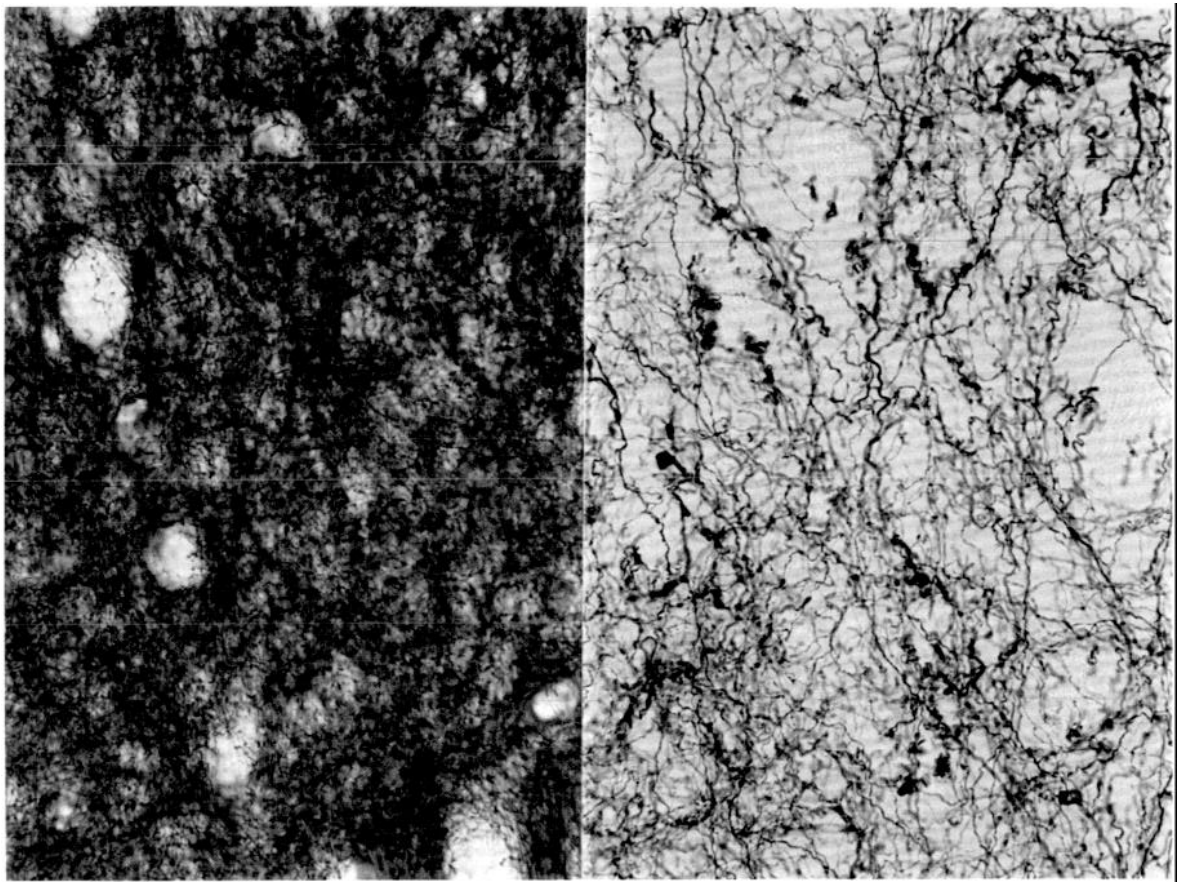

FIGURE 1A.
Coronal sections (40 µm) through the neostriatum of 80-day-old male Sprague-Dawley CD rats treated with IO mg/kg x 4 (2-hr intervals) of MA (expressed as free base) or saline IP and examined 72 hr later. TH-IR is shown at 400x magnification. Note on the right the swollen and twisted fibers in the MA-treated, TH-stained section.

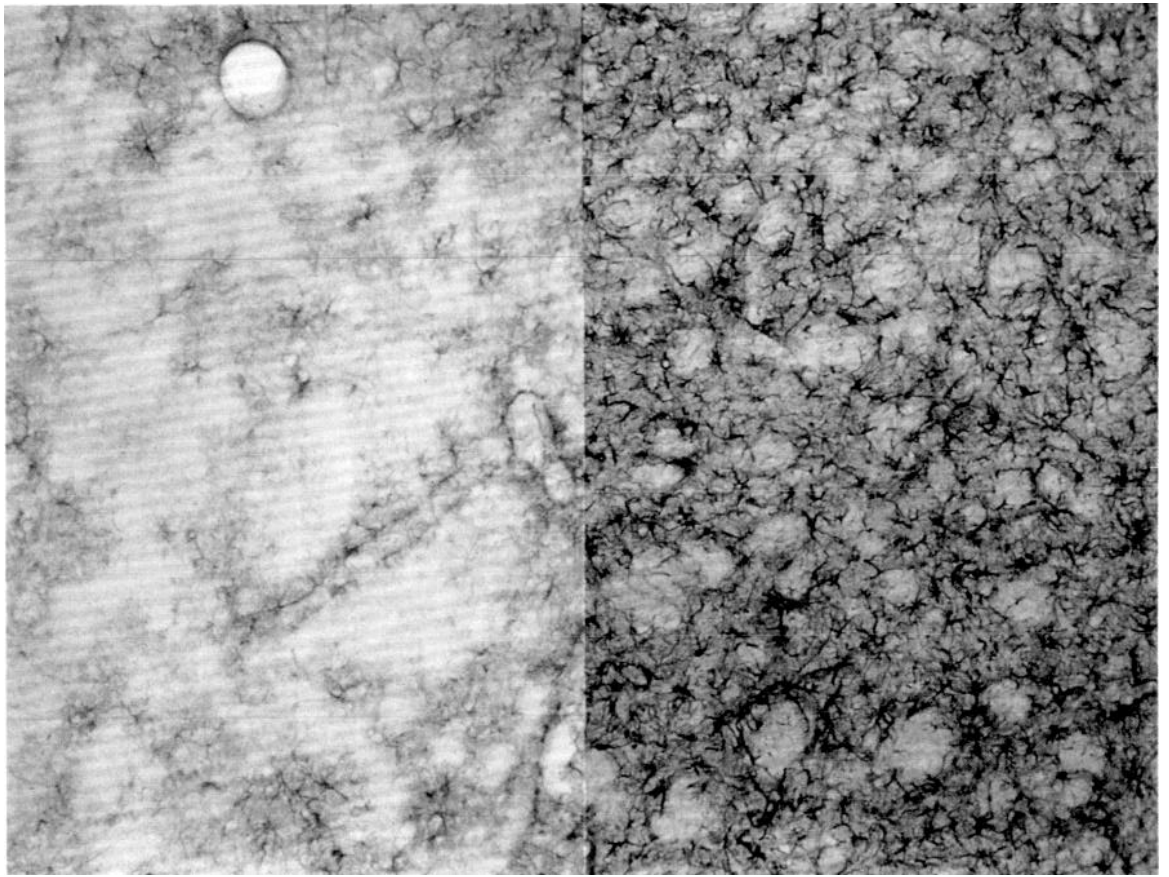

FIGURE 1B.
Coronal sections as in figure IA. GFAP-IR shown at 100x magnification. Controls are on left, MA treated on right. Note the extensive astrocytic hypertrophy (astrogliosis) in response to MA treatment.

be seen in subsequent studies, this observation proved to be consistent. For GFAP-IR, there was a marked increase, indicating extensive gliosis in response to the TH-IR reduction (figure 1B). The observation of extensive gliosis confirms that the TH-IR reduction represents not simply a TH depletion but actual nerve terminal degeneration, since reactive gliosis is not triggered by drugs causing only transmitter depletion but instead is elicited by neurotoxins (O'Callaghan 1991).

Figure 2 shows the effects of the same dose at 40 days. No reduction in TH-IR is evident. However, a smaller ventral-lateral zone of gliosis is still evident in the neostriatum. This observation suggested that an effect other than degeneration of DA terminals must be occurring to trigger gliosis. Alternatively, it could be postulated that TH-IR reductions below the threshold of visual detection might account for the residual gliosis observed. This requires further investigation; however, the TH-stained fibers seen in the 40-day-old animals under high magnification showed none of the neuropathological features observed when TH is reduced (i.e., no swollen or twisted fibers). This argues against TH-IR reduction as the basis of the GFAP-IR increase seen at this age. Finally, figure 3 shows TH- and GFAP-stained sections from a 20-day-old rat. No decrease in TH-IR (figure 3, left) or increase in GFAP-IR (figure 3, right) is evident.

The authors concluded that there is a developmental dissociation in the pattern of effects induced by MA during development, with TH-IR susceptibility shifting from resistance to sensitivity between 40 and 60 days, and GFAP-IR responsiveness shifting from no effect to reactivity between 20 and 40 days of age. These observations led the authors to focus on the mechanism of MA-induced neurotoxicity in adult rats.

MECHANISM OF METHAMPHETAMINE-INDUCED NEUROTOXICITY

The mechanism of MA-induced neurotoxicity is not completely understood. A prerequisite for striatal DA nerve terminal degeneration is DA overrelease (Axt et al. 1990; Schmidt et al. 1985; Wagner et al. 1983). It has been shown that inhibition of presynaptic DA reuptake reduces or prevents MA-induced DA depletion in the neostriatum (Marek et al. 1990*b;* 1990*a;* Muraki et al. 1992). As there are no data currently available on TH-IR or GFAP-IR after DA reuptake inhibition, the authors

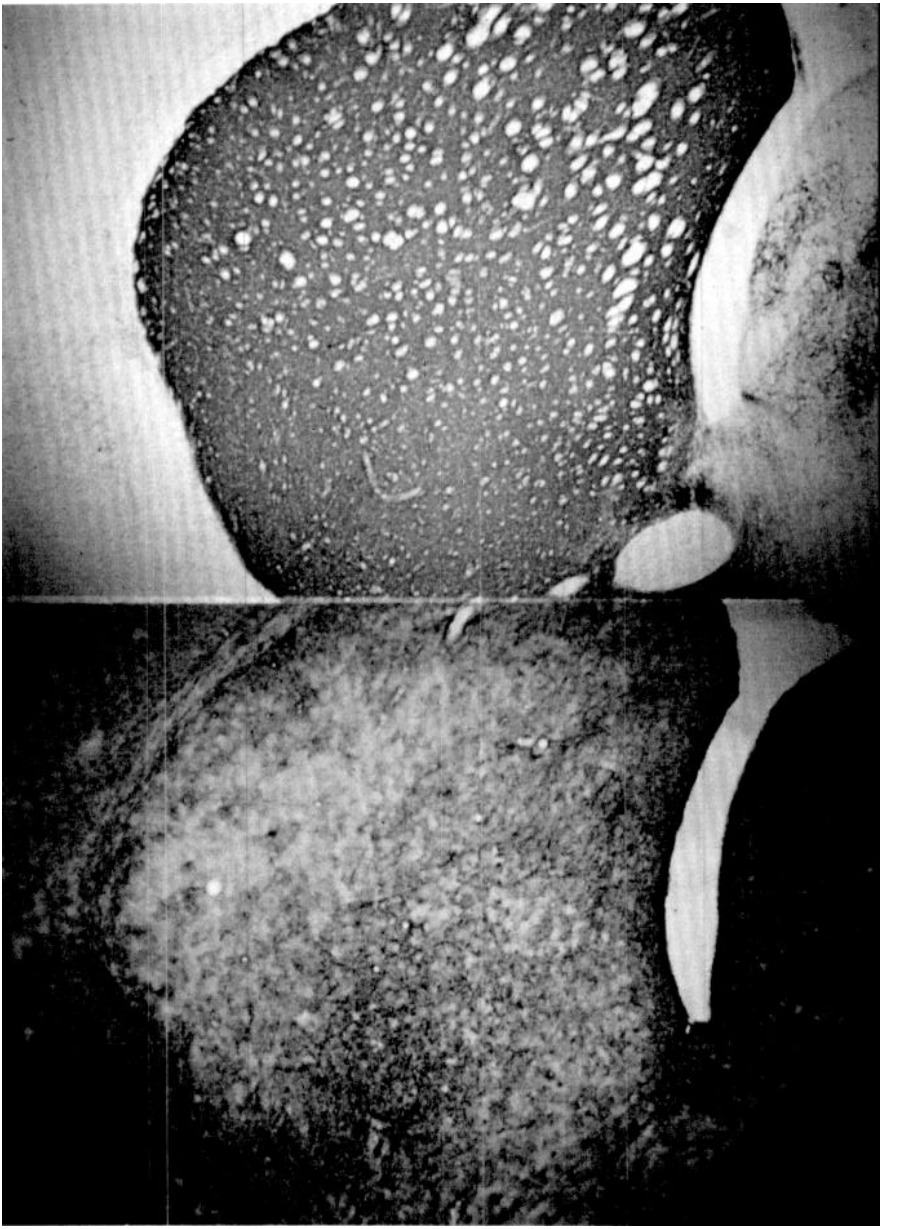

FIGURE 2. *Coronal section (40 µm) through the neostriatum of 40-day-old male Sprague-Dawley CD rats treated with 10 mg/kg x 4 of MA (see figure 1A for details). Top: TH-IR of MA-treated rat showing no TH reduction in the neostriatum at a magnification of 20x. Bottom: Adjacent section of MA-treated rat showing ventral zone of GFAP-IR increase at a magnification of 20x.*

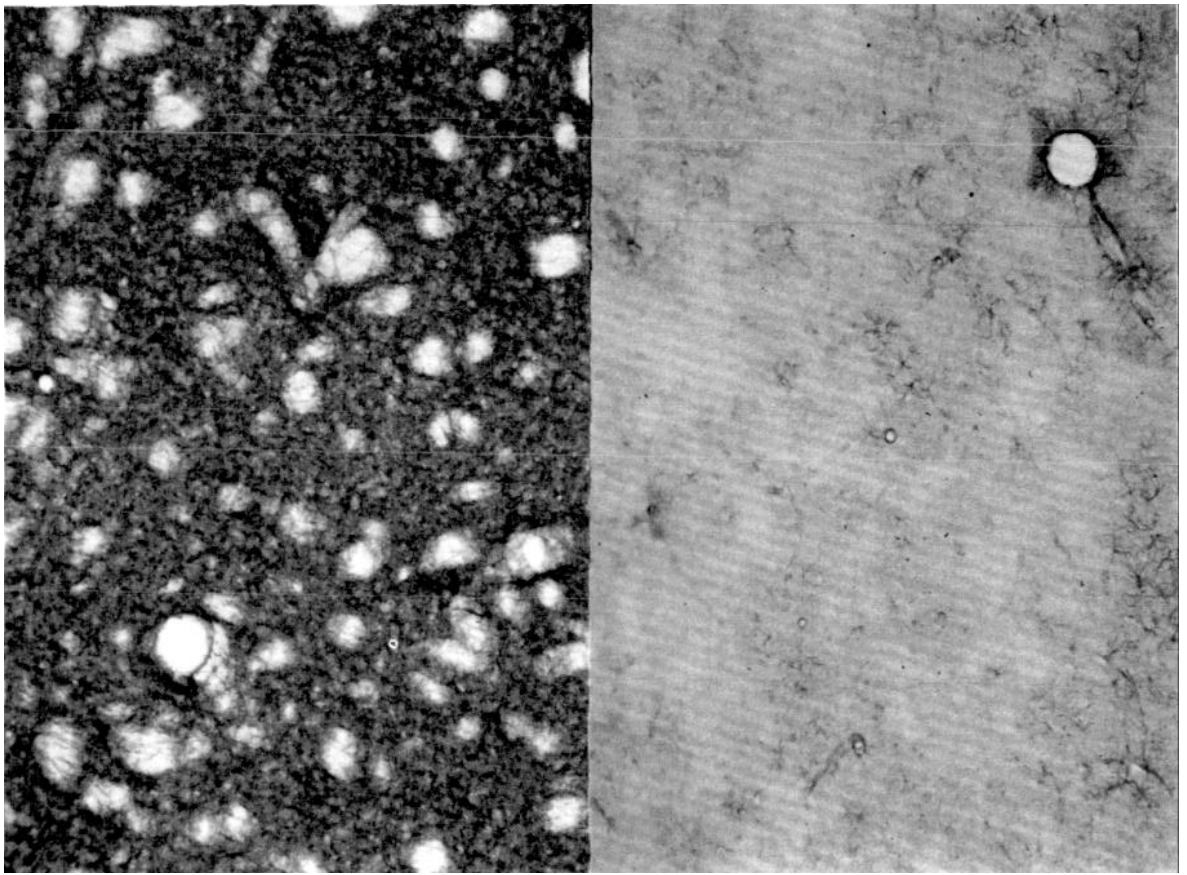

FIGURE 3.
Coronal section (40 µm) through the neostriatum of 20-day-old male Sprague-Dawley CD rats treated with IO mg/kg x 4 of MA (see figure IA for details). Left: TH-IR of MA-treated rat showing no effect of the treatment at a magnification of 100x. Right: GFAP-IR of same MA-treated rat from an adjacent section also showing no change from normal at a magnification of 100x.

examined the effects of MA (10 mg/kg x 4 as before) and the DA reuptake inhibitor amfonelic acid (AFA) (20 mg/kg) administered following the last MA injection on immunohistochemical changes in some animals and biochemical changes in others (Pu et al. 1994). Rats treated only with MA show the typical pattern of TH-IR reduction and GFAP increase in the neostriatum. By contrast, the MA-AFA-treated animals show no TH-IR change (figure 4, top). However, this group does exhibit a residual zone of GFAP-IR increase, and that zone turns out to be in the ventral-lateral portion of the neostriatum (figure 4, bottom). This is the same zone affected in 40-day-old rats when TH-IR was unaffected. Perhaps this is simply a coincidence or perhaps it is indicative of a second effect of MA on a subset of neostriatal nerve terminals that are either especially susceptible or are affected by damage to distal neurons with input to this region of the neostriatum. (As will be seen later, the authors will argue for the latter.) The biochemical determinations of DA concentrations in the neostriata of MA- and MA-AFA-treated rats is shown in figure 5. As can be seen, the results are entirely consistent with the immunohistochemical findings. More importantly, these data show quantitatively that there is no residual DA reduction that could explain the GFAP-IR increase seen in the ventral-lateral region of the neostriatum. If DA terminal degeneration is not the whole story in MA-induced neurotoxicity, what else might be involved?

Sonsalla and associates (Sonsalla et al. 1989, 1991) have shown that glutamate (GLU) interacts in the induction of DA striatal depletion as administration of MK-801, a GLU receptor antagonist, blocked DA depletion. To determine what the immunohistological effects of this inhibition are on 5-HT or GFAP-IR, the authors conducted an experiment in which adult rats were treated with 10 mg/kg x 4 of MA as before, and 15 minutes prior to each MA dose a dose of 1 or 2 mg/kg of MK-801 was given. It had previously been shown that MK-801, unlike AFA, is most effective at interfering with MA's effects if treatment is given 15 minutes prior to MA exposure (Marshall et al. 1993; Weihmuller et al. 1991, 1992). As before, rats were examined 72 hours posttreatment. As can be seen in figure 6, rats treated with MA alone show the typical pattern of TH-IR reduction and GFAP-IR increase. Rats treated with MA-MK-801, on the other hand, show no TH or GFAP-IR changes. Actually, this pattern, although the dominant one, was not seen in every animal. A few animals showed partial TH-IR reductions, and in these (and only in these) rats a concomitant GFAP-IR increase was observed (not shown). In addition, MK-801 pretreatment also prevented any 5-HT-IR reduction compared to that seen after MA alone (not shown). These data confirm

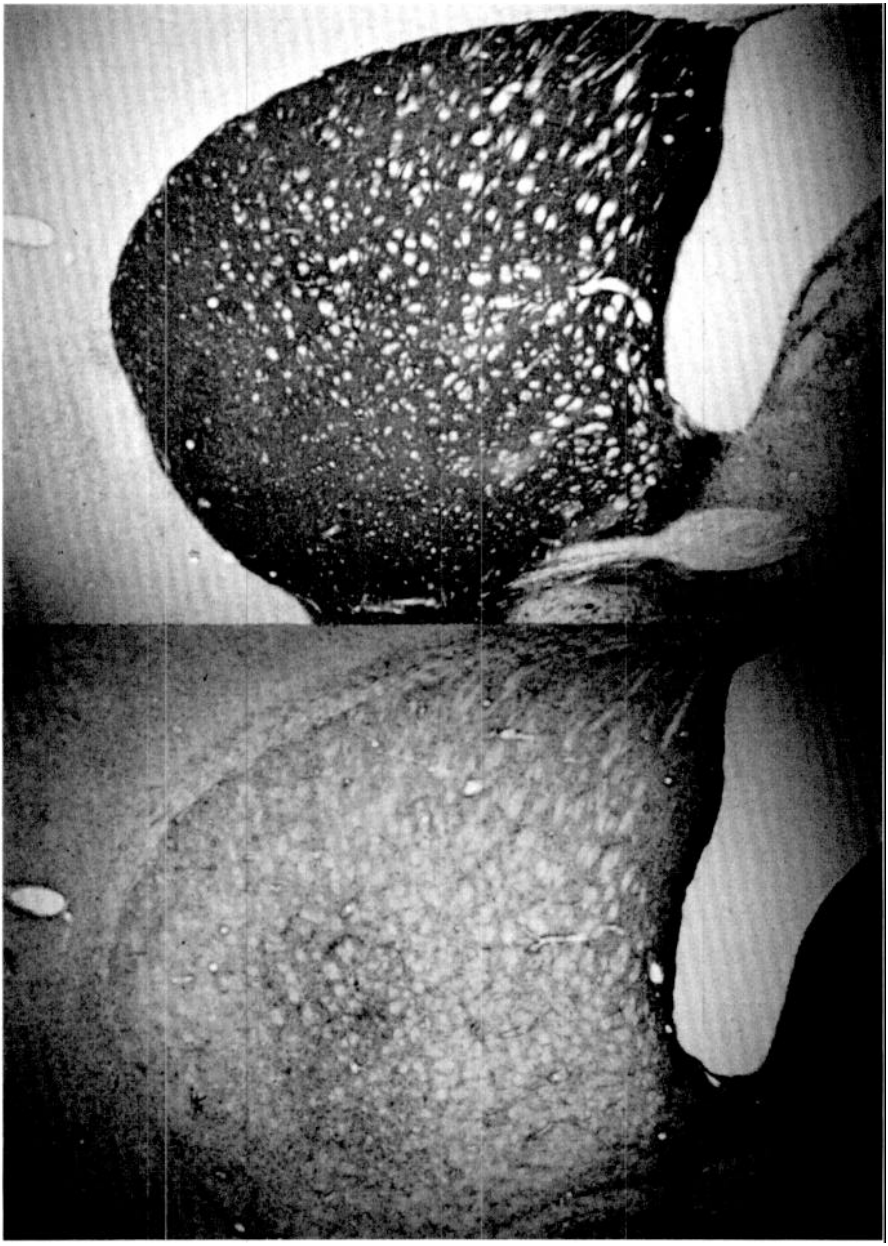

FIGURE 4. *Coronal section (40 pm) through the neostriatum of adult male Sprague-Dawley CD rats treated with 10 mg/kg x 4 of MA and AFA 20 mg/kg x 1 IP at fourth MA treatment (see figure 1A). Top: TH-IR of MA-AFA-treated rat showing protection of TH terminals from depletion. Bottom: GFAP-IR of adjacent section from same rat showing a ventral-lateral zone of residual astrogliosis. Magnification in both panels is 20x.*

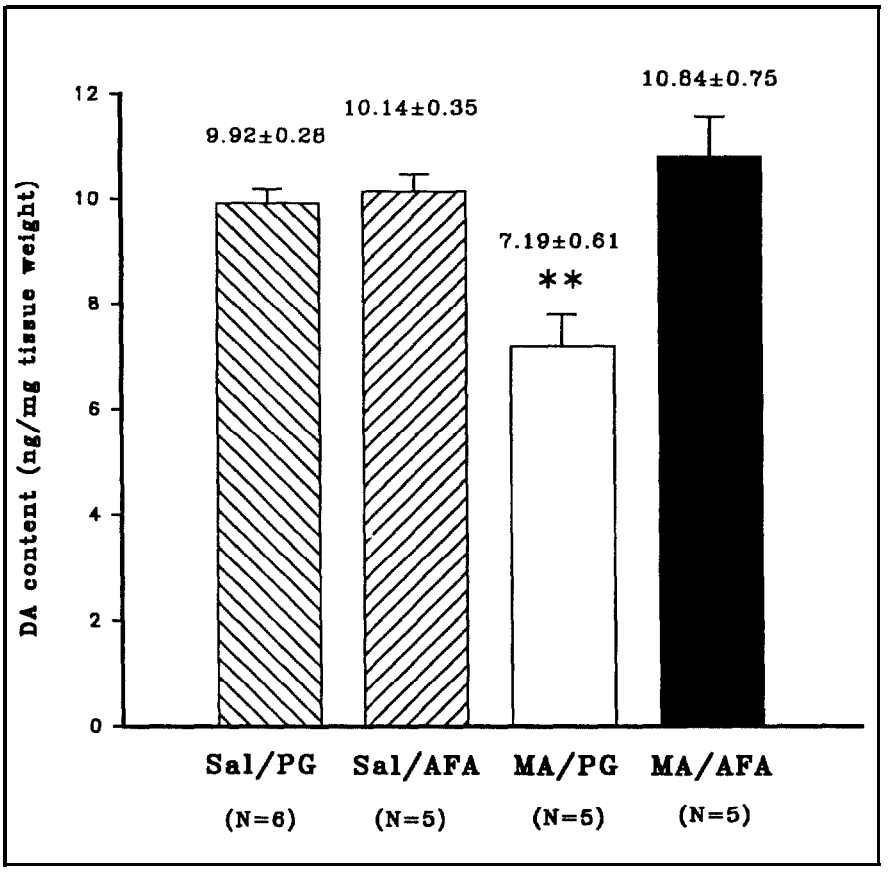

FIGURE 5. *Protective effect of AFA on MA-induced reduction of DA. DA concentration in striatum of rats treated with saline and propylene glycol (Sal/PG; vehicle for AFA), saline and AFA (Sal/AFA), MA and propylene glycol (MA/PG), or MA and AFA (MA/AFA).*

KEY: ** = $p > 0.01$ compared to all other groups based on analysis of variance and Duncan pairwise comparisons.

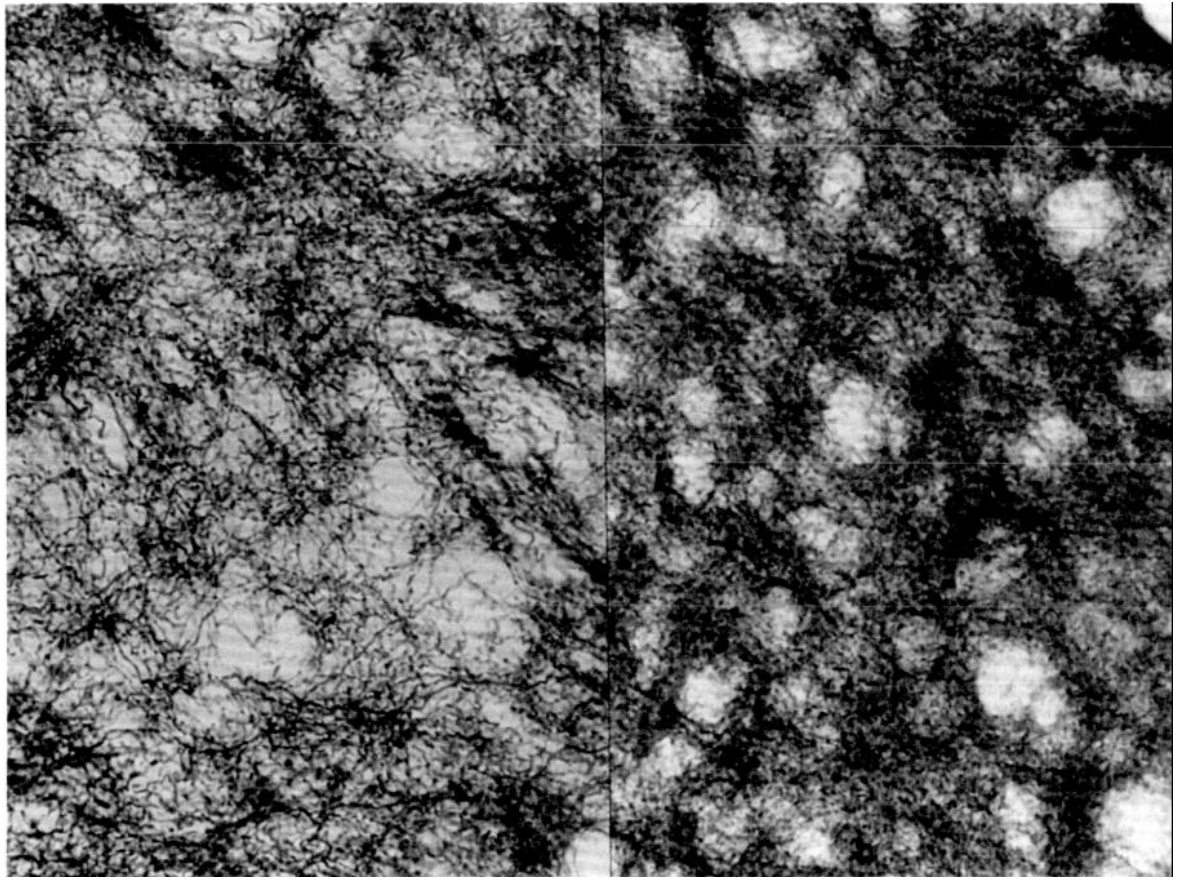

FIGURE 6A.
Coronal section (40 µm) through the neostriatum of adult male Sprague-Dawley CD rats treated with IO mg/kg x 4 of MA (specifics as in figure IA). Sections stained for TH-IR at a magnification of 400x. Left: TH-IR of MA-treated rat showing TH depletion. Right: TH-IR of MA+MK801 (1 or 2 mg/kg x 4) treated rat showing normal TH staining. Magnification is 400x.

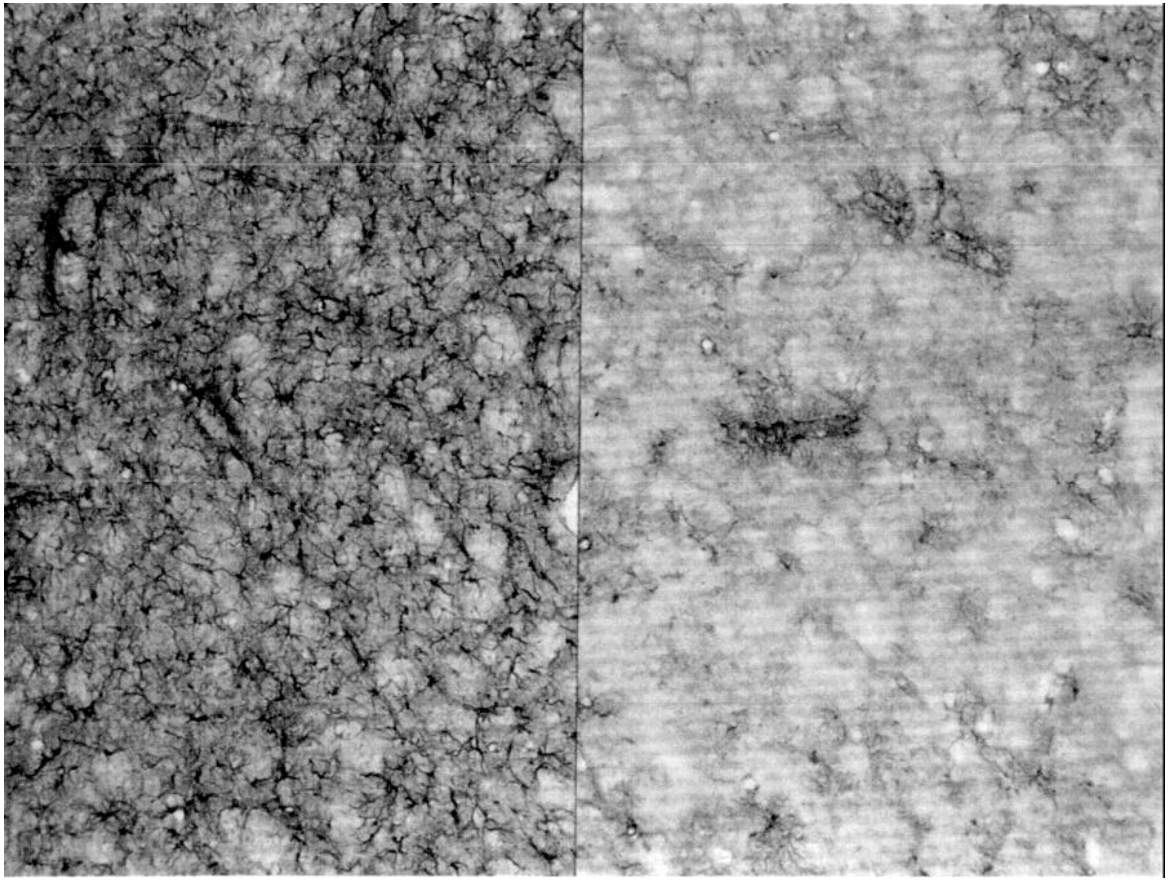

FIGURE 6B
Coronal section (40 µm) through the neostriatum of adult male Sprague-Dawley CD rats treated with 10 mg/kg x 4 of D-MA (specifics as in figure 1A). Sections stained for GFAP-IR at a magnification of 100x Left: MA-treated rat showing astrogliosis typical of the astrocytic response to nerve terminal damage. Right: MA+MK801-treated rat showing normal GFAP staining.

and extend earlier findings (Marshall et al. 1993; Sonsalla et al. 1989, 1991; Weihmuller et al. 199 1, 1992) that there is an indirect effect of GLU receptors on MA-induced neurotoxicity. The data also show that interference with this contribution, unlike that of DA reuptake inhibition, is more effective at preventing the associated reactive gliosis effect of MA. While the exact mechanism of MK-801's effects on MA-induced neurotoxicity are not known, it has been demonstrated that MA induces GLU release (Nash and Yamamoto 1992) and that GLU overrelease induces enhanced DA release (Clow and Jhamandas 1989; Krebs et al. 1991). Thus, it may be that MA acts through several processes to increase DA overrelease through action within the DA presynaptic terminal (and for which MA uptake and DA reuptake processes are required), and through GLU overrelease which stimulates N-methyl-D-aspartate (NMDA) receptors on DA terminals that further augment DA release. The latter, which may be described as a gluta-minergic enhancement of DA release mechanism, appears to be an integral part of the sequence of events since NMDA receptor inhibitors interrupt MA neurotoxicity at this step of the process (Pu and Vorhees, in press; Weihmuller et al. 1992).

POSSIBLE GLUTAMINERGIC NEUROTOXICITY INDUCED BY METHAMPHETAMINE

While all of these observations fit together coherently, they cannot be the whole story because there is still the problem of residual gliosis in 40-day-old rats and in AFA posttreated rats. What additional source of effects could be leading to this effect? To investigate this, the authors turned to evidence on neuronal degeneration visualized using silver stains. Ricaurte and associates (Ricaurte et al. 1982) have shown that MA-treated rat brains stained by the Fink-Heimer method show degenerating neurons in a narrow wedge of cortical cells in the somatosensory region of the parietal cortex. Furthermore, Ryan and associates (Ryan et al. 1990) have shown that d-amphetamine induces a pattern of silver-stain degeneration in somatosensory cortex and neostriatum in Sprague-Dawley rats. Track tracing and other methods have shown that the neostriatum has corticostriatal projections (Dinopoulos et al. 1989; McGeorge and Faull 1989; Webster 1961). These projections are topologically organized, such that more anterior cortical cell bodies project their axons to more anterior regions of the neostriatum, and more posterior cortical cell bodies project to more

posterior regions of the neostriatum. There is a comparable lateral cortical to lateral neostriatum, and medial cortical to medial neostriatum, pattern. Focal lesion studies have shown that regions very close to the ventral-lateral neostriatum degenerate when the somatosensory cortex is lesioned. Current evidence also supports the view that most of the corticostriatal projections are of glutaminergic neurons. Also, the silver-stain degeneration experiments cited above do not provide any evidence as to the neurotransmitters involved; the convergence of evidence from several sources suggested that there are possibly glutaminergic neurons in the somatosensory region of the cortex that are sensitive to the effects of MA and which likely project to the region of the neostriatum where residual gliosis has been seen.

Immunohistochemical analysis of GLU-containing neurons is not as straightforward as for the molecules described above (Ottersen and Storm-Mathisen 1984). Two limitations are that the method (Ottersen and Storm-Mathisen 1984) does not stain GLU-containing axons, and background staining is higher than with other antibody-linked stains. In this experiment, adult rats were treated with 10 mg/kg x 4 of MA as before, and 72 hours later the brains examined for GLU-IR. As can be seen in figure 7, a reduction in GLU-IR staining was evident in the MA-treated rats (figure 7, top) compared to controls (figure 7, bottom), and this effect was restricted to a narrow band of cortical tissue in the somatosensory region. Moreover, the effect was most pronounced in cortical layers 2 and 3 (figure 8). This observation is consistent with the silver-stain degeneration studies noted earlier.

In an effort to gather converging evidence, previously GFAP-stained sections of MA-treated rats were checked to see if gliosis was present in this region. No effects were noticed initially; however, such an effect might have been overlooked because the region of interest is focal. In 3 out of 10 rats examined a gliosis-responsive band was seen in the same region showing the GLU-IR reduction in cortical layer 3 (figure 9). The authors surmised that the GFAP-IR increase was only seen in layer 3 because this is the most affected layer seen in the GLU-IR-stained sections. Unfortunately, the different perfusing requirements for GFAP and GLU-IR are such that it is impossible to perform the most desirable experiment and examine both stains in the same brains in alternate sections. Nevertheless, the coincidental match of effects between the GLU-IR and GFAP-IR in the somatosensory cortex is such that it supports the view that MA induces damage to glutaminergic neurons in this region. Given that this region of the cortex sends projections to the

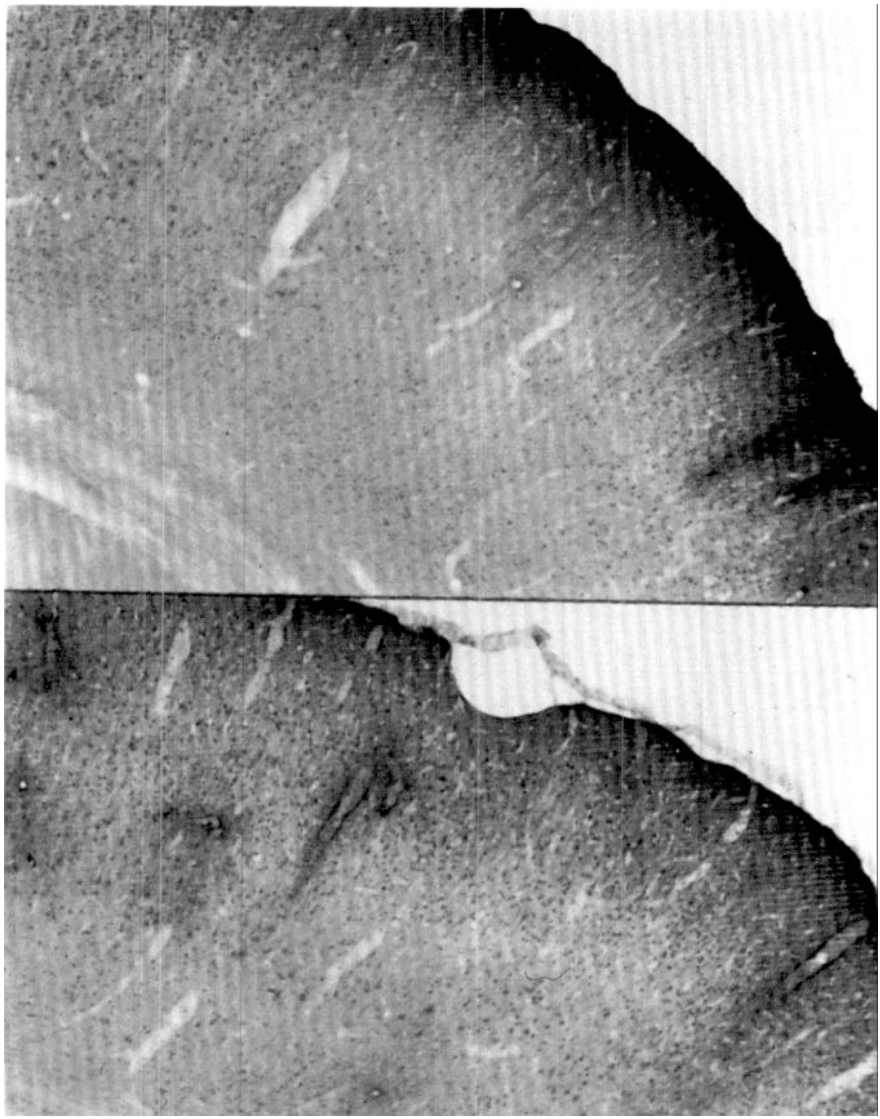

FIGURE 7. *Coronal section (40 pm) through the cortex (somatosensory and surrounding regions) of adult male Sprague-Dawley CD rats treated with 10 mg/kg x 4 of D-MA as before and stained for GLU-IR. Bottom: GLU-IR of control rat showing dark cell bodies containing the label scattered throughout most layers of the cortex. Top: GLU-IR of MA-treated rat showing region-specific reduction of cell bodies containing the GLU label. The affected region is in the sompatosensory cortex covering the middle one-third from the longitudinal fissure to the rhinal sulcas. Magnification in both panels is 40x.*

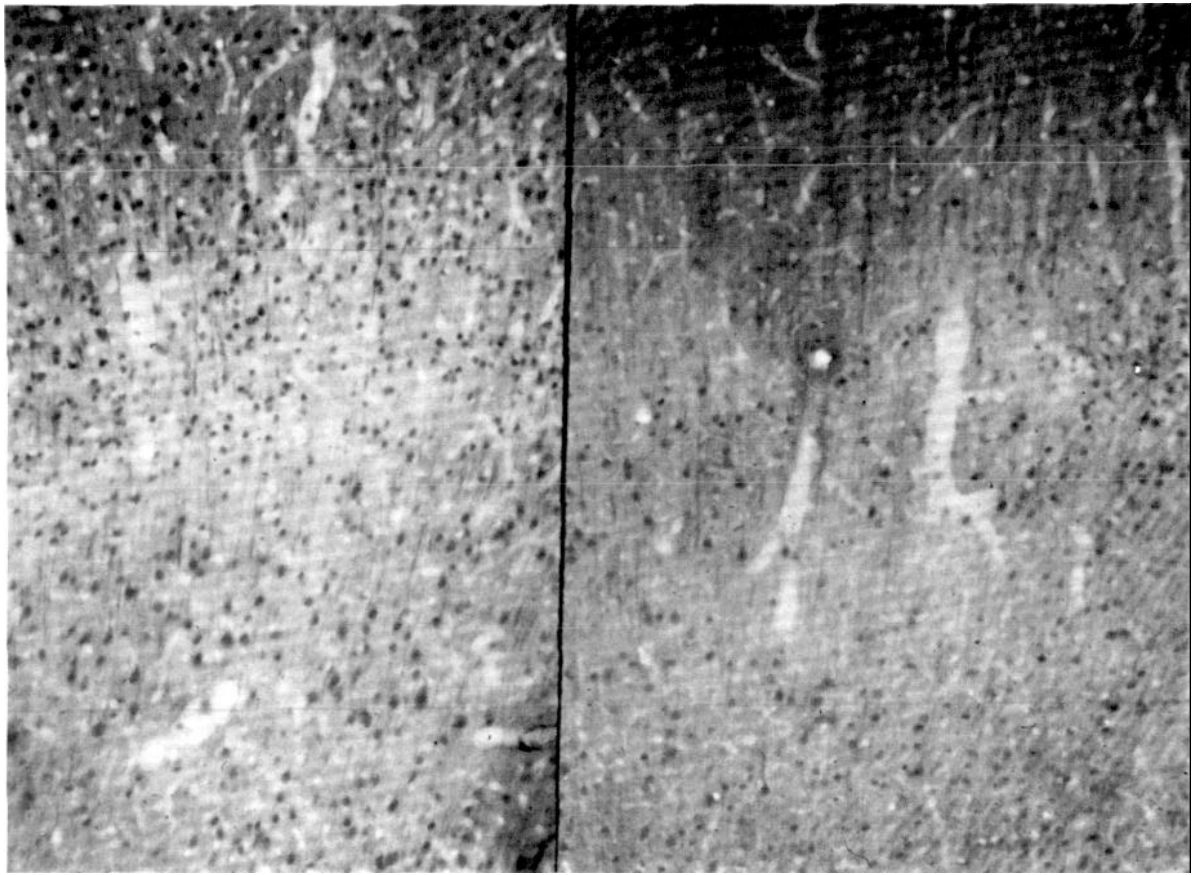

FIGURE 8.
Coronal section (40 µm) through the cortex of same animals as in figure 7. Left: GLU-IR of saline control showing dark cell bodies containing the GLU label. Right: GLU-IR of MA-treated rat showing depletion of GLU-labeled cell bodies especially from the upper cell layers (the boundary to unaffected areas may be seen in the right third of this panel). Magnification in both panels is 100x.

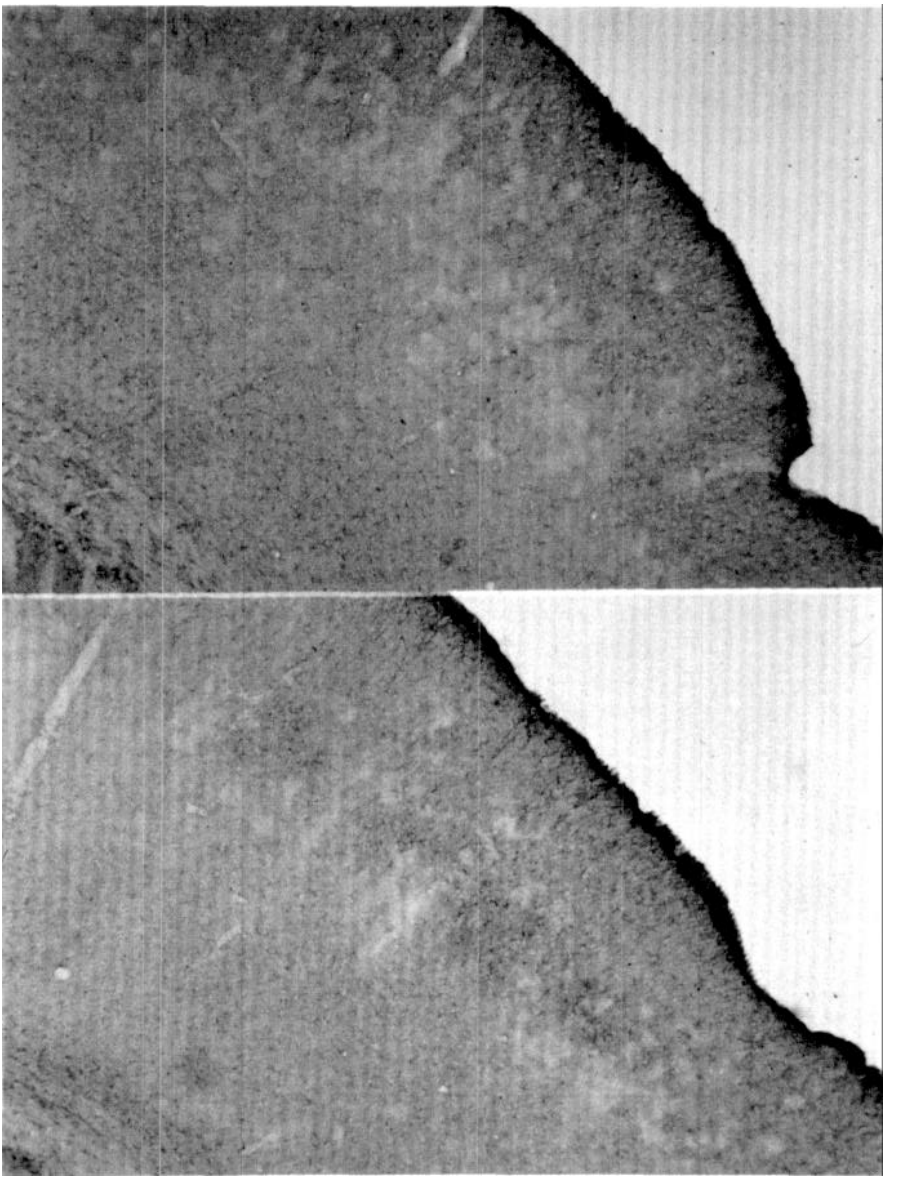

FIGURE 9. *Coronal section (40 pm) through the cortex of adult male Sprague-Dawley CD rats stained for GFAP. Top: Same region (somatosensory cortex) as shown infigure 7 in a saline control rat. Note absence of darkened astrocytic zones. Bottom: Similar section in rat treated with 10 mg/kg x 4 of MA showing patchy band of darkened astrogliotic cells in layers found in figure 7 to show GLU-IR depletion following MA treatment. Magnification in both panels is 400x.*

ventral-lateral region of the neostriatum, it is hypothesized that it is the damage to this cortical zone that is responsible for the residual zone of increased GFAP-IR that has been observed in the neostriatum in 40-day-old rats and in adults posttreated with AFA. If correct, this suggests that the glutaminergic effect of MA may appear earlier in development than do the classic markers of MA-induced neurotoxicity. Accordingly, the authors intend to examine developing animals for GLU-IR.

ACKNOWLEDGMENT

The authors' development of this manuscript was made possible by support from Public Health Service grant no. DA06733 from the National Institute on Drug Abuse of the National Institutes of Health.

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Ontogeny of Nociception and Antinociception

Gordon A. Barr

INTRODUCTION

The issue of how pain is processed at various levels of the neuroaxis has been the subject of intense study for decades, and significant progress has been made in researchers' understanding of how painful sensations are perceived, processed, and dampened. Yet little is known of how pain processing differs between immature and adult organisms. The question of whether or not infants even experience pain has been controversial. Not long ago the prevailing medical opinion was that human infants did not perceive pain due to the immature state of their nervous system and in particular the lack of myelination of neurons. This assumption led directly to the inappropriate withholding of analgesics for painful surgical treatments (Yaster 1987). Recent studies have shown that the requisite pathways for the transmission of pain from the periphery to the central nervous system (CNS) develop perinatally, but a detailed understanding of how these input systems become organized is still lacking.

Although it is clear today that the human neonate can feel pain, the use of postoperative analgesia remains less common for the infant patient than for the adult patient (Schechter 1989). This is in part because so little is known of the pharmacological mechanisms of analgesic drugs at these early ages, except that they act differently in the young organism than they do in the adult. This information is particularly important because of the growing recognition that adequate and continued alleviation of pain is a critical factor in the survival of human infants after invasive medical procedures (Anand and Carr 1989; Anand et al. 1987).

This chapter reviews the literature on the ontogeny of nociception and of antinociception, identifying issues that are important in the study of each of these processes. It is not meant to be a comprehensive review of the literature; rather it is designed to provide a statement of what is known of nociceptive and antinociceptive processes during development and to describe important issues that must be considered in these types of studies. Most of the reviewed literature has been conducted using the infant rat as subject. Where different, the species will be specified. It

must be kept in mind, however, that chronological age and state of maturation are not equivalent and at birth different species differ in their degree of development.

NOCICEPTION

It is not possible to assess whether or not animals or nonverbal human infants feel pain. What is described below are data on the maturation of behavioral, electrophysiological, and anatomical events within the pain pathways as they are currently understood. (For a discussion of these issues see Light 1992.)

Behavioral Studies

Basic research on the ontogeny of nociception has focused on the postnatal infant rat. What is known is that the infant rat responds behaviorally to a noxious stimulus in much the same way as does the adult. The flexor-withdrawal reflex in response to pinching or heating the hindpaw is present at birth in the rat (Fitzgerald and Gibson 1984) and is likely to be present by gestational day (GD) 19 (normal gestation in the rat is 21 to 22 days), since pressure or pinching the skin activates dorsal horn cells in the lumbar spinal cord at that age (Fitzgerald 1991). In contrast, the reflexive withdrawal response to mustard oil painted on the hindpaw, which activates C fibers, does not develop until about 10 days of age (see below) (Fitzgerald et al. 1987). The behavioral response of the 3-day-old rat to a formalin injection into the hindpaw, which in the adult is mediated by C fibers (Dickenson and Sullivan 1987), is quite similar to that of the adult (Abbott et al. 1982; Dubuisson and Dennis 1977; McLaughlin et al. 1990). Both the infant and the adult respond by paw lifting, paw licking, and expressing recuperative behaviors. There are similar parallels for other noxious stimuli. In a crude measure of nociception, withdrawal of appendages when immersed in warm water or subjected to a pressure stimulus, the intensity-latency function was relatively constant over the first 14 days of life in the rat (Hughes and Barr 1988).

There are differences between the behavioral responses of the infant and those of the adult. The infant rat does not respond to an intraperitoneal injection of hypertonic saline or acetylcholine until the end of the third week (Bronstein et al. 1986). The late development of the withdrawal reflex following application of mustard oil to the hindpaw is paralleled by

its failure to induce c-fos expression of 3-day-old rats (Fitzgerald et al. 1987; Williams et al. 1990). These are all C-fiber- mediated processes suggesting the earlier functional development of Aδ- and C fibers (see below), although as stated above formalin injection to the hindpaw produces a behavioral response by birth (McLaughlin et al. 1990; Yi and Barr 1995).

Studies of pain perception in human infants show that nociception develops gradually through the last trimester. Using an empirically developed facial activity scale that seems specific and sensitive to painful stimuli, Craig and coworkers described the maturation of the responses to presumably painful and nonpainful stimuli in premature and term babies (Craig et al. 1993). They recorded the baseline level of responding, the reaction to a heel swab or heel lance, and the responses during a subsequent recovery period. Preterm human infants of gestational age 25 to 27 weeks did not show significant differential responding across the three stimuli. Slightly older infants, 28 to 30 weeks, did. At the gestational age of 37 to 41 weeks the infants showed significantly more painlike reactions to the lance than did the younger infants. Infants between 28 and 36 weeks did not differ from each other.

Slightly different results were reported by Andrews and Fitzgerald (1994). Calibrated von Frey hairs were applied to the foot and leg of human infants (preterm to neonates 27.5 to 42.5 weeks postconception). All infants responded with a flexion-withdrawal reflex, but unlike the facial response, there was an increase in threshold with age. This may be due to the nature of the two responses since the withdrawal reflex occurred in response to tactile as well as to noxious stimulation and there are likely differences in their level of neural processing of the two responses (Andrews and Fitzgerald 1994). Thus, human infants are first responsive to noxious mechanical stimuli at about weeks 28 to 29 of gestation and there are changes in reactivity near parturition. Whether or not this is true of other noxious stimuli, especially chemical irritants, is not known.

Anatomical Studies

The anatomical foundations for nociceptive processing develop during gestation in the rat, and physiological processing of nociceptive information apparently occurs prenatally. However, nociception is not fully mature until close to weaning. Fitzgerald and colleagues have done perhaps the most elegant and exhaustive work on both the anatomic and

physiologic maturation of the nociceptive pathways. The immature rat appears to respond first to natural noxious stimulation at GD 19. Nociceptors in the peripheral tissues are present prenatally and the fine fiber nociceptive afferents, corresponding to Aδ- and C fibers in the adult, are functional by GD 17. Aδ- and C fibers, which relay pain signals from the periphery to the dorsal horn of the spinal cord, reach lamina II on fetal day 19.5 and demonstrate an adultlike somatotopic distribution through the dorsal horn at birth. Substance P (SP) and fluoride-resistant acid phosphate (FRAP), markers for C fiber afferents (Dodd et al. 1984; Nagy et al. 1981), are found in the small primary afferents before birth (Fitzgerald and Gibson 1984; Pickel et al. 1982; Semba et al. 1982) and within 12 hours of birth, respectively (Fitzgerald and Gibson 1984; Mattio et al. 1981). SP receptors are functional in the dorsal horn at birth (Suzue et al. 1981). Substantia gelatinosa interneurons mature rapidly but postnatally (Jancso and Kiraly 1980; Pickel et al. 1982). Maturation of these afferents continues throughout early postnatal life. For example, adult concentrations of SP and FRAP are reached only after the first week (Fitzgerald and Gibson 1984). Thus the anatomical foundation for the processing of pain at the level of the spinal cord is in place, but not fully mature, by late in gestation in the rat.

Maturation of the ascending spinothalamic tract, which transmits the information about noxious input to the thalamus, is also present early. Injection of small quantities of wheat-germ agglutin in horseradish peroxidate (WGA-HRP) into the ventral posterolateral nucleus of the thalamus in pre- and postnatal rats resulted in retrograde labeling of cells in the superficial dorsal horn by fetal day 18, the youngest age injected. The distribution of the cells in the dorsal horn changed with age (figure 1). There is no information as to whether or not this pathway is functional in the fetus (Miya and Barr 1991).

Electrophysiologic Data

Despite the early anatomical maturation of the primary afferents, the electrophysiological properties of the fine-diameter nociceptive fibers continue to undergo significant maturation throughout the first postnatal week (Fitzgerald 1988). Aδ fibers, which respond to high threshold mechanical stimulation, are functional earlier than are C fibers since dorsal horn cells respond to pinching of distal hindlimb at GD 19 (Fitzgerald 1991) but the capsaicin-sensitive burst of spikes elicited by

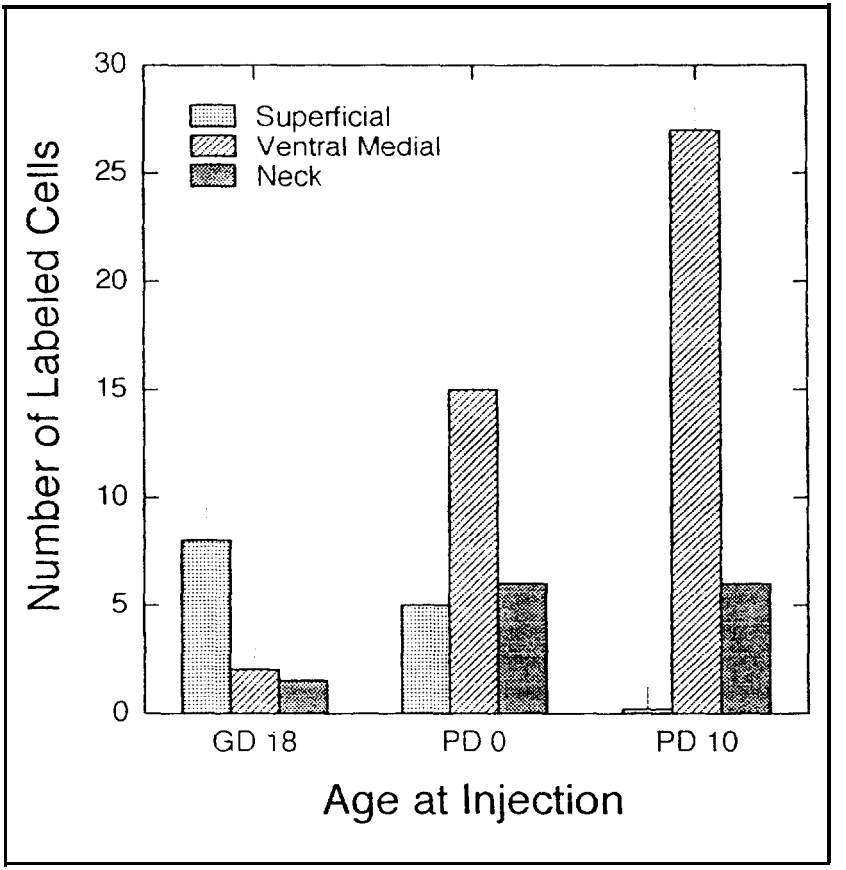

FIGURE 1. *This figure depicts the distribution of retrogradely labeled cells (WGA-HRP) in the dorsal horn of the spinal cord following injection into the ventral posterior lateral nucleus of the thalamus. Pups were returned to their dam following recovery from surgery. Fetuses were returned to the uterus. Survival time was 48 hr before perfusion. Labeling was contralateral to the injection site and labeled neurons were never noted below the central canal. Superficial refers to the marginal region of the dorsal horn. The neck region is lamina V, whereas ventral medial is an area dorsal to the central canal.*

SOURCE: Miya 1993.

electrical stimulation or by mustard oil does not appear until postnatal day (PD) 9 (Fitzgerald 1988). Furthermore, neurogenic edema, a C fiber mediated event in the adult (Kenins 1981), occurs only after the first postnatal week (Fitzgerald and Gibson 1984). C fibers are not totally incapable of being stimulated since high-intensity electrical stimulation can activate C fibers by birth (Garcia-Arraras et al. 1986; Otsuka and Konishi 1974), at least in superficial laminae (Fitzgerald 1985). Thus it appears that there is an early prenatal maturation of the peripheral receptors and afferents but that the central mechanisms of nociception are delayed and dependent on the type of stimulation and fiber type.

c-Fos Studies

One of the most intriguing developments in cellular neuroscience is the discovery and characterization of immediate early genes, which, as transcriptional factors, translate ligand-induced events to gene-expressed phenotypic changes. The Fos protein is expressed in the dorsal horn of 0- to 1-day-old pups in response to formalin injection to the hindpaw but not to mustard oil application, a finding consistent with the physiological data (Fitzgerald and Gibson 1984; Williams et al. 1990). Recent studies have elaborated on those data (Yi and Barr 1995). Awake 0-, 1-, 2-, 3-, and 14-day-old rat pups were subjects and the hindpaw was immersed in moderately hot water, pinched, or dilute formalin was injected into the plantar pad. On the day of birth, all three stimuli elicited expression of the Fos protein in superficial dorsal horn cells indicating that nociceptive primary afferents are functional at this age. Staining was predominantly in the medial superficial dorsal horn, which is the major termination site of these primary afferents. There was no staining on the side contralateral to the site of stimulation and no staining in control animals.

The developmental course of responding was similar for the thermal and formalin stimuli; there were significant but low numbers of stained nuclei before 3 days of age and a substantial increase in the number of stained cells between 2 and 3 days of age (figure 2). This level was consistent to 14 days. The data for the mechanical stimulus differed. There was an early increase in staining followed by a decline and subsequent rise again at 14 days of age. The early high number of stained nuclei likely represents an increased afferent drive in the youngest animals using the mechanical stimulus. Measured more directly, the expression of the Fos protein was related to the intensity of stimulation: greater injection volumes of formalin or application of the thermal stimulus for a longer duration of time increased the number of stained nuclei (figures 3 and 4).

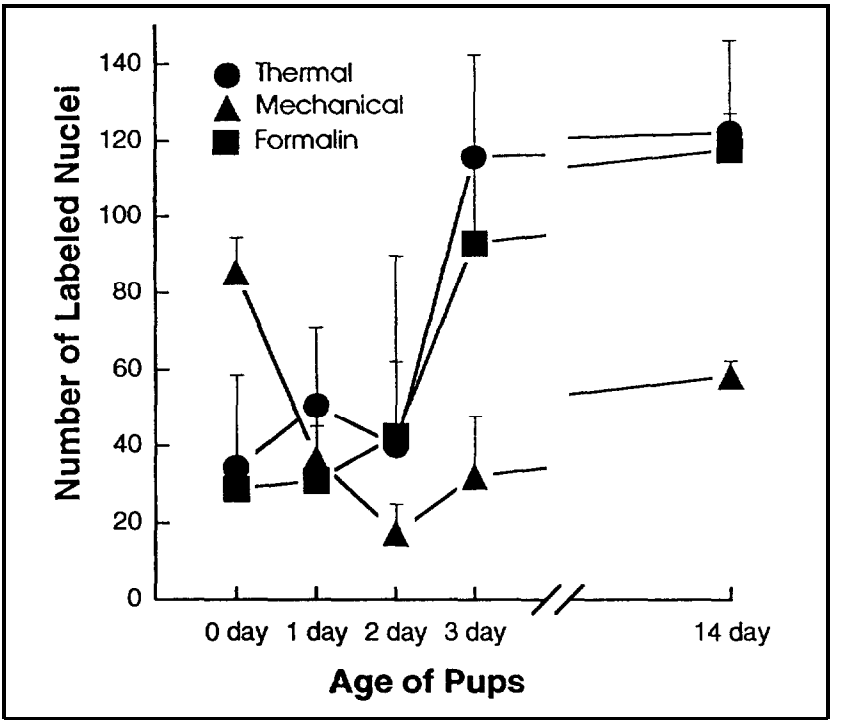

FIGURE 2. *The hindpaws of pups were injected with dilute formalin, pinched, or submerged in moderately hot water. Fos-immunoreactive nuclei were counted in the lumbar dorsal horn of the spinal cord. For both formalin injections and thermal stimulation there was an increase in the number of labeled nuclei from PD2 to PD3. For the pinch, the number of labeled cells showed an inverted U-shaped function from PD0 to PD14.*

SOURCE: Adapted from Yi and Barr 1995.

Less is known about pain processing for other limbs and segmental levels. In preliminary experiments, it was found that the cervical spinal cord neurons express the Fos protein by 24 hours after birth when formalin is injected into the forepaw (Yi and Barr, unpublished results), a finding not surprising since c-fos is expressed by birth in the lumbar cord. The questions of when and how the noxious information reaches the brain has not been addressed. The lateral spinothalamic tract is anatomi-

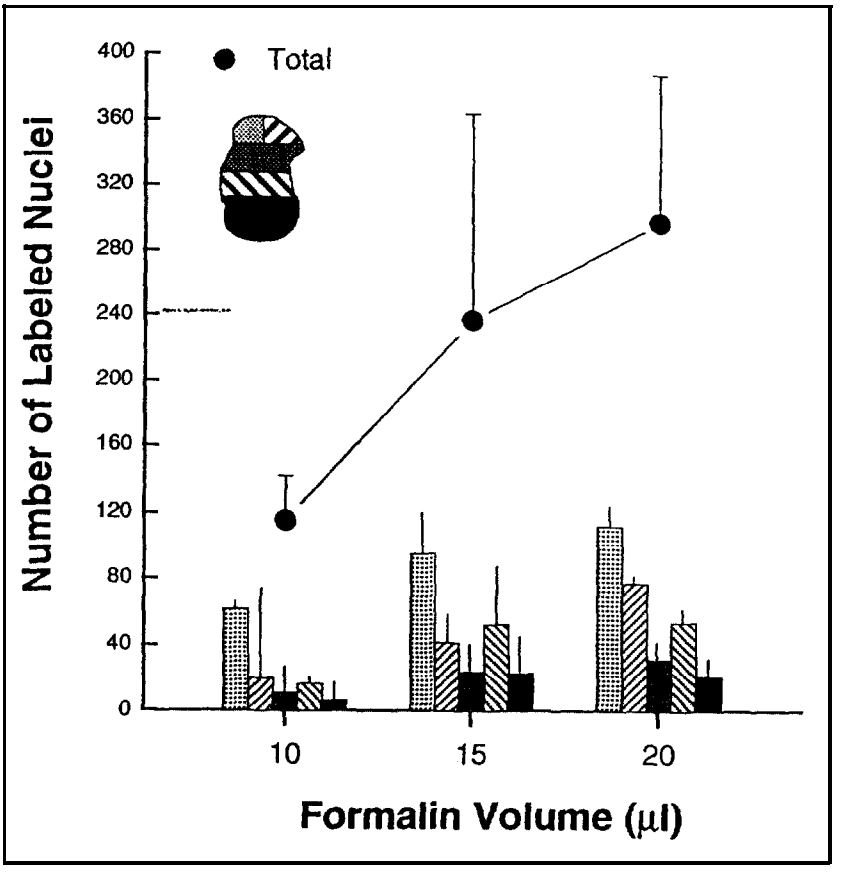

FIGURE 3. *This figure demonstrates that increased noxious stimulation increased the number of stained nuclei in 3-day-old pups. The filled circles represent the number of positive cells in a lumbar spinal cord section, whereas the bars correspond to different levels of the cord as depicted in the upper left comer of the graph.*

SOURCE: Adapted from Yi and Barr 1995.

cally present if immature by fetal day 18; it is not known when it is functional. In the adult, staining in brain has been described following application of noxious stimuli (Anton et al. 1991; Bullitt 1989, 1990; Keay and Bandler 1993; Lee and Beitz 1993). In the infant, formalin injections (15 microliters (µL), 10 percent) into the forepaw or hindpaw

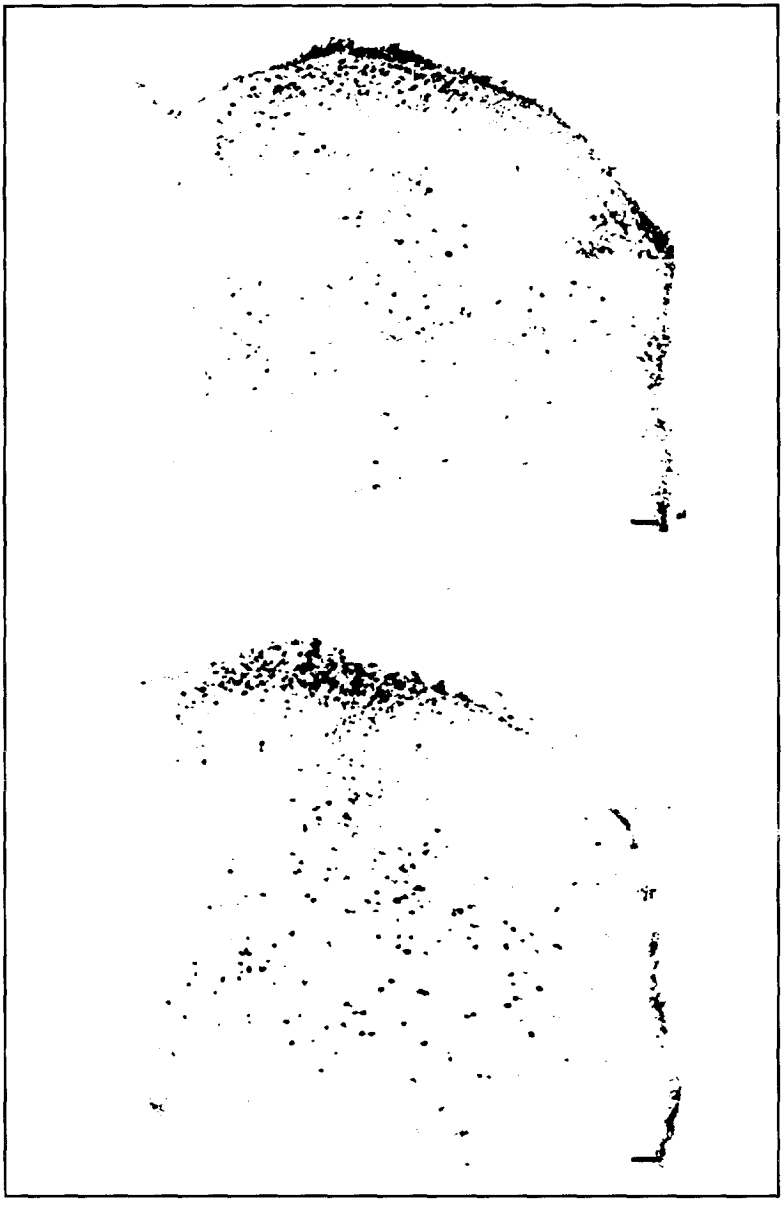

FIGURE 4. *This photomontage demonstrates the increased number and density of staining with increased volume of formalin in the 3-day-old pup. The bar is 50 mm.*

SOURCE: Yi and Barr 1995.

resulted in c-fos expression in several brain regions (Yi and Barr, unpublished data). These include the nucleus raphe magnus and lateral reticular formation, the periaqueductal gray of the midbrain, medial thalamic sites, and subregions of the hypothalamus. Although this work is preliminary, at least for some neural sites the infant rat responds as does the adult to noxious stimulation of the limbs.

The existent data on the ontogeny of nociception are presented in figure 5. Many important issues remain. It has been suggested, based on brain growth curves, that the infant rat is born more altricial than the human infant and that its first postpartum week is roughly equivalent to the third trimester human infant. To the extent that those equivalencies can be made, the maturation of pain perception in rats and humans is comparable and appears to correspond to brain development rather than to time of parturition in each species. This needs to be confirmed. Second, virtually all data are from experiments conducted on the hindlimb and, in the rat, the lumbar segments of the spinal cord. It is not known whether these data are representative of other segmental levels of the spinal cord or of the trigeminal system. These may be more or less responsive at earlier ages.

ANTINOCICEPTION

This section on the development of opiate-induced antinociception presents data on the maturation of analgesia at different levels of the neural axis and elaborates on some critical issues in the study of analgesia in the infant animal. Although this review focuses on opiate-induced antinociception, the discussion of these issues applies to other analgesics as well.

Since the early reports of opiate-induced analgesia in infants first appeared, many studies have demonstrated that opiates can produce analgesia in immature animals and human infants (Auguy-Valette et al. 1978; Kupferberg and Way 1963; Yaster 1987). What has been lacking, however, is any systematic examination of the neural mechanisms that mediate this analgesia and how these mechanisms are similar to or different from analgesia induced by the same drugs in the adult. The exact pattern of the development of analgesia depends on the type and intensity of the noxious stimulus, the somatotopic region stimulated, and the opiate that is given. These are addressed one at a time.

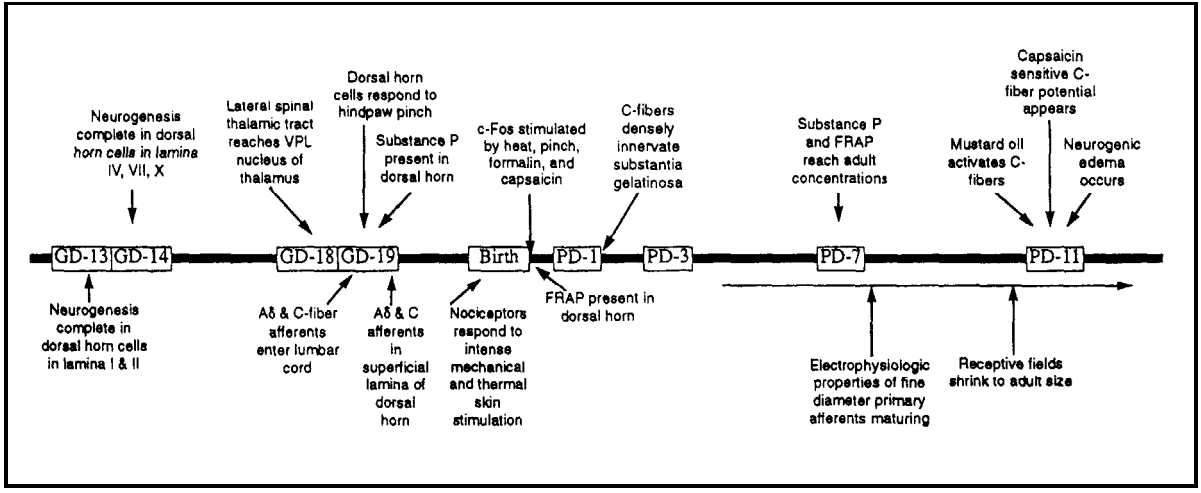

FIGURE 5. *This figure summarizes some of the major developmental landmarks in the processing of noxious stimulation in the infant rat. The events depicted are not an exhaustive list. (References have been cited in the text.)*

Stimulus Factors

To assess the maturation of the mechanisms that subserve analgesia, care must be taken to specify the intensity and type of stimulus, the age of the animal, and the body part tested. Erroneous conclusions have been made when these variables were not taken into consideration. Figures 6 and 7 illustrate these points. When the stimulus is applied to the rostral body parts, analgesia elicited from brain sites occurs earlier than when the stimulus is applied to caudal body parts. As the animal grows up and the neural circuitry responsible for this analgesia matures, this rostral to caudal gradient disappears (figure 6, top). Simultaneously, equal doses of analgesics are less efficacious with more intensely noxious stimuli (figure 6, bottom). The more intense the stimulus, the more widespread activation of the spinal cord second-order neurons in both adult and infant (as noted above with increased c-fos expression with increased formalin volume) and the more analgesic drug required. Taken together this implies that the developmental pattern of analgesia is dependent on the parameters chosen (figure 7). Antinociception appears early in life when a low-intensity stimulus is applied to the forepaw; a high-intensity stimulus applied to the hindpaw or tail results in the late appearance of antinociception.

This theoretical difference in the age of analgesia onset depicted in figure 7 is in actual fact not trivial, and can be as great as 10 days to 2 weeks. This became apparent in a recent study on the neural mechanisms of analgesia in infant rats. When the mu-specific agonist [D-ala^2,N-Me-Phe^4,G1y^5-ol]-enkephalin (DAMGO) was injected into the ventral lateral periaqueductal gray, the inhibition of the withdrawal response was fully dependent on which appendage was tested and the intensity of the stimulus (figure 8). When a low-intensity stimulus, in this example 44 ^{o} C, was applied to the forepaw, analgesia occurs in the infant rat quite robustly at least by 3 days of age, confirming earlier findings (Blass et al. 1993; Giordano and Barr 1987; Kehoe and Blass 1989). If, however, different parameters are chosen and the stimulus intensity is increased and applied to the hindpaw or tail, then analgesia occurs later, also as previously reported (Barr et al. 1992; Giordano and Barr 1987; Pastemak et al. 1980; Zhang and Pastemak 1981). The critical question, therefore, is not ***when*** analgesia develops because "when" is parameter dependent. And although understanding the consequences of varying parameters on the appearance of analgesia is

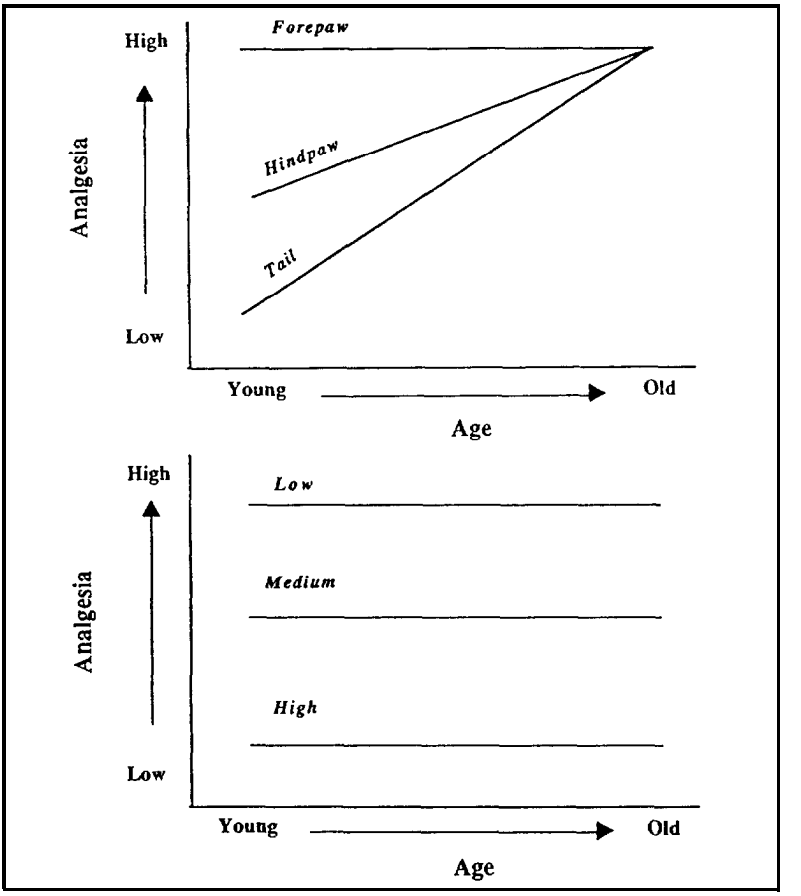

FIGURE 6. ***Top.*** *This schematic diagram demonstrates the pattern of analgesia seen when opiates are injected into the brain. Younger animals are more analgesic in forepaw withdrawal tests than they are in the hindpaw or tail withdrawal tests. Although the hindpaw is depicted as more sensitive than is the tail, these two appendages vary considerably in their susceptibility to opiates. The forepaw, however, is always more responsive in the very young animal. As the animal matures this pattern is less obvious and all three appendages are typically equiresponsive.* ***Bottom.*** *For animals of all ages, analgesics are typically less effective against more intensely noxious stimulation (depicted by the parallel lines).*

SOURCE: Adapted from Barr 1992.

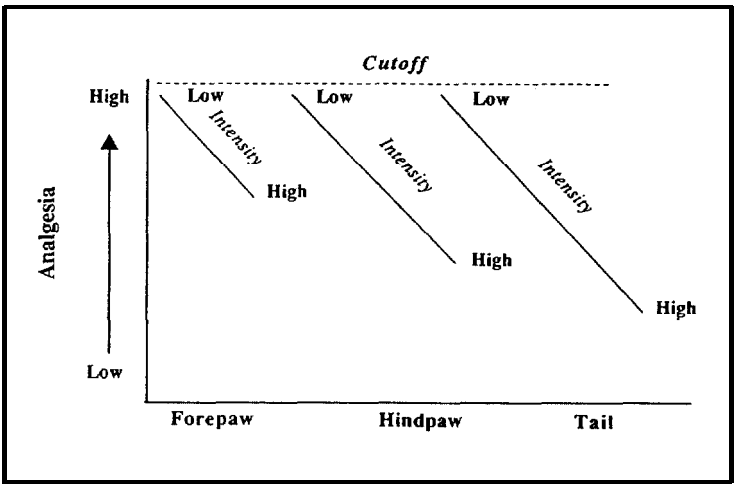

FIGURE 7. *These are hypothetical interactions between stimuli of increasing intensity applied to the three appendages. It is possible to erroneously conclude that analgesia occurred in very young animals if mildly noxious stimuli were applied to the forepaw or that analgesia was late occurring if intensely noxious stimuli were applied to the hindpaw or tail.*

SOURCE: Adapted from Barr 1992.

critical to understanding mechanisms, the important question is *how* the physiological systems become organized to mediate analgesia at different ages.

There are ample data in the adult to suggest that analgesics are differentially effective against different types of noxious stimuli and that different neurotransmitters in the primary afferents may process different types of noxious stimuli (Kuraishi et al. 1983, 1991). There are comparable analgesia data for the infant. Figure 9 depicts the differential development of analgesia following ventral periaqueductal gray stimulation by morphine for both mechanical and thermal noxious stimuli (Tive and Barr 1992). Parameters were adjusted such that baseline response latencies were roughly equated. Analgesia developed quite early, at 3 days of age, to the thermal stimulus but not until 14 days of age when the mechanical stimulus was used.

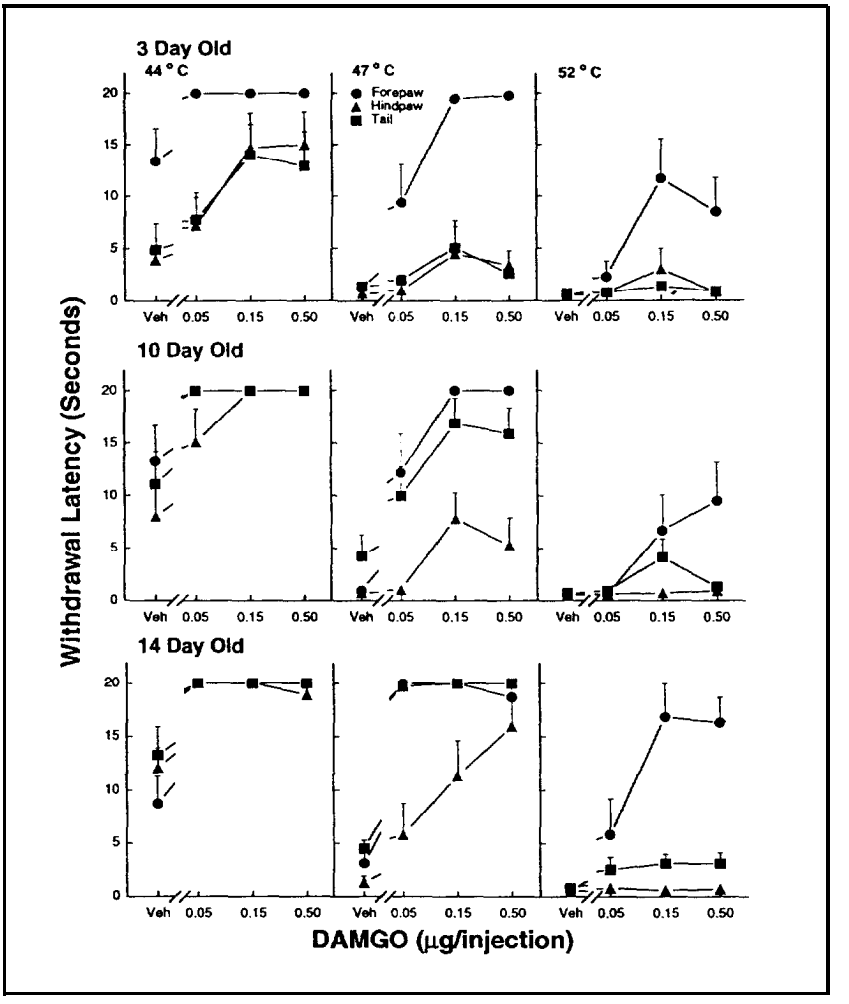

FIGURE 8. *These are data from an experiment in which DAMGO was injected into the ventral aspects of the periaqueductal gray. Pups were tested for their withdrawal response from heated water 10 minutes after injection. The three appendages were tested in a counterbalanced order. When the temperature of the water was 44 °C, analgesia was seen in the forepaw at all doses and in the caudal appendages at the two higher doses. In contrast, analgesia to the forepaw was attenuated at the highest water temperature and never appeared in the hindpaw and tail. These results are in concordance with the model presented in figure 7. In this paradigm the tail withdrawal latency was more affected than was the hindpaw.*

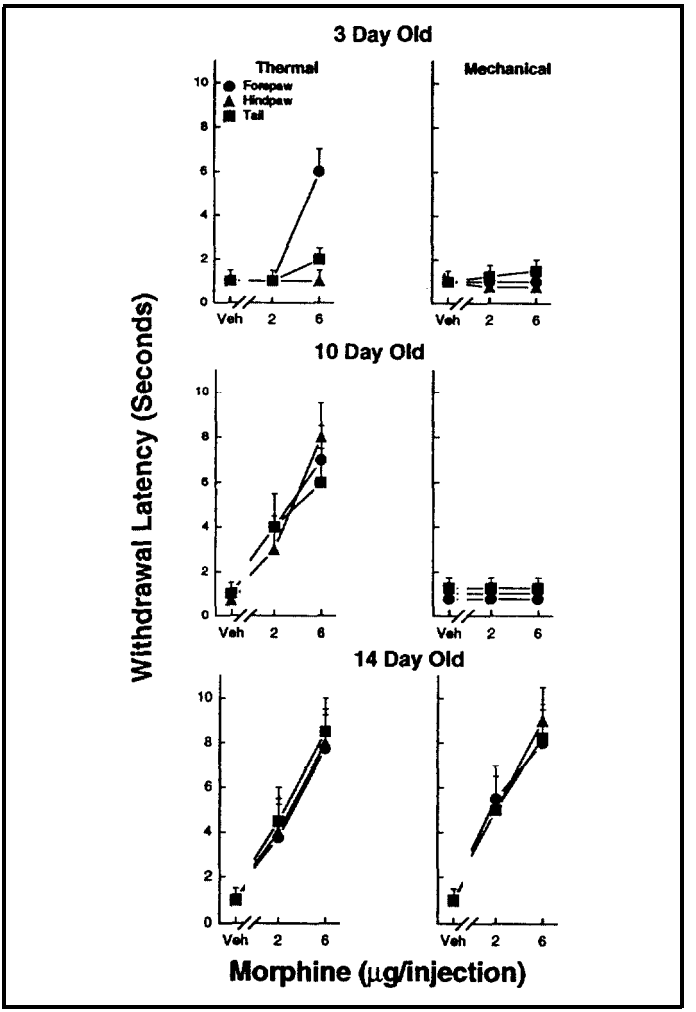

FIGURE 9. *These data depict the results of morphine injection into the ventral periaqueductal gray. A single intensity of noxious stimulation was used for each modality. Withdrawal baselines for the two stimuli did not differ. As can be seen, analgesia appeared when the thermal stimulus was applied at least a week prior to when a mechanical stimulus was used. Opposite results were obtained when glutamate was injected.*

SOURCE: Adapted from Tive and Barr 1992.

Physiological Factors

First, and perhaps most basic, there are pharmacokinetic factors that change during development. Absorption, distribution, and metabolism of drugs differ in the neonate compared to the adult; for example, the blood-brain barrier is more permeable early in life (Auguy-Valette et al. 1978; Kupferberg and Way 1963).

Ligands with different affinities for mu, delta, and kappa opioid receptors can induce analgesia. Each receptor type develops at a different rate in different regions of the brain. Analgesia therefore depends on the specific receptor targeted, the regional maturation of that receptor, and its interactions with other brain systems. In a review of the maturation of opioid receptors, Leslie and Loughlin (1992), demonstrated that each receptor type has its own unique ontogenetic course within a single subregion of the CNS. For example, within the nucleus accumbens, a forebrain structure rich in opioid receptors, the mu opioid receptor is present at high densities at birth and declines in number over the first 2 weeks of life. During this time, kappa opioid receptor numbers are consistently high whereas delta opioid receptors are negligible at birth and appear in increasing density during the entire prepubertal period (figure 10).

There are functional differences in the analgesic effects of opiates that parallel these differences; for example, morphine and ketocyclazocine, two drugs that, although not receptor specific, prefer mu and kappa opioid receptors respectively and differ in their antinociception profile during development. When tested against a thermal stimulus, morphine was more effective in producing analgesia in a forepaw test, whereas ketocyclazocine was more effective in the tailflick test (figure 11; Giordano and Barr 1987).

There are also regional differences in the development of each receptor type. Again, using the review by Leslie and Loughlin (1992) and the example of the mu opioid receptor, the rat pup is born with high densities of this receptor in the matrix of the caudate nucleus, nucleus accumbens, and olfactory tubercle. The receptor numbers in the caudate increase with age, whereas those in the ventral striatum decline. These data are depicted in figure 12. Regional differences in receptor development can result in regional differences in analgesia. To take an obvious example,

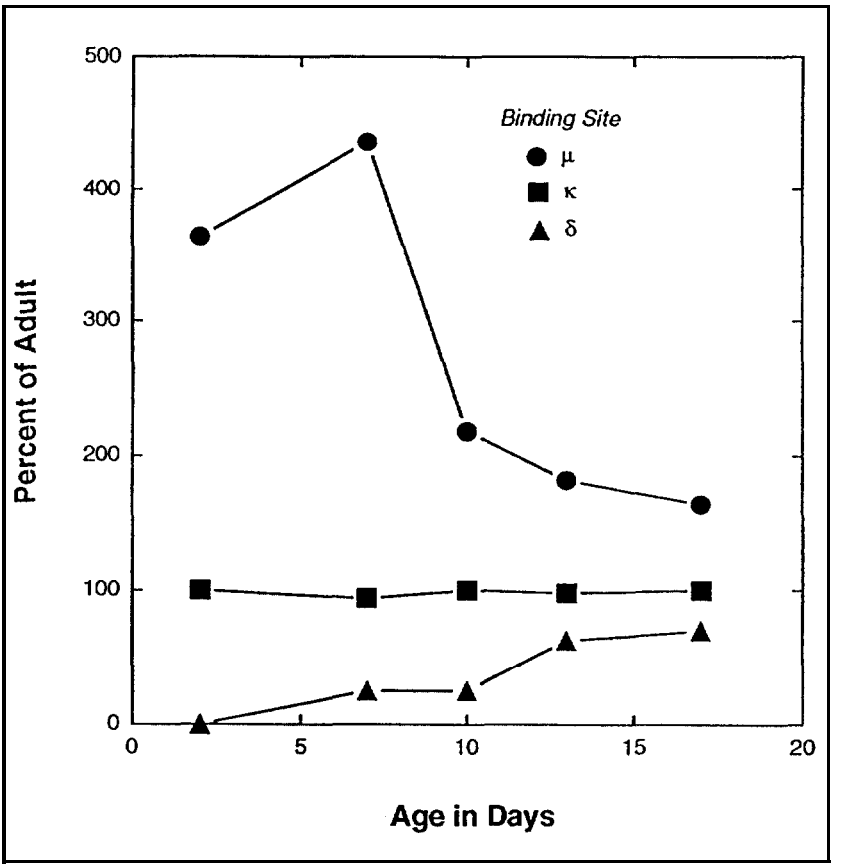

FIGURE 10. *This figure demonstrates that specific opioid receptors develop at different rates. These data are for the nucleus accumbens but equally distinct maturation would be noted in several regions of the CNS.*

SOURCE: Modified from Leslie and Loughlin 1992.

when morphine was administered to the spinal cord there were striking differences in the pattern of analgesia compared to the results when morphine was administered to the lateral ventricle of the brain (figure 13; Barr et al. 1992). Whereas morphine administered intracerebroventricularly (ICV) resulted in the rostral-to-caudal soma-totropic pattern of analgesia described above, analgesia following intrathecal administration induced analgesia in all three appendages at 3 days of age.

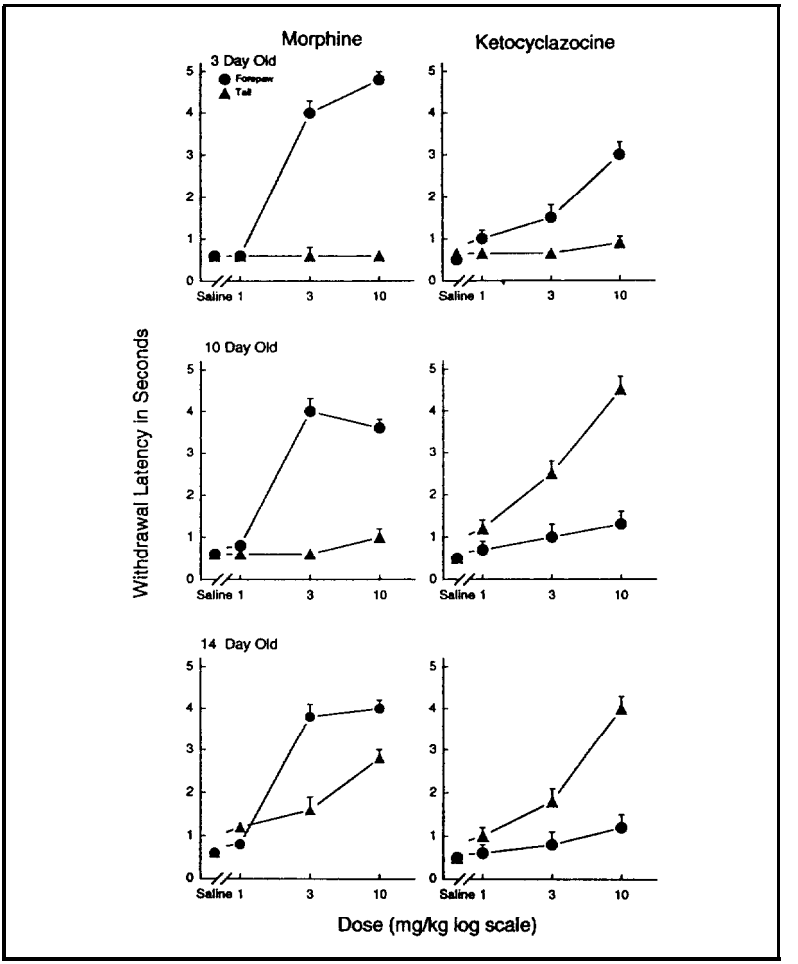

FIGURE 11. *When opiates that prefer different receptor types (morphine: μ; ketocyclazocine: κ) are injected intraperitoneally the resultant analgesic differs. In this case morphine induced analgesia in the thermal forepaw withdrawal test whereas ketocyclazocine was more effective in the thermal tailflick test. These drugs are not specific to these receptors but are representative of the different types of results that can be obtained with different ligands. These differences could be due to a different maturational rate for each receptor, to regional and maturational differences in site of action of the drug, or to both.*

SOURCE: Giordano and Barr 1987.

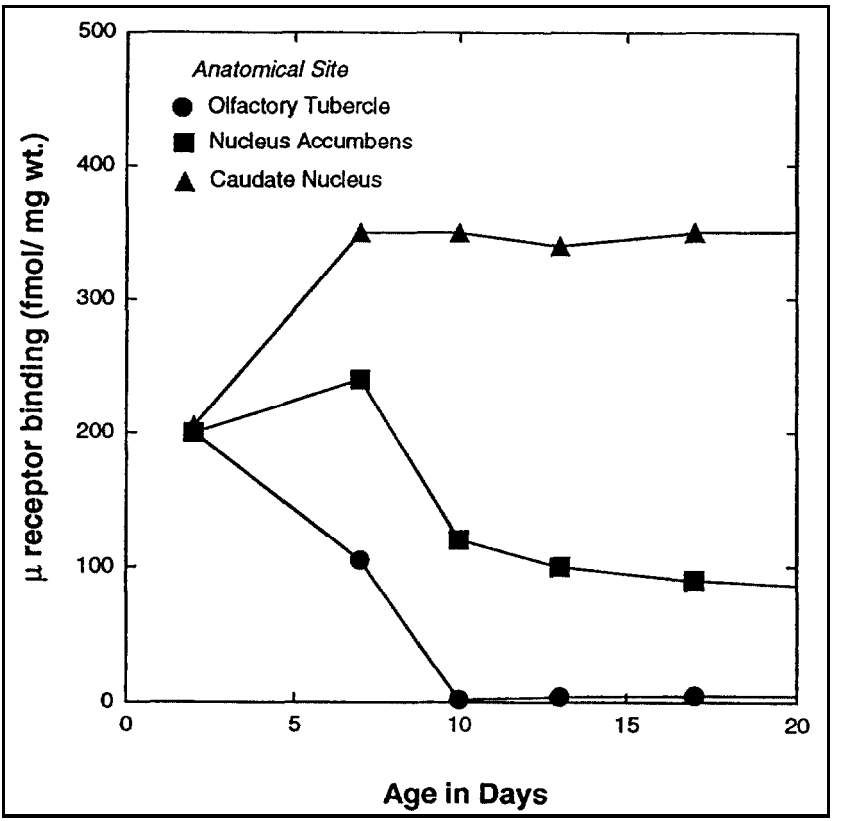

FIGURE 12. *This figure demonstrates that a single opioid receptor can develop at different rates in different regions of the CNS. These data are for the mu opioid receptor but a equally distinct difference could be made for delta or kappa opioid receptors.*

SOURCE: Modified from Leslie and Loughlin 1992.

The importance of these data is that they provide clues as to the mechanisms of analgesia in the maturing animal. These data are schematically represented in the drawing shown in figure 14.

Furthermore, the maturation of a neural system that mediates any function is dependent on the developmental integrity of all of its components. This is true of all developmental functions. Stimulation of the periaqueductal gray produces analgesia in the adult and this analgesia depends on

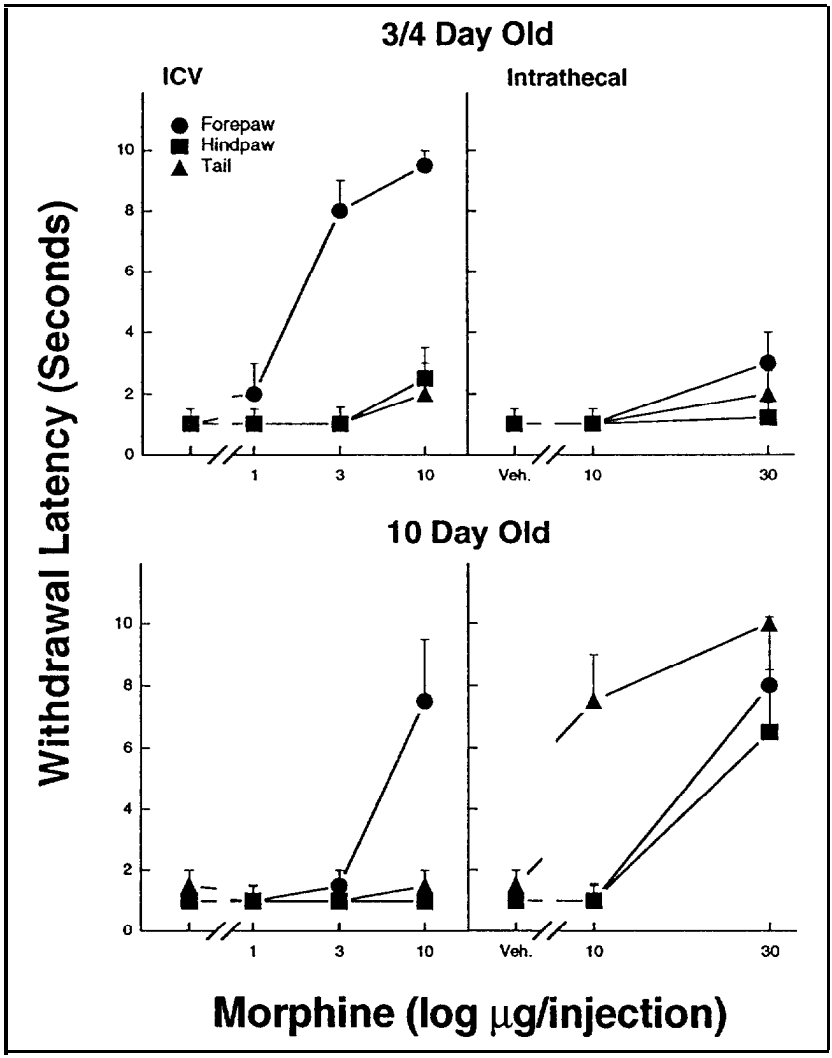

FIGURE 13. *These data show the different pattern of analgesia obtained when morphine was injected into the lateral ventricle or the intrathecal space of the lumbar spinal cord. Analgesia following brain injection followed the rostral-to-caudal pattern of analgesia described in figures 8 and 9. In contrast the pattern of analgesia following spinal injection was quite distinct.*

SOURCE: Barr et al. 1992.

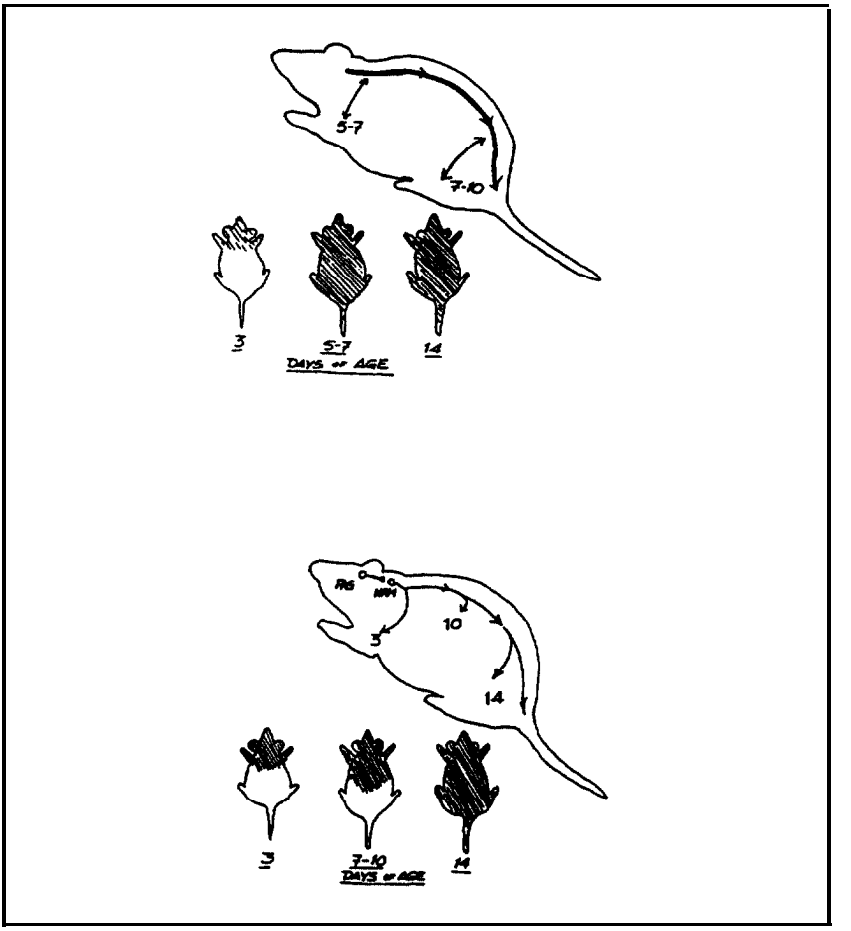

FIGURE 14. *These two drawings depict the interactive effects of different receptor types in different brain regions. An opioid receptor (presumable the mu type) matures within the brain and interacts with descending pathways (e.g., periaqueductal gray to rostral ventral medulla to spinal cord). Analgesia is dependent on development of the opioid receptor and the maturation of the descending paths. Analgesia would develop slowly in a rostral-to-caudal pattern. In contrast, a different opioid receptor (μ, δ, or κ type) might act directly to inhibit the primary afferents or second-order neurons in the dorsal horn of the spinal cord. The development expression of analgesia would depend largely on the development of the receptor site only. Thus the pattern of analgesia might be more constant throughout the body.*

the functional integrity of opioid receptors in the periaqueductal gray, their projections to the rostra1 ventral medulla, and the subsequent projections from the rostral ventral medulla to the dorsal horn (figure 15). If any of those are delayed in developing then analgesia is likewise later maturing. This is the developmental equivalent of the rate-limiting step. The behavioral consequences are clear. As shown above, when DAMGO is injected directly into the periaqueductal gray, analgesia develops in a rostral-to-caudal pattern (figure 9). Analgesia in the forepaw appears prior to that of the hindpaw or tail. This could be due to maturation within the periaqueductal gray of its connections with medullary structures such as the nucleus raphe magnus or one of the more lateral ventral medullary sites (e.g., nucleus gigantocellularis, pars alpha), or of the descending inhibitory pathways from the rostral ventral medulla to the dorsal horn of the spinal cord. To test this, DAMGO was injected directly into the nucleus raphe magnus. The degreee of analgesia that resulted was similar to that following periaqueductal gray stimulation (figure 16); thus the delayed rostral-to-caudal maturation of opiate-induced antinociception is likely due to in part to projections from the periaqueductal gray to the rostral ventral medulla but largely because of the maturation of the descending inhibitory paths from the medulla to the dorsal horn of the lumbar spinal cord. This development occurs during the first 3 weeks of postnatal life. Anatomically these descending pathways develop after birth, including their neurotransmitter content (Bregman 1987; Leong et al. 1984). Transection of the spinal cord does not change spinal reflex activity within the first several weeks of life (Prendergast and Shusterman 1982; Weber and Stelzner 1977) and stimulation of the thoracic dorsal lateral funiculus, which contains these descending fibers, did not inhibit lumbar dorsal horn responding to pinching of the skin before PD 10 to 12 and was not fully effective until 22 to 24 days of age.

It should be clear from this chapter that the neural organization of pain signaling and its modulation by analgesics is complex and that the analysis of these systems in the developing organism requires attention to a number of variables. At the very least, pain modulation is a function of specific receptors and receptor types, neural level of processing, somato-topic organization, type and intensity of stimulus and, in developmental studies, the state of maturation. Without attention to these variables, conclusions about the ontogeny of analgesia are incomplete.

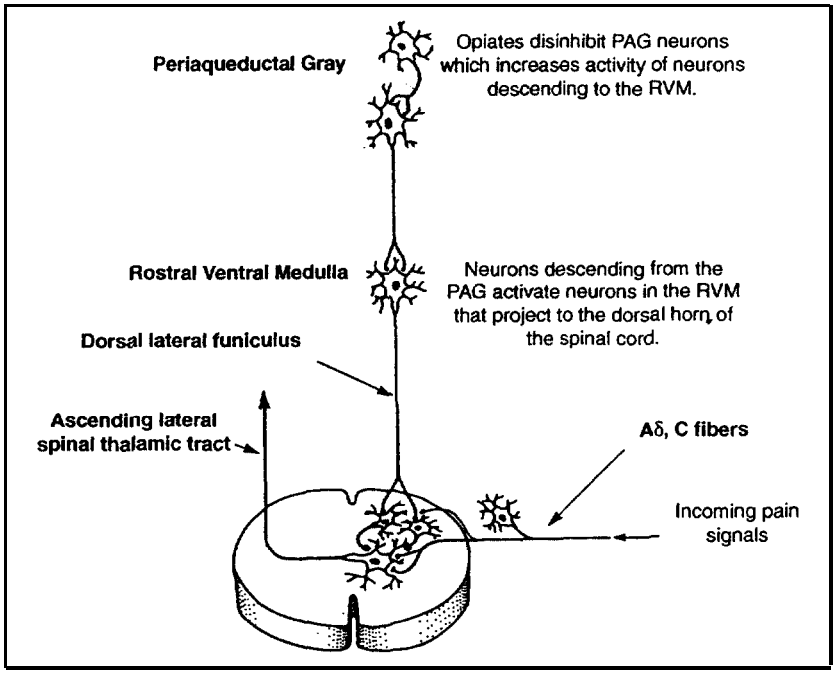

FIGURE 15. *This scheme was described by Basbaum and Fields (1984) to explain putative neurocircuits that might mediate analgesia from supraspinal sites. Although clearly oversimplified given the advances since it was first proposed, this model has been an extremely useful heuristic on which studies done in the adult and infant have been based.*

SOURCE: Adapted from Pinel 1993.

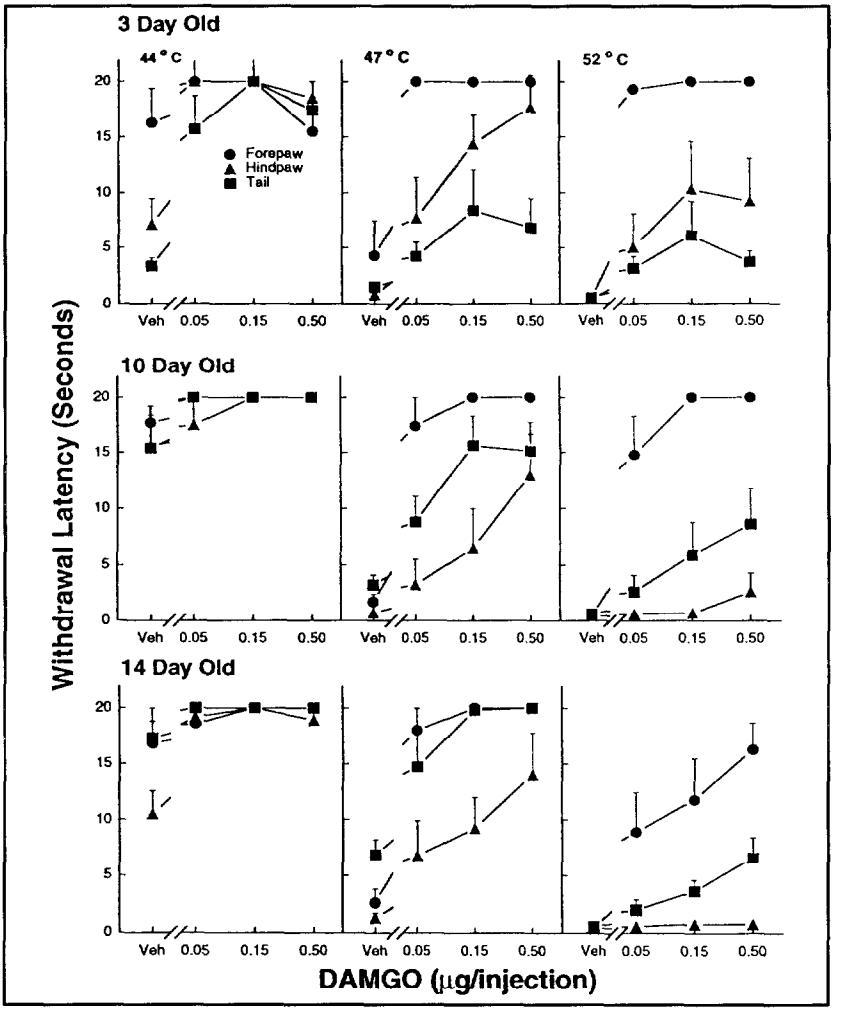

FIGURE 16. *These results are from an experiment in which DAMGO was injected directly into the nucleus raphe magnus. Details are the same as for figure 9. Comparing these data to those in figure 9, it is clear that although there are differences, the rostral-to-caudal pattern of analgesia is still present, suggesting that this pattern is not due to maturation within the periaqueductal gray or of its connections with the raphe nucleus, but rather more likely to development of the descending paths from this nucleus and the surrounding reticular formation.*

ACKNOWLEDGMENTS

This chapter was prepared with support from National Institute on Drug Abuse grant DA-07345. The following people were instrumental in many of the studies described here: James Giordano, Ph.D.; Harry E. Hughes, Ph.D.; Dorene Y. Miya, Ph.D.; William Paredes, B.A.; Leslie A. Tive, Ph.D.; and Duckhyun K. Yi, M.S.

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Perinatal Benzodiazepine Modulation of $GABA_A$ Receptor Function: Influence on Adaptive Responses

Carol K. Kellogg

INTRODUCTION

Prenatal exposure to benzodiazepines (BZDs) such as diazepam (DZ) results in altered neural and behavioral responses to environmental challenges in adult exposed animals (Kellogg 1992). In other words, adult rats exposed in utero to DZ make inappropriate behavioral responses and have altered neural responses to environmental stimuli that threaten the organism's stability and homeostasis. Thus, the early exposure appears to influence adaptive responses. These effects will be discussed later in the chapter, but initially, a possible biologic mechanism that could account for these effects of early DZ exposure will be presented. Understanding the biologic mechanisms whereby developmental exposure to these compounds can influence such functions later in life will assist in understanding the neural organization underlying adaptive (coping) responses. Many forms of illness, including behavioral disorders, are thought to result from altered stress responsiveness (Chrousos and Gold 1992), and information on the neural organization of adaptive responses would assist in understanding the etiology of these disorders. The final organization of the nervous system and the behavioral capacity of an organism involve the interaction during development among three major influences: the genome, the prenatal and postnatal chemical environment, and the external (sensory, maternal, peer) environment (Oppenheim and Haverkamp 1986). Certainly drugs that reach the developing organism become part of that organism's internal chemical environment and, therefore, the possibility that drugs could exert an influence on neural development must be considered.

BENZODIAZEPINES AND THE DEVELOPING BRAIN

DZ and other BZDs are highly lipid-soluble substances that readily reach the fetal compartment following maternal administration (Mandelli et al. 1975). DZ and its active metabolites have been detected in neonatal rat brain following prenatal exposure over gestational days 13 to 20 (Simmons et al. 1983). The BZDs are anxiolytic and anticonvulsant compounds that exert their pharmacologic effects in the adult via modulation of the action of the inhibitory neurotransmitter γ-aminobutric acid (GABA) at the $GABA_A$ receptor (Braestrup and Squires 1978). The BZD site mediating the pharmacologic effects is referred to as the "central-type BZD receptor." Benzodiazepines such as DZ can also interact in the brain at a site referred to as the "peripheral-type BZD receptor," considered to be located mainly on mitochondria (Anholt et al. 1984, 1986). The role of the peripheral-type receptor in brain function is not understood, but action at this site may underlie some of the effects of prenatal exposure to DZ (Kellogg 1992). However, it is more likely that the multiple effects of the early DZ exposure on later adaptive responses are mediated via action of the drug at the $GABA_A$ receptor.

Certain GABA-containing neurons differentiate very early in development (Lauder et al. 1986), making these neurons some of the first transmitter-identified neurons to differentiate. GABA-immunoreactive cells are detectable in the rat cerebral cortex by embryonic day 14 (Cobas et al. 1991), and all presumptive GABA-containing neurons are settled in this region by birth (fetal day 21-22) in the rat (Balcar and Johnston 1987). Immunoreactivity for a subunit of the $GABA_A$ receptor was found to appear in the developing cerebral cortex slightly earlier than staining for GABA itself (Cobas et al. 1991). Consistent with the early appearance of GABA receptors, GABAergic signals appear with the expression of GABAergic cells (Fiszman et al. 1993). However, since marked synaptogenesis in rat cerebral cortex does not take place until the second postnatal week (Balcar and Johnston 1987), the role of early-appearing GABA neurons and receptors is unclear.

Central-type BZD binding sites have been identified in developing rat brain by the third week of gestation (Braestrup and Nielsen 1978) and in human fetal brain at 12 weeks conceptual age (Brooksbank et al. 1982). The modulatory effect of BZDs on $GABA_A$ receptor function as well as other allosteric interactions around the $GABA_A$ receptor are present in rat brain from early developmental stages. For example, DZ facilitates GABA-mediated chloride uptake in synaptoneurosomal preparations of

rat forebrain by gestational day 20 (Kellogg and Pleger 1989), and GABA modulates BZD binding in rat brain as early as day 16 of gestation (Mallorga et al. 1980).

GABA has been proposed to have a trophic role in the developing brain (Wolff et al. 1993). Therefore, modulation of GABA responses at the $GABA_A$ receptor could have lasting consequences by altering neural organization in early development. Electrophysiologic measurements carried out on fetal mouse cerebral cortex cultures indicated the presence of tonic inhibition (Crain and Bornstein 1974). GABA antagonists reversed the inhibition, implying that GABA receptors mediated the inhibition. More recent work has shown that exposure of fetal rat neuronal cultures to DZ decreases the uptake of 2-deoxyglucose, indicating that DZ decreased neural activity, presumably via enhancement of the action of GABA (Daval et al. 1988). Other studies, however, have demonstrated that GABA can act as an excitatory transmitter during early development (Cherubini et al. 1991).

Whether GABA is excitatory or inhibitory on the developing brain, the presence of DZ or other BZDs in the fetal chemical environment could, via modulation of the action of GABA, alter ionic currents in the developing brain. By modulating ionic currents, developmental exposure to BZDs could, via indirect trophic mechanisms, have a widespread influence on activity-directed developmental events (Spitzer 199 1). GABA has also been proposed to exert direct trophic effects on the nervous system (Wolff et al. 1993), and BZDs could alter these effects.

The actual effect of developmental exposure to any drug will depend upon several factors: (1) the presence and distribution of specific recognition sites for the drug, (2) the maturational state of effector systems that translate the interaction of a drug with its binding site into a response, (3) the maturational state of the receptive cell, and (4) the maturational state of specific neural circuitry. While many questions remain unanswered, the hypothesis will be presented that the final consequence of early BZD exposure could be altered neural organization of specific behaviors. Since adaptive behaviors are influenced by early DZ exposure, this hypothesis presupposes that the $GABA_A$ receptor may play a role in the neural organization underlying such responses.

NEURAL INHIBITION AND ADAPTIVE BEHAVIOR

Environmental challenges (stressors) can place physical, social, and/or psychological demands on organisms. These demands elicit adaptive responses that serve to help the organism meet the demands and maintain normalcy and thereby survive (Selye 1973). The most widely studied adaptive responses have been hormonal and sympathetic responses to stressors (Chrousos and Gold 1992). According to Selye's stress concept, these are nonspecific responses that organisms make to a variety of stressors; albeit, it is now understood that there is some specificity even in these responses. But the demands of stressors also elicit critically important behavioral adaptive responses. The decision to flee, freeze, or fight, for example, contributes significantly to survival potential. Behavioral adaptive responses can be specific for a given stressor and can be modified based upon experience. Thus, organisms must possess neural mechanisms that aid them in selecting the most optimally adaptive behavioral response to meet the challenge.

While the neural mechanisms that influence the hormonal and sympathetic adaptive responses have been widely studied (Axelrod and Reisine 1984), much less is understood about the neural systems that mediate adaptive behaviors. However, there has been an emerging interest in the role of inhibitory systems in choice behaviors (Levine and Leven 1991). Roberts (1991) has suggested that inhibitory projection and local-circuit neurons (particularly phasic inhibitory interneurons) play crucial roles in information processing in the nervous system. Appropriate firing of inhibitory neurons prevents total synchrony of neural output, thereby preventing either paroxysmal discharge or total neural inactivity. Disinhibition, rather than active inhibition of excitatory cells, appears to be the key to regulating output. According to Roberts' hypothesis (Roberts 1991), increased activity in phasic inhibitory interneurons in response to increased environmental demands would lead to disinhibition of tonically tiring inhibitory neurons, which then would enhance neural desynchronization, thereby permitting variability in behavior. Whether inhibitory interneurons are activated by enhanced activity in cortical excitatory cells depends on the temporal and spatial properties of local circuits in the cortex (Thomson and Deuchars 1994). However, computer simulation of simple behavior patterns has indeed demonstrated that mutual inhibition among command systems directing incompatible behaviors can provide a mechanism that produces adaptive behavior (Edwards 199 1).

As mentioned earlier, the major inhibitory transmitter in mammalian brains is GABA (Roberts 1986). This substance has been postulated to be the transmitter at 25 percent of all synapses in the cerebral cortex (Hendry et al. 1987) and has been identified in a variety of types of synapses (Ribak and Roberts 1990). The receptor system that mediates rapid timescale inhibition by GABA is the $GABA_A$ receptor, a multimeric complex that is the site of action for a variety of endogenous and exogenous compounds, including BZDs (Burt and Kamatchi 1991). While this receptor complex is thought to be composed of 4 to 5 peptide subunits that form a chloride channel, 16 to 18 subunits have been cloned that conceivably could be used in constructing a receptor. The pharmacologic and functional nature of $GABA_A$ receptors is influenced by the particular composition of subunits (Mohler et al. 1992; Sigel et al. 1990; Verdoom et al. 1990). In situ hybridization analysis of the distribution of messenger ribonucleic acid (mRNA) for various $GABA_A$ receptor subunits suggests that the $GABA_A$ receptor can vary from cell to cell as well as region to region (Persohn et al. 1992). While the expression of various subunits has been determined in developing rat brain (Bovolin et al. 1992; Killisch et al. 1991; MacLennan et al. 1991), the particular composition of $GABA_A$ receptors in any region during fetal development is not understood. The nature of the complex in specific brain regions during fetal development may determine the effect of fetal exposure to compounds that act at this receptor.

$GABA_A$ RECEPTOR RESPONSE TO ENVIRONMENTAL CHALLENGE

Clearly, if GABA, acting via the $GABA_A$ receptor, is crucial in maintaining creativity in neural circuits and permitting flexible and appropriate behavioral responses, then changes in GABA function should be detected in response to environmental demands. Several studies have demonstrated changes in adult animals in binding to specific recognition sites on the $GABA_A$ receptor complex in response to a variety of challenges (Lippa et al. 1978; Medina et al. 1983; Miller et al. 1987; Soubrie et al. 1980; Weizman et al. 1989); however, the direction of changes in binding depends upon the type of stressor and on the degree of experience an animal has had with the challenge. A number of studies have now demonstrated that function of the $GABA_A$ receptor is altered by environmental challenges and that the changes in function occur very rapidly in response to an acute challenge, within a few seconds. An acute episode of forced swimming enhanced t-butylbicyclophosphorothionate

(TBPS) binding (a marker of chloride channel function) in the cerebral cortex and enhanced chloride-facilitated BZD binding (an index of the coupling between the chloride channel and BZD binding site [Havoundjian et al. 1986, 1987; Trullas et al. 1987]). The same challenge also increased GABA-stimulated chloride uptake (a measure of the effector response to GABA) in the cerebral cortex (Schwartz et al. 1987).

Forced swimming also changed $GABA_A$ receptor function in the cerebellum and hippocampus, whereas the psychologic challenge of cohort removal order affected receptor function in the cerebral cortex and hippocampus but not the cerebellum (Trullas et al. 1987). Social conflict reportedly enhanced levels of mRNA encoding a, and γ_2 receptor subunits in mouse cerebral cortex but not in the hippocampus or cerebellum (Kang et al. 1991). The direction of all these changes in $GABA_A$ receptor function and expression is consistent with Roberts' suggestion that environmental challenges should increase inhibitory tone (Roberts 1991). However, the increase in TBPS binding observed following footshock (Concas et al. 1988) was associated with a decreased latency to isoniazid-induced seizures and thus interpreted to indicate a stressor-related decrease in $GABA_A$ receptor function (Serra et al. 1991). These studies have all demonstrated that acute environmental challenges induce changes in $GABA_A$ receptor function, even though there is some disagreement over the functional implications of the changes observed.

If the $GABA_A$ receptor is involved in the neural processing of environmental information that is crucial to behavioral selection, then differences in $GABA_A$ receptor function should correlate with specific behavioral response to a given stimulus. None of the above-mentioned challenges (environmental restraint, forced swimming, nor footshock) could be met with variety in behavioral response. Primus and Kellogg (1991*a*), however, demonstrated selective changes in function of the cortical $GABA_A$ receptor complex in adult rats that were related to the nature of the environment in which a male rat encounters another male rat. In the social interaction test, the amount of social interaction measured in pairs of male rats (strangers to each other) changes as a function of their familiarity with a neutral environment, such that social interaction is greater in a familiar than in an unfamiliar environment (File 1980). This environmental-related behavioral response is illustrated in figure 1 (60 days old). Analysis of $GABA_A$ receptor function following behavioral testing of young adult rats indicated that the sensitivity of the receptor complex in the cerebral cortex to GABA (as indicated by the

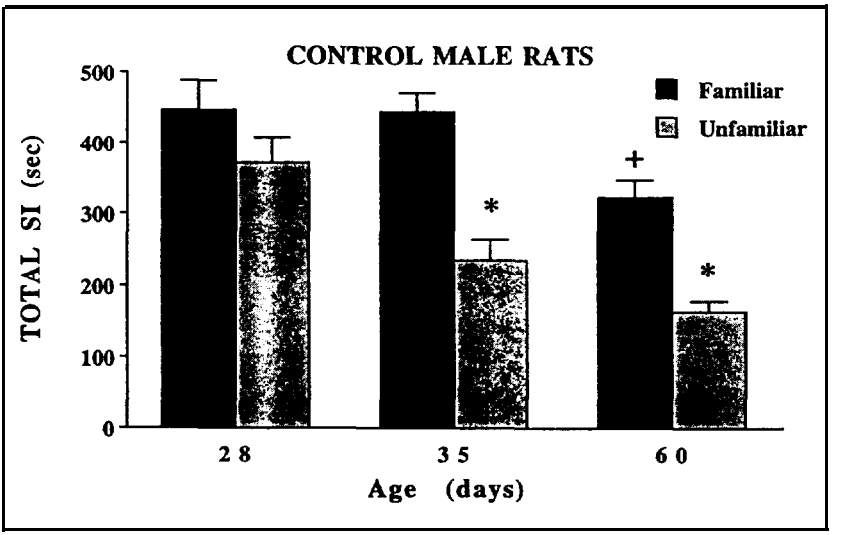

FIGURE 1. *Total social interaction (in seconds) between male rats as a function of experience with the testing environment and adolescent age. All rats were prehandled daily for 5 days before testing. On the 2 days prior to testing, rats to be tested in the familiar environment were transported to the test room and individually exposed to the test chamber. Animals to be tested in an unfamiliar environment were transported to the test room but remained in their home cage. On the test day, two male rats (strangers to each other) were placed in the chamber together for 7.5 minutes. Animals were tested at 28 days (juvenile), 35 days (early adolescent), or 60 days (young adults) of age.*

KEY: * = significant difference between environments at respective age; + = a significant difference from 28 and 35 days, familiar environment.

SOURCE: Primus and Kellogg 1989.

effective concentration that elicits 50% of maximal response (EC,) for GABA-stimulated chloride uptake) was enhanced when the animal encountered a stranger in either the familiar or unfamiliar environment (table 1). However, there were marked and specific effects of the

TABLE 1. *Effect of testing environment on GABA-stimulated ^{36}chloride uptake in adult rat cortical synaptoneurosomes.*

	Handled Control	Familiar	Unfamiliar
EC_{50}(μM)	12.02±0.64	*9.52±1.15	*9.23±0.58
(nM/mg protein) Maximal uptake	29.65±1.16	33.46±4.04	35.19±1.73

KEY: * = Significantly different from handled control, $p < 0.05$.

SOURCE: Primus and Kellogg 1991*a*.

different environments on chloride-facilitated BZD binding (table 2), implying different demands on the organism from the different environments (Primus and Kellogg 1991*a*). The direction of all changes is consistent with increased inhibitory tone in the cerebral cortex in response to increasing environmental challenge. These results suggest that the neural processing associated with specific behavioral responses to an environmental challenge includes selective changes in $GABA_A$ receptor function. Because these studies were carried out on homogenized tissue, it is not clear whether or not the differential responses represent changes in specific populations of receptors in the cerebral cortex.

SPECIFIC ADAPTIVE BEHAVIORS AND CORTICAL $GABA_A$ RECEPTOR RESPONSIVENESS EMERGE OVER ADOLESCENTDEVELOPMENT

The $GABA_A$ receptor, the site of action of DZ and other BZDs, then appears to be a participant in an organism's response to environmental challenge. Thus a biologic mechanism underlying the effects of early DZ exposure on neural and behavioral responses to challenges begins to emerge. But to support an action at this site by early developmental exposure to DZ as responsible for the later effects on adaptive responses, the developmental profile of adaptive behaviors and $GABA_A$ receptor responsiveness must be understood. Evaluation of environment-related

TABLE 2. *Effect of testing environment on characteristics of benzodiazepine binding in adult rat cerebral cortex.*

	Handled Control	Familiar	Unfamiliar
Saturation binding*			
K_d(nM)	3.2±0.2	2.5±0.3 †	2.0±0.06 ‡†
B_{max}(fmol/mg protein)	773.0±61.0	688.0±102.0	913.0±65.0‡
Chloride facilitation §			
% Maximal enhancement	16.9±3.2‡	13.4±0.8	16.0±0.9‡
EC_{50}(mM)	44.9±12.1	26.4±3.3†	12.4±2.1‡†

KEY: * = Using [^{3}H]flunitrazepam, at 0 mM NaCl; † = Significantly different from handled control; ‡ = Significantly different from familiar environment; § = At 1 nM [^{3}H]flunitrazepam.

SOURCE: Primus and Kellogg 1991*a*.

social interaction showed that the novel environment had no effect on the animals' behavior until after the onset of puberty (Primus and Kellogg 1989; figure 1), even though exposure to a novel environment elicited hormonal adaptive responses in juvenile animals (Guillet and Kellogg 1988). The emergence of environment-specific social interaction was observed to depend upon the organizational influence of gonadal androgens during adolescent development, whereas the maintenance of appropriate social interaction behavior in the adult was not dependent upon the presence of androgens (Primus and Kellogg 1990*a*). The timely appearance of adaptive behaviors and related neural systems in parallel with reproductive behaviors could be viewed as contributing to the eventual reproductive success of the organism.

Decreased social interaction in the novel environment is considered to be a response to an anxiogenic stimulus (File 1980). Accordingly, acute treatment with anxiolytic drugs increases social interaction of adult male rats tested in a novel environment. Acute treatment with DZ, however, did not modulate the behavioral responsiveness to the novel environment

in early adolescent male rats, as it did in young adult males (figure 2). Furthermore, gonadal hormones influenced the age of appearance of the anxiolytic effect of DZ (Primus and Kellogg 1990*b*). Since DZ added in vitro to cortical synaptoneurosomal preparations modulates GABA-mediated chloride uptake from late gestation (Kellogg and Pleger 1989), the failure of DZ to modulate environment-specific social interaction behavior before late adolescence/young adulthood would not seem to be due to failure of the drug to act at the molecular level.

Consistent with the influence of adolescence on environment-related social behavior, responsiveness of the $GABA_A$ receptor in the cerebral

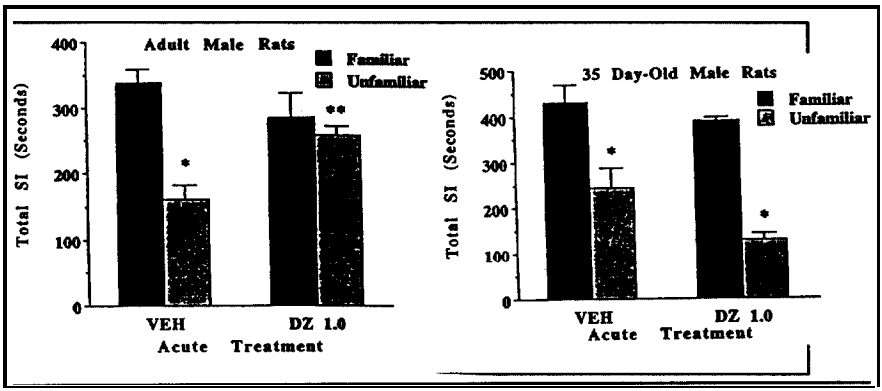

FIGURE 2. *Effect of acute exposure to DZ (1.0 mg/kg) on environment-related social interaction between male rats as a function of adolescent age. Social interaction was measured as described in the legend to figure 1. During the 5 days prior to testing, each rat received daily injections of either DZ or saline-Tween 80 vehicle. This procedure produces tolerance to any sedative effects of DZ without interfering with the anxiolytic effects.*

KEY: * = a significant difference between environments for respective treatment group; ** = a significant difference from vehicle-treated rats, unfamiliar environment. An anxiolytic effect of DZ is defined as a significant effect on interaction in the unfamiliar environment with no effect on interaction in the familiar environment.

SOURCE: Primus and Kellogg 1990*b*.

cortex to environmental challenge was also observed to emerge over adolescent development. Forced swimming enhanced maximal GABA-stimulated chloride uptake relative to basal levels in control young adult rats but not in 35-day-old (early adolescent) rats (Kellogg et al. 1993; figure 3). Overall, the maximal response to GABA decreased from 35 to 60 days in control rats. The influence of adolescent development on $GABA_A$ receptor responsiveness was more clearly observed by evaluating chloride facilitation of BZD binding. Whereas chloride facilitation of BZD binding was selectively altered in young (60-day-old) adult rats by familiar versus unfamiliar environments, there was no impact of either of the environments on this measure in late juvenile rats (Primus and Kellogg 1991*b;* figure 4). Furthermore, castration of juvenile male rats altered the environment-related response profile of the $GABA_A$ receptor in the animals as adults (figure 4). Gonadal steroids, therefore, seem to exert an influence on the maturation of receptor as well as behavioral responsiveness. This developmental change in responsiveness of the $GABA_A$ receptor in the cerebral cortex to environmental stressors could reflect changes in the nature of the cortical receptors or it could reflect recruitment of neural circuitry that includes the cortical $GABA_A$ receptor. The latter explanation would also account for the lack of an anxiolytic effect of DZ before late adolescence.

If the $GABA_A$ receptor complex, particularly in higher brain structures, plays an important role in information processing that underlies adaptive choice behavior, then a delay in the stressor-induced responsiveness of this system until late adolescence makes certain intuitive sense. Organisms are programmed for two functions: survival and reproduction.

Young mammals are generally cared for by a single parent who often needs to leave them alone to forage for food. And the young should remain where the parent leaves them. Variability in behavior in the young would not serve their survival. Indeed, just as a parallel appearance of reproductive and adaptive behaviors might serve reproductive success, the delayed appearance of variability in adaptive behavior until maturity might aid survival to an age of reproductive maturity.

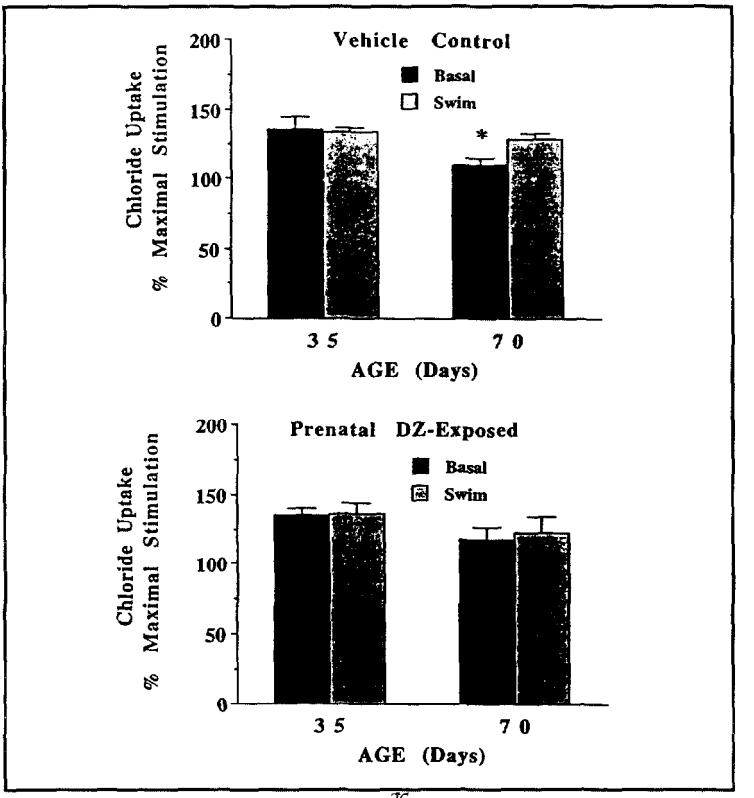

FIGURE 3. *GABA-stimulated ^{36}choloride uptake in cortical synaptoneurosomes from male rats as a function of adolescent age and environmental challenge. Uptake was measured across 10 concentrations of GABA (1-1,000 µM) and is presented as percent maximal stimulation. All animals had been exposed in utero to DZ (2.5 mg/kg to pregnant dam) or vehicle (40 percent propylene glycol and 10 percent ethanol) over gestational days 14 through 20. Challenged rats were subjected to forced swimming for 10 min at a water temperature of 23 to 25 °C. All animals were prehandled daily for 5 days prior to testing.*

KEY: * = a significant difference between basal and swim-challenged rats at respective age.

SOURCE: Kellogg et al. 1993.

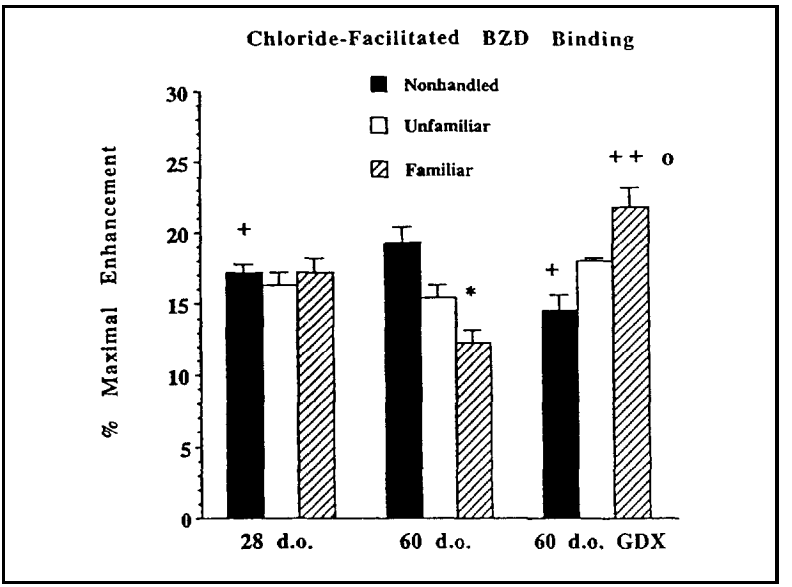

FIGURE 4. *Chloride-facilitated BZD binding to rat cortical membrane preparations as a function of adolescent age, gonadal status, and environmental challenge. Chloride facilitation of [^{3}H] flunitrazepam binding at 1 nanomolar (nM) was determined over six concentrations of Cl-(12.5-500 millimolars (mM)). Nonspecific binding was determined using 10 µM clonazepam. The data are presented as percent maximal enhancement by Cl-. Challenged rats were tested for social interaction as described in the legend to figure 1. Control animals were previously unmanipulated. Gonadally intact male rats were tested at 28 (juvenile) and 60 (young adult) days of age (d.o.). Male rats castrated at 19 days of age were tested at 60 days (60 d.o. GDX).*

KEY: * = significant difference from nonhandled and unfamiliar 60 d.o. animals; + = significant difference from 60 d.o. nonhandled animals; ++ = significant difference from 60 d.o. familiar animals; o indicates significant difference from 60 d.o. GDX nonhandled and unfamiliar animals.

SOURCE: Primus and Kellogg 1991*b*.

EARLY DEVELOPMENTAL FACTORS INFLUENCE THE ADOLESCENT APPEARANCE OF ADAPTIVE BEHAVIORS AND NEURAL RESPONSES

While it is clear that reproductive behaviors mature over adolescence along with the maturation of gonadal function, it is also clear that the adolescent maturation of these behaviors is influenced by earlier developmental effects of fetal gonadal hormones on neural organization (Arnold and Breedlove 1985). Similarly, adaptive responses that appear during adolescence and that may be linked to $GABA_A$ receptor responsiveness are also influenced by perinatal actions of drugs that act at the $GABA_A$ receptor. For example, in the test of environment-related social interaction, adult male rats exposed in utero to DZ showed increased interactions in the novel environment and decreased interactions in the familiar environment, resulting in no environment-related difference in social interaction (Kellogg et al. 1991; figure 5). These results suggest that the prenatally exposed animals suffered from impaired processing of information concerning their environment. Reproductive functions appear to be intact in these animals, so the impairment in their adaptive behavior would not appear to relate to inadequate gonadal function during adolescence. Rather, the impact on adaptive behavior could be related to an effect of the early drug exposure on a neural target of the gonadal hormones.

Early exposure to DZ also interfered with the adolescent development of cortical $GABA_A$ receptor responsiveness to stressors, as indicated by the failure of forced swimming to alter GABA-mediated chloride uptake in prenatally exposed adult male rats (Kellogg et al. 1993; figure 3). In control rats, the maturation of the stressor responsiveness of the $GABA_A$ receptor seemed to relate to a decrease in the maximal response to GABA under basal conditions, which occurred over 35 to 60 days of age; the decrease was less pronounced in prenatally exposed animals. Prenatal exposure of mice to lorazepam has also been reported to alter muscimol-stimulated chloride uptake in 42-day-old animals (Chesley et al. 1991).

Furthermore, other neural systems that are responsive to environmental challenges are also affected by early DZ exposure. The noradrenergic projection to the hypothalamus is activated by a variety of stressors (Ceccatelli et al. 1989; Gaillet et al. 1991; Tilders and Berkenbosch 1986), and acute BZD exposure attenuates the response in this projection system (Corrodi et al. 1971). This system is markedly affected by

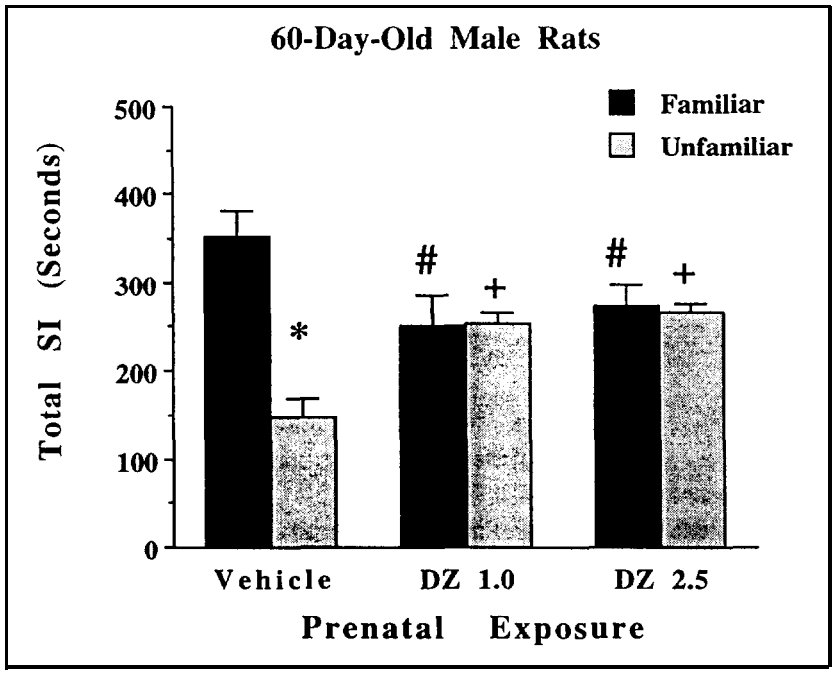

FIGURE 5. *Total social interaction (seconds) in young adult male rats as a function of environmental challenge and prenatal DZ exposure. Animals were exposed in utero to DZ (1.0 or 2.5 mg/kg to pregnant dam) or vehicle (40 percent propylene glycol, 10 percent ethanol) over gestational days 14 through 20. Testing for social interaction was as described in the legend to figure 1.*

KEY: * = a significant difference between environments for respective treatment group; # = significant difference from familiar environment, vehicle group; + = significant difference from unfamiliar environment, vehicle group.

SOURCE: Kellogg et al. 1991.

prenatal DZ exposure as indicated by the dose-related effect on norepinephrine (NE) levels and on the depolarized release of NE in the hypothalamus (Kellogg and Retell 1986; Simmons et al. 1984*a;* figure 6). Early DZ exposure also altered the stressor-induced response of the hypothalamic noradrenergic projections and altered hormonal responses to stressors (Simmons et al. 1984*b).* The effects of early DZ exposure on

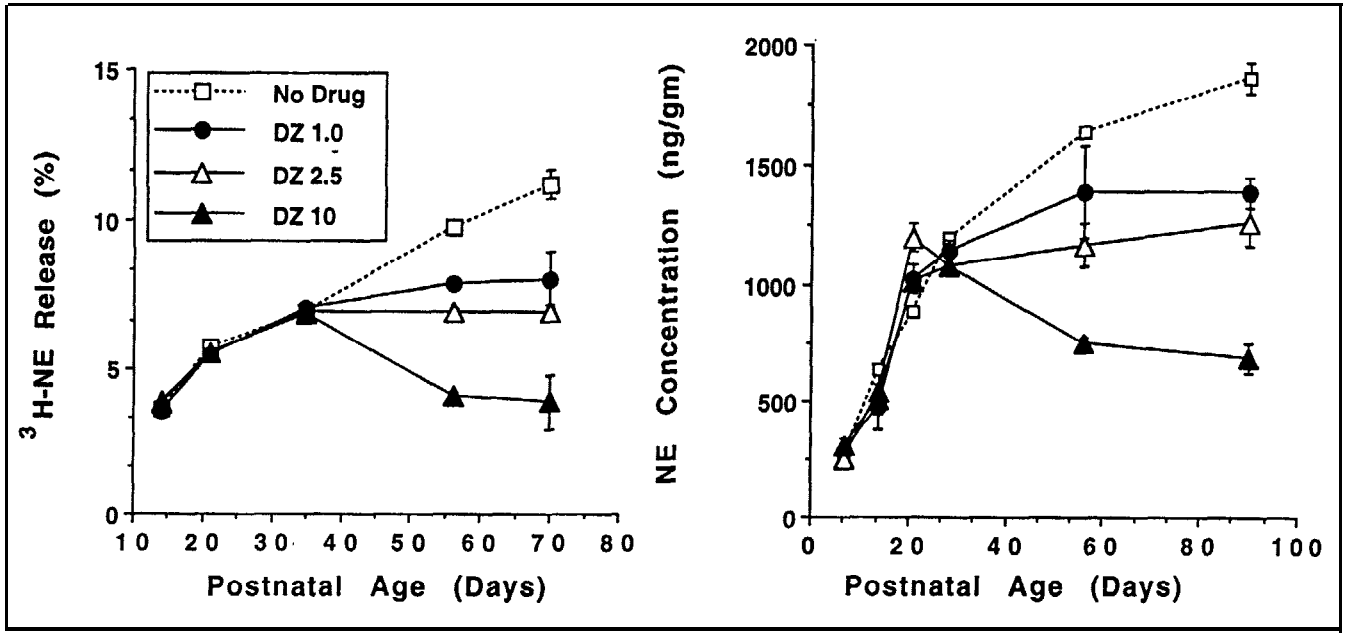

FIGURE 6. *Effect of prenatal exposure to DZ on NE projections to the hypothalamus, Pregnant dams were injected with DZ (1.0, 2.5, or 10 mg/kg) over gestational days 13 through 20. Controls were unmanipulated. Animals (males and females) were tested at varying postnatal ages (from 7 to 90 days of age). Data are expressed as in vivo NE levels of nanograms per gram (ng/g) in right figure or in vitro release of [^{3}H]NE in left figure.*

SOURCES: Kellogg and Retell 1986; Simmons et al. 1984*a*.

hypothalamic NE projections were prevented by coexposure to a central-type BZD antagonist. The impact of prenatal DZ exposure appears to be limited to selective NE projection sites and corticotrophin-releasing factor-containing cells as indicated by immunocytochemical analysis (Inglefield et al. 1993). Of particular interest, the effect of the early exposure on hypothalamic NE projections was not apparent until late in adolescent development (see figure 6). Recent work has shown that this projection system in naive control animals undergoes changes in function over adolescence (Choi and Kellogg 1992). Environmental challenges also alter dopamine (DA) utilization, particularly in the prefrontal cortex (Deutch and Roth 1990; Thierry et al. 1976), and prenatal exposure to DZ interferes with stressor-induced changes in select DA projection systems (Deutch et al. 1989). The effects of the early exposure on select DA systems were clearly present in 90-day-old rats, but not distinguishable from implantation controls at 60 days (Gruen et al. 1990). Thus, early developmental BDZ exposure appears to target stressor-responsive neural systems and, when evaluated, these systems appear to be destined to undergo changes during adolescent development.

A BIOLOGIC MECHANISM UNDERLYING THE EFFECTS OF EARLYDEVELOPMENTALDZEXPOSURE

As demonstrated, early developmental exposure to DZ, presumably acting via interaction at the $GABA_A$ receptor in the developing organism, affects integrated responses to environmental challenges, but the effects of the early exposure are not readily apparent until late in adolescent development. Developmental drug exposure could influence later behavioral responses selectively, after all drug has been cleared from the organism, only if the drug altered or reorganized some aspect of brain function. Clearly, DZ administered prenatally (at doses that markedly affect adaptive responses later in life) is cleared from the exposed organism between 10 and 20 days postnatal age (Simmons et al. 1983). Thus, effects of the early exposure that appear after the onset of puberty (around 30 to 32 days of age in male rats) cannot be attributed to action from persisting drug.

It now seems evident that the $GABA_A$ receptor in adult organisms plays an important role in adaptive behavioral responses. Furthermore, responsiveness of cortical $GABA_A$ receptors to environmental challenges normally emerges over adolescent development. The effects of early exposure to BZDs, which modulate the action of GABA at the $GABA_A$

receptor, suggest, therefore, that $GABA_A$ receptors play a role in the neural organization underlying integrated adaptive responses. However, since so many of the effects of early exposure to DZ do not become apparent until late in adolescent development, the neural targets of DZ action in utero must be recruited or undergo some change during adolescence.

A trophic role for GABA during early development has been proposed (Wolff et al. 1993), and by modulating the response to $GABA_A$ early DZ exposure could directly influence neural organization. On the other hand, DZ could also influence the action of other trophic factors via its interaction at the $GABA_A$ receptor. Sex steroids, for example, are known to exert organizational effects on the developing brain, stimulating axonal and dendritic growth and synapse formation (Matsumoto 1991). Puberty also seems to be a developmental stage associated with synapse formation, possibly influenced by steroid action. Clearly, steroids influence adolescent development of neural and behavioral adaptive responses. The early organizational actions of steroids are thought to be mediated via steroids binding to intracellular receptors and influencing genetic expression. Recent studies, however, have shown that metabolites of specific sex steroids can also exert direct membrane effects; in particular, several steroids have been shown to interact directly with the $GABA_A$ receptor and modulate the action of GABA and other compounds that act at the complex (Paul and Purdy 1992). An organizational action of steroids mediated via the $GABA_A$ receptor would provide a mechanism whereby the sex steroids could exert a separate influence on nonreproductive behaviors, in particular behaviors whose developmental profile parallel that of reproductive behaviors. Perhaps action at a membrane receptor is also a mechanism whereby steroids could influence later neural circuitry.

As shown earlier, prenatal exposure to DZ affects adaptive but not reproductive behaviors later in life. The presence of DZ in the brain during early development could interfere with the action of steroids at the $GABA_A$ receptor, and this interference could then selectively affect nonreproductive behaviors and underlying neural mechanisms without altering reproductive functions.

The delayed appearance of the effects of early DZ exposure in experimental animals until late in adolescent development mirrors the timecourse for the appearance of many clinical behavioral disorders such as schizophrenia and depression (Iacono and Beiser 1989). Early

developmental insults at the molecular level may indeed contribute to the underlying etiology of such disorders. The perinatal action of BZD may disrupt control of neuronal excitability or direct trophic activity mediated by the $GABA_A$ receptor. In any case, the altered $GABA_A$ receptor function apparently disrupts developmental influences of the genome and/or external environment resulting in altered coping responses later in life. Since this receptor complex is the site of action of a variety of endogenous and exogenous agents (Schwartz 1988), it could represent a major point of vulnerability in neural development.

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ACKNOWLEDGMENT

This chapter was prepared with support from National Institute on Drug Abuse grant no. DA-07080.

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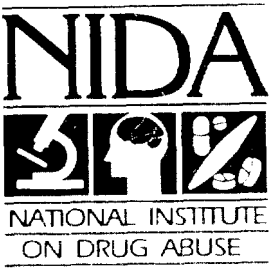

NIH Publication No. 95-4024
Printed 1995